Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in Asia: a randomised, double-blind, placebo-controlled trial

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Summary

Background Rotavirus vaccine has proved effective for prevention of severe rotavirus gastroenteritis in infants in developed countries, but no efficacy studies have been done in developing countries in Asia. We assessed the clinical efficacy of live oral pentavalent rotavirus vaccine for prevention of severe rotavirus gastroenteritis in infants in Bangladesh and Vietnam.

Methods In this multicentre, double-blind, placebo-controlled trial, undertaken in rural Matlab, Bangladesh, and urban and periurban Nha Trang, Vietnam, infants aged 4–12 weeks without symptoms of gastrointestinal disorders were randomly assigned (1:1) to receive three oral doses of pentavalent rotavirus vaccine 2 mL or placebo at around 6 weeks, 10 weeks, and 14 weeks of age, in conjunction with routine infant vaccines including oral poliovirus vaccine. Randomisation was done by computer-generated randomisation sequence in blocks of six. Episodes of gastroenteritis in infants who presented to study medical facilities were reported by clinical staff and from parent recollection. The primary endpoint was severe rotavirus gastroenteritis (Vesikari score ≥11) arising 14 days or more after the third dose of placebo or vaccine to end of study (March 31, 2009; around 21 months of age). Analysis was per protocol; infants who received scheduled doses of vaccine or placebo without intervening laboratory-confirmed naturally occurring rotavirus disease earlier than 14 days after the third dose and had complete clinical and laboratory results were included in the analysis. This study is registered with ClinicalTrials.gov, number NCT00362648.

Findings 2036 infants were randomly assigned to receive pentavalent rotavirus vaccine (n=1018) or placebo (n=1018). 991 infants assigned to pentavalent rotavirus vaccine and 978 assigned to placebo were included in the per-protocol analysis. Median follow up from 14 days after the third dose of placebo or vaccine until final disposition was 498 days (IQR 480–575). 38 cases of severe rotavirus gastroenteritis (Vesikari score ≥11) were reported during more than 1197 person-years of follow up in the vaccine group, compared with 71 cases in more than 1156 person years in the placebo group, resulting in a vaccine efficacy of 48·3% (95% CI 22·3–66·1) against severe disease (p=0·0005 for efficacy >0%) during nearly 2 years of follow-up. 25 (2·5%) of 1017 infants assigned to receive vaccine and 20 (2·0%) of 1018 assigned to receive placebo had a serious adverse event within 14 days of any dose. The most frequent serious adverse event was pneumonia (vaccine 12 [1·2%]; placebo 15 [1·5%]).

Interpretation In infants in developing countries in Asia, pentavalent rotavirus vaccine is safe and efficacious against severe rotavirus gastroenteritis, and our results support expanded WHO recommendations to promote its global use.

Funding PATH (GAVI Alliance grant) and Merck.

Lancet 2010; 376: 615–23

Published Online
August 6, 2010
DOI:10.1016/S0140-6736(10)60755-6

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Introduction

Rotavirus is the most common cause of severe gastroenteritis in infants and young children in the world.[1] In 2004, WHO calculated that six countries in Asia accounted for 215 896 of the estimated 527 000 deaths attributable to rotavirus worldwide, and that 196 000 of all deaths were in developing countries in Asia with high rates of childhood mortality.[1] Although improvements in sanitation might shift the occurrence of rotavirus infection from younger to older children (ie, when a child has more physiological reserve to survive severe gastroenteritis),[2] successful vaccination is the best option for reduction of disease burden and mortality in Asian populations, for whom the occurrence of rotavirus remains high in early life.

Two new, live, oral rotavirus vaccines have been developed and shown to be safe and effective against severe rotavirus gastroenteritis in developed populations.[3–5] In 2005, WHO's Strategic Advisory Group of Experts (SAGE) reviewed efficacy data for these vaccines and urged the manufacturers and global public health community to obtain efficacy data for these vaccines in Asia and Africa, because of a history of poor capability of live oral vaccines for protection of the poorest children in developing countries, particularly in Asia.[6] SAGE noted that additional efficacy studies would not need to be large, but should be representative of regional populations and should assess rotavirus vaccines in the context of each country's Expanded

Program on Immunization. In response to this mandate, the PATH Rotavirus Vaccine Program and Merck partnered with investigators in Asia to assess the efficacy of pentavalent rotavirus vaccine in populations in developing countries. We aim to assess clinical efficacy of this vaccine for prevention of severe rotavirus gastroenteritis in infants in Bangladesh and Vietnam.

Methods

Participants and study design

Our multicentre, double-blind (with sponsor blinding), placebo-controlled trial was designed to assess the efficacy of three doses of pentavalent rotavirus vaccine (RotaTeq; Merck, Whitehouse Station, NJ, USA) against severe rotavirus gastroenteritis in infants in representative low-income populations in Asia. The investigation was done from March 29, 2007, to March 31, 2009, in rural Matlab, Bangladesh, and from Sept 28, 2007, to March 31, 2009, in urban and periurban Nha Trang, Vietnam.

Infants between 4 and 12 weeks of age were eligible for enrolment if they had no symptoms of active gastrointestinal disease and could be adequately followed up for assessment of safety by home visit or telephone contact (1 and 2 weeks after each dose of study vaccine or placebo). Breastfeeding was not restricted. There were no enrolment restrictions based on HIV status, and HIV testing was not done at the study sites. The investigation was designed to identify severe gastroenteritis in participants upon presentation to medical facilities in the study areas.

The trial was approved by the investigators' institutional review boards and the Western Institutional Review Board (Olympia, WA, USA). Written informed consent was obtained from parents or guardians of all participants. The study was done in accordance with the principles of the Declaration of Helsinki, and in compliance with good clinical practice guidelines.

Procedures

Infants were randomly assigned in a 1:1 ratio to receive three oral doses of pentavalent rotavirus vaccine 2 mL or placebo at around 6 weeks, 10 weeks, and 14 weeks of age, according to site-specific childhood immunisation schedules. Doses were given with other routine paediatric vaccines, including oral poliovirus vaccine. Pentavalent rotavirus vaccine contains five human–bovine reassortant rotaviruses, with the WC3 bovine strain as backbone and viral surface proteins corresponding to human rotavirus serotypes G1, G2, G3, G4, and P1A[8].[3] Every dose of vaccine had an estimated potency of 2×10^7 infectious units per reassortant rotavirus in about 2 mL of buffered liquid. Placebo contained the same constituents as the active vaccine but without viral antigens.

Infants who received vaccine or placebo were visited once a month to remind parents to bring their child to a clinic or hospital if their child developed symptoms of gastroenteritis. We documented all serious adverse events occurring within 14 days of every dose and deaths or vaccine-related serious adverse events occurring at any time during the study. Serious adverse events were classified with the US regulatory definition, in line with ICH guidance, and identified by monthly query and parental reporting at any time or identification by study staff in hospitals or clinics. Intussusception at any time was assessed with an additional detailed protocol. All these events were monitored by an independent, unmasked, data and safety monitoring board that met about twice a year during the course of the investigation. The board also provided guidance about enrolment and severity scoring.

For assessment of immune responses to vaccination, a small amount of venous blood was obtained immediately before the first dose of study vaccine or placebo was given and about 14 days after the third dose was given in a subset of around 300 participants (around 150 per site).

Rotavirus antigen in stool was detected by enzyme immunoassay.[3] Wild-type rotavirus was confirmed by RT-PCR for identification of the VP6 genotype. Identification of rotavirus P and G genotypes was done by RT-PCR as previously described.[7] Antirotavirus IgA and serum neutralising antibodies were measured as described elsewhere.[3,8–10] Enzyme immunoassay, IgA, and serum neutralising antibody assays were done at the Children's Hospital Medical Center (Cincinnati, OH, USA), and RT-PCR assays were done at Merck Research Laboratories (Wayne, PA, USA).

Randomisation and masking

Unique allocation numbers were designated at Merck as pentavalent rotavirus vaccine or placebo with computer-generated block randomisation, with block sizes of six. Vaccine and placebo packages were then labelled with allocation numbers and provided to sites in identical presentations. Sites were instructed to assign allocation numbers to participants in sequential order as they were enrolled. Participants were enrolled by study staff, who remained masked to treatment assignment throughout the trial. Only the data and safety monitoring board and an associated Merck statistician, who was not involved in the rest of the trial, were unmasked to treatment assignment; the statistician from Merck who analysed the data and the Merck and PATH protocol teams were masked to treatment assignment.

Primary and secondary outcomes

The primary outcome was severe rotavirus gastroenteritis, irrespective of serotype, occurring 14 days or more after the third dose of vaccine or placebo until end of study. Gastroenteritis was defined as three or more watery or looser-than-normal stools within a 24 h period, or forceful vomiting. Stool samples were obtained from participants with gastroenteritis who reported to a medical facility. Information about past symptoms was obtained retrospectively through interview with the parent or guardian, and occurrence

of persisting signs and symptoms was collected prospectively by medical staff caring for the participant via direct observation and interviews with the parent or guardian. Severity was defined by use of the 20-point modified Vesikari clinical scoring system, with a score of 11 or more classified as severe.[11]

Secondary outcomes were efficacy of vaccination against rotavirus gastroenteritis of any severity; disease scoring 15 or more or 19 or more with the Vesikari clinical scoring system; severe disease by individual serotype; severe disease and disease of any severity between doses; severe gastroenteritis of any cause; and rotavirus gastroenteritis with a score of 17 or more with the 24-point Clark clinical scoring system.[3] Post-hoc analyses were efficacy against severe rotavirus gastroenteritis 14 days or more after the third dose of vaccine or placebo in the first year of life, in the second year of life, and by country.

Other secondary outcomes in each group consisted of proportion of participants with a serious adverse event within 14 days of any dose; the proportion of participants with seroresponse (defined as at least a three-fold titre rise from baseline to after the third dose of placebo or vaccine) for antirotavirus IgA and for serum neutralising antibodies against human rotavirus serotypes G1, G2, G3, G4, and P1A[8]; and geometric mean titres for th s.

Statistical analysis

Vaccine efficacy was defined as $(1–R_{vaccine}/R_{placebo})\times100\%$, where R is the person-time incidence rate for the respective groups. A-priori assumptions were that the number of cases in each group followed a Poisson distribution; the statistical analysis conditioned on the total number of participants with severe rotavirus

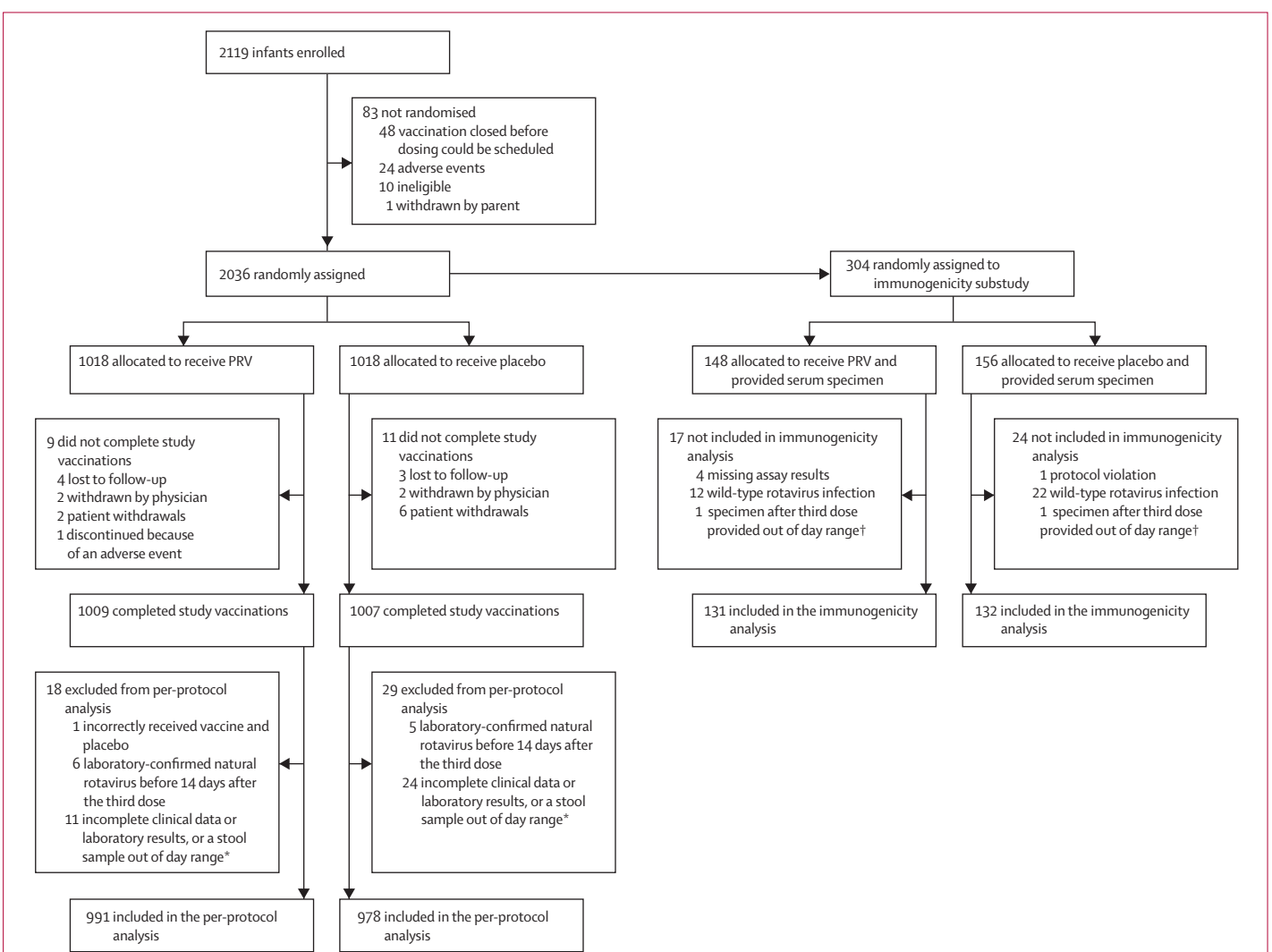

Figure 1: **Trial profile**
*Stool sample obtained more than 14 days after onset of illness. †Participants who had serum collected before day 9 or after day 33 after dose 3.

gastroenteritis from both treatment groups, such that the numbers of participants with severe rotavirus gastroenteritis in the vaccine group had a binomial distribution.[12] For participants with more than one episode of severe rotavirus gastroenteritis, only the first episode was counted. Exact inference was used, and follow-up time was accounted for in the calculations.

The main analyses of efficacy were based on the per-protocol participant population; infants who received scheduled doses of vaccine or placebo without intervening laboratory-confirmed naturally occurring rotavirus disease earlier than 14 days after the third dose were included in the analysis. Participants with at least one episode that could not be classified with certainty as rotavirus gastroenteritis or non-rotavirus gastroenteritis because of incomplete data—and with no later episodes that could be confirmed as disease—were excluded from the per-protocol analysis. An intention-to-treat analysis for efficacy against severe rotavirus gastroenteritis was also done, which included all participants who received at least one dose of vaccine or placebo, including protocol violators, and with case assessment starting immediately after dose 1.

On the assumption of a true efficacy of 70%, a severe rotavirus gastroenteritis capture rate of 3·5%, and a 20% patient non-assessment rate, 2036 infants were enrolled to provide around 93% statistical power to detect vaccine efficacy against severe rotavirus gastroenteritis of more than 0%.

95% CIs for rate reduction ($R_{placebo}$–$R_{vaccine}$) were derived with the method of Miettinen and Nurminen.[13]

Our analysis of immunogenicity was also based on the per-protocol patient population; participants with intervening laboratory-confirmed wild-type rotavirus disease were excluded from this analysis. Seroresponse rates were calculated with corresponding 95% CIs on the basis of binomial distributions, and geometric mean titres with normal distributions. Analyses were done with SAS version 8.0.

This study is registered with ClinicalTrials.gov, number NCT00362648.

Role of funding source

The study was designed by Merck investigators, with substantial input from PATH staff and site investigators. Merck had direct oversight or participation in every stage of the study. Merck also participated in pharmacovigilance, organised and led the data and safety monitoring board meetings, and did the data analysis. Staff from PATH independently monitored study execution at sites and participated in pharmacovigilence, data analysis, and data and safety monitoring board meetings. All authors had full access to the data, and the corresponding author had final responsibility for the decision to submit for publication.

	Bangladesh		Vietnam		Overall	
	PRV	Placebo	PRV	Placebo	PRV	Placebo
Number of infants randomly assigned and doses received						
Dose 1*	568	568	450	450	1018	1018
Dose 2	566	565	447	444	1013	1009
Dose 3	563	565	446	442	1009	1007
Age (weeks)						
Dose 1						
Mean	8·3 (1·3)	8·2 (1·3)	9·7 (1·2)	9·7 (1·3)	8·9 (1·5)	8·8 (1·5)
Median	8·1 (5·9–11·7)	8·1 (5·9–11·7)	9·9 (7·3–12·0)	9·7 (7·4–11·9)	8·9 (5·9–12·0)	8·7 (5·9–11·9)
Dose 2						
Mean	12·9 (1·5)	12·7 (1·5)	14·3 (1·5)	14·3 (1·5)	13·5 (1·7)	13·4 (1·7)
Median	12·7 (10·0–19·0)	12·6 (9·9–18·4)	14·4 (11·7–19·4)	14·3 (11·7–19·6)	13·4 (10·0–19·4)	13·3 (9·9–19·6)
Dose 3						
Mean	17·4 (1·6)	17·3 (1·7)	19·4 (1·6)	19·3 (1·7)	18·3 (1·9)	18·2 (1·9)
Median	17·1 (14·0–23·1)	17·3 (13·9–23·0)	19·4 (16·1–25·9)	19·3 (16·1–25·6)	18·3 (14·0–25·9)	18·1 (13·9–25·6)
Sex						
Boys	305 (54%)	279 (49%)	249 (55%)	247 (55%)	554 (54%)	526 (52%)
OPV co-administered						
Dose 1	565 (99%)	568 (100%)	401 (89%)	411 (91%)	966 (95%)	979 (96%)
Dose 2	561 (99%)	559 (99%)	414 (93%)	407 (92%)	975 (96%)	966 (96%)
Dose 3	557 (99%)	560 (99%)	394 (88%)	389 (88%)	951 (94%)	949 (94%)
Record of OPV birth dose†	140 (25%)	171 (30%)	0	0	140 (14%)	171 (17%)

Data are mean (SD), median (IQR), or number (%) unless otherwise stated. PRV=pentavalent rotavirus vaccine. OPV=oral poliovirus vaccine. *All participants randomly assigned to study groups received at least one dose of vaccine or placebo. †Received within 28 days of birth.

Table 1: **Baseline characteristics of trial participants**

	Pentavalent rotavirus vaccine			Placebo			Vaccine efficacy, % (95% CI)	Rate reduction* (95% CI)
	Cases (n)	Person-years	Incidence*	Cases (n)	Person-years	Incidence*		
RVGE of any severity†	65	1185·6	5·5	109	1143·4	9·5	42·5% (21·1 to 58·4)	4·1 (1·8 to 6·4)
Severe RVGE (score of ≥11 by VCSS)†	38	1197·3	3·2	71	1156·9	6·1	48·3% (22·3 to 66·1)‡	3·0 (1·2 to 4·8)
Severe RVGE scoring ≥15 by VCSS†	8	1215·2	0·7	26	1184·8	2·2	70·0% (31·8 to 88·3)	1·5 (0·6 to 2·6)
Severe RVGE (score of ≥11 by VCSS, after dose 1)§	39	1416·9	2·8	72	1364·9	5·3	47·8% (21·9 to 65·6)	2·5 (1·1 to 4·1)
Severe GE of any cause (score of ≥11 by VCSS)†	81	1002·6	8·1	107	967·3	11·1	27·0% (1·6 to 46·0)	3·0 (0·3 to 5·8)
RVGE (score of ≥17 by CCSS)†	4	1219·8	0·3	7	1216·0	0·6	43·0% (–124·1 to 87·8)	0·2 (–0·3 to 0·9)

There were no cases of rotavirus gastroenteritis (RVGE) scoring 19 or more by the Vesikari clinical scoring system (VCSS). CCSS=Clark clinical scoring system. GE=gastroenteritis. *Per 100 person-years. †Per-protocol analyses excluded participants who received fewer than three doses, incorrectly received vaccine and placebo, had no follow-up, or had laboratory-confirmed naturally occurring rotavirus before the start of the efficacy follow-up period. Participants whose classification could not be established because of incomplete clinical or laboratory data or with stool samples obtained out of day range were excluded from the per-protocol analysis. Out of day range refers to participants who had stool sample collected more than 14 days after start of clinical episode. ‡p=0·0005 for efficacy greater than 0%. §Intention-to-treat analysis.

Table 2: **Efficacy of pentavalent rotavirus vaccine for prevention of rotavirus gastroenteritis and gastroenteritis for complete follow-up in Asia**

Results

Figure 1 shows the trial profile. 2119 infants were enrolled, of whom 2036 (1136 in Bangladesh and 900 in Vietnam) were randomly assigned to study group and received at least one dose of vaccine or placebo. 991 participants in the vaccine group and 978 participants in the placebo group were included in the per-protocol analysis. Table 1 shows baseline characteristics of study participants. Median follow-up time from 14 days after the third dose of placebo or vaccine until final disposition was 498 days (IQR 480–575) for all randomised participants (excluding those with protocol violations; 554 days [IQR for vaccine 495–631 and placebo 493–631] in Bangladesh and 496 days [IQR for both groups 480–496] in Vietnam). Median age at study end was 21 months.

174 cases of rotavirus gastroenteritis were reported in the primary efficacy period, of which 109 (63%) were severe (Vesikari score ≥11), and were used in the primary analysis. For nearly 1200 person-years of follow-up, overall vaccine efficacy against severe disease (Vesikari score ≥11) was more than 48% (table 2). With a cutoff Vesikari score of 15 or more, efficacy was around 70%. Vaccine efficacy was also shown against rotavirus gastroenteritis of any severity and severe gastroenteritis of any cause (table 2). In the intention-to-treat analysis, efficacy against severe rotavirus gastroenteritis (Vesikari score ≥11) for the entire study follow-up period was more than 47% (table 2).

Vaccine efficacy against severe rotavirus gastroenteritis was around 43% in Bangladesh compared with nearly 64% in Vietnam (table 3). However, more severe cases were prevented per 100 person-years in Bangladesh than were prevented in Vietnam (table 3, figure 2). With a criterion of Vesikari score 15 or more, point estimates for efficacy against rotavirus gastroenteritis for Bangladesh and Vietnam were similar in both countries, at 71·0% (95% CI 18·1–91·6) in Bangladesh and 68·1% (–28·0 to 94·4%) in Vietnam. In Bangladesh and Vietnam, vaccine efficacy against severe rotavirus gastroenteritis (Vesikari score ≥11) was slightly higher in the first year of life than it was in the second year of life (table 3).

Between-dose efficacy could not be reliably estimated because there were few cases of severe rotavirus gastroenteritis before the three-dose vaccination series was completed. No cases occurred in vaccine or placebo groups between 14 days after dose one and dose two, and only one infant in each group had the disorder from 14 days after dose two and dose three.

For participants who had complete molecular testing results, most cases of severe rotavirus gastroenteritis (79 [92%] of 86 in Bangladesh, and all [100%] of 20 in Vietnam) were caused by viruses with G or P genotypes contained in the vaccine. By individual rotavirus genotype, estimates of efficacy against severe rotavirus gastroenteritis were consistent, although less precise because of low numbers: G1 (46·2%, 95% CI –13·5 to 75·7), G2 (29·2%, –159·0 to 82·3), G3 (67·0%, –8·9 to 92·2), G9 (48·7%, –7·3 to 76·8), P1A[8] (49·7%, 19·2 to 69·3), P1B[4] (40·9%, –79·4 to 82·4), and P2A[6] (60·5%, –141·3 to 96·2).

IgA seroresponse after receipt of pentavalent rotavirus vaccine was high (115 [87·8%] of 131 participants; table 4). 50 (78·1%) of 64 Bangladeshi infants (95% CI 66·0–87·5) had an IgA seroresponse, as did 65 (97·0%) of 67 Vietnamese infants (89·6–99·6). This predisposition to higher immunogenicity in recipients of pentavalent rotavirus vaccine in Vietnam compared with those in Bangladesh was also noted in the geometric mean titres of IgA (29·1 units per mL [95% CI 18·6–45·7] in Bangladesh *vs* 158·5 units per mL [107·0–234·6] in Vietnam). Geometric mean titres of serum neutralising antibodies against specific rotavirus serotypes ranged from 23·0 for serotype G2 to 95·5 for serotype G1 (table 4). However, seroresponse rates for serum neutralising antibodies (ie, a three-fold or higher rise from baseline to after dose 3) were low in these settings (table 4), in which infants had high concentrations of serum neutralising antibodies before the first dose of vaccine or placebo for most of the serotypes that we tested (data not shown).

Table 5 shows serious adverse events within 14 days of any dose of vaccine or placebo. The most common

	Pentavalent rotavirus vaccine			Placebo			Vaccine efficacy, % (95% CI)	Rate reduction* (95% CI)
	Cases (n)	Person-years	Incidence*	Cases (n)	Person-years	Incidence*		
Entire study period (14 days after third dose to end of follow-up)†								
Overall	38	1197·3	3·2	71	1156·9	6·1	48·3% (22·3 to 66·1)‡	3·0 (1·2 to 4·8)
Bangladesh	33	712·1	4·6	56	692·1	8·1	42·7% (10·4 to 63·9)	3·5 (0·8 to 6·2)
Vietnam	5	485·2	1·0	15	464·7	3·2	63·9% (7·6 to 90·9)	2·2 (0·4 to 4·4)
First year of life (14 days after third dose to age 365 days)								
Overall	19	605·9	3·1	38	594·3	6·4	51·0% (12·8 to 73·3)	3·3 (0·8 to 5·9)
Bangladesh	17	345·6	4·9	31	342·4	9·1	45·7% (–1·2 to 71·8)	4·1 (0·2 to 8·4)
Vietnam	2	260·3	0·8	7	251·9	2·8	72·3% (–45·2 to 97·2)	2·0 (–0·4 to 5·1)
Second year of life (age 366–730 days)								
Overall	19	586·4	3·2	33	555·6	5·9	45·5% (1·2 to 70·7)	2·7 (0·2 to 5·4)
Bangladesh	16	355·7	4·5	25	337·5	7·4	39·3% (–18·3 to 69·7)	2·9 (–0·7 to 6·9)
Vietnam	3	230·7	1·3	8	218·1	3·7	64·6 %(–47·7 to 93·9)	2·4 (–0·6 to 6·1)

Per-protocol analyses excluded participants who received fewer than three doses, incorrectly received vaccine and placebo, had no follow-up, or had laboratory-confirmed naturally occurring rotavirus before the start of the efficacy follow-up period. Participants whose classification could not be established because of incomplete clinical or laboratory data or with stool samples obtained out of day range were not assessed. *Per 100 person-years. †There was follow-up beyond the second year of life, although no cases were reported. ‡p=0·0005 for efficacy greater than 0%.

Table 3: **Efficacy of the pentavalent rotavirus vaccine for prevention of severe rotavirus gastroenteritis (Vesikari score ≥11) in Asia by country and follow-up period**

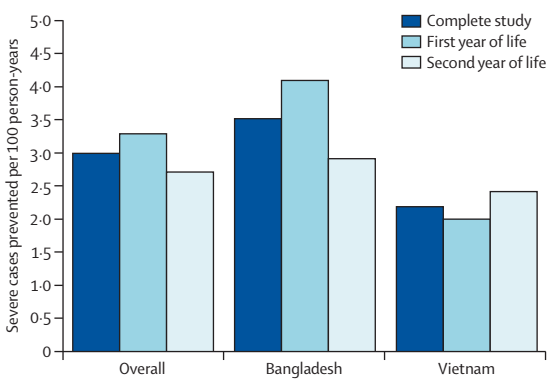

Figure 2: **Cases of severe rotavirus gastroenteritis prevented by pentavalent rotavirus vaccine per 100 person-years (rate reduction)**
95% CIs for the estimates are shown in table 3.

serious adverse event in both groups was pneumonia. Seven participants died during the study, three (0·3%) in the pentavalent rotavirus vaccine group and four (0·4%) in the placebo group. The most common causes of death were drowning and sepsis, with one instance of each in the vaccine and placebo groups. In Vietnam, there was one abdominal ultrasound-confirmed case of intussusception arising 97 days after the third dose of placebo. An air enema reduction was done and the infant recovered fully. All serious adverse events were regarded by site investigators as unrelated to study intervention.

Discussion

In our double-blind, placebo-controlled, trial in the developing Asian countries of Bangladesh and Vietnam, pentavalent rotavirus vaccine was efficacious for prevention of severe rotavirus gastroenteritis in infants for nearly 2 years of follow-up. Moderately high vaccine efficacy in the first year of life led to a substantial reduction of severe rotavirus gastroenteritis. Efficacy was slightly reduced in the second year of life, but, combined with a lower background incidence of the disorder than was reported in the first year (table 3), still led to a significant reduction in incidence of severe rotavirus gastroenteritis.

Our study is the first clinical efficacy trial of an already licensed rotavirus vaccine in developing countries in Asia. Although pentavalent rotavirus vaccine significantly reduced the burden of severe disease, our reported efficacy measurements were lower than were those reported in trials of this vaccine in more industrialised countries in the USA, Europe, and Latin America.[3,14–16] Although many factors could have led to the lower estimate of vaccine efficacy, our estimates might not be comparable with those reported in previous trials in the developed world. Studies were designed differently and used different clinical scoring systems. Furthermore, inclusion criteria were broad in this trial, and severity scores might have been less precise than in other trials because clinical measurement procedures were done only during stay in a medical facility, and relied on parental recall.

Higher efficacy point estimates were consistently measured in Vietnam than in Bangladesh, apart from efficacy against severe rotavirus gastroenteritis with a Vesikari score of 15 or more. These differences might be attributable to a low degree of precision in this study, because it was not designed to make statistical comparisons between countries. Alternatively, these differences could be a result of the different socioepidemiological circumstances of the study populations in the two countries' study sites. For example, before the start of the study, the infant mortality rate in the study areas was two times higher in Bangladesh than it was in Vietnam

	Seroresponse				Geometric mean titre (95% CI)†	
	PRV (N=131)		Placebo (N=132)		PRV (N=131)	Placebo (N=132)
	n	% (95% CI)	n	% (95% CI)		
IgA	115	87·8% (80·9–92·9)	24	18·2% (12·0–25·8)	69·3 (49·9–96·2)	2·9 (2·2–3·9)
SNA to serotype G1	42	32·1% (24·2–40·8)	3	2·3% (0·5–6·5)	95·5 (76·6–119·0)	19·9 (17·1–23·2)
SNA to serotype G2	13	9·9% (5·4–16·4)	1	0·8% (0·0–4·1)	23·0 (19·7–26·8)	12·5 (11·0–14·3)
SNA to serotype G3	37	28·2% (20·7–36·8)	4	3·0% (0·8–7·6)	30·8 (25·2–37·7)	10·1 (8·7–11·7)
SNA to serotype G4	24	18·3% (12·1–26·0)	0	0% (0–2·8)	51·4 (43·1–61·2)	15·7 (13·7–18·0)
SNA to serotype P1A[8]	36	27·5% (20·0–36·0)	7	5·3% (2·2–10·6)	78·9 (65·1–95·5)	18·0 (14·8–21·9)

N is number of participants in immunogenicity subset, n is number of participants with a three-fold or higher rise in seroresponse rates from baseline. PRV=pentavalent rotavirus vaccine. SNA=serum neutralising antibodies. *Excludes protocol violators, participants with invalid data based on laboratory determinations, participants with rotavirus-positive stool antigen enzyme immunoassay results, and participants with samples taken out of a specified day range. Out of day range refers to participants who has serum collected before day 9 or after day 33 after dose 3. †Geometric mean titres for IgA are units per mL, and are dilution units for SNAs.

Table 4: **Antirotavirus IgA and SNA seroresponse rates and geometric mean titres after the third dose in recipients of pentavalent rotavirus vaccine and placebo in the immunogenicity subset***

(29·7 deaths per 1000 livebirths in Bangladesh[17] *vs* 14·7 deaths per 1000 livebirths in Vietnam[18]). Incidence of severe rotavirus gastroenteritis (Vesikari score ≥11) in the first year of life was substantially higher in Bangladesh than in Vietnam, suggesting a higher force of infection in Bangladesh. Equally, about 75% of children in developing countries have their first rotavirus infection before the age of 12 months,[19] a time in their early childhood when they are most susceptible to diarrhoeal disease morbidity and mortality. Finally, the difference in rates of severe rotavirus gastroenteritis measured between the sites might have been attributable to different case-capture sensitivity and the health-care-seeking behaviours of the participants' carer givers, although this should not have been different between vaccine and placebo groups.

Although there is no known immune correlate of protection for rotavirus, serum antirotavirus IgA response or serum neutralising antibody response might be a metric of vaccine efficacy.[3,14,20] Serum IgA seroresponse and geometric mean titres after the third dose of vaccine or placebo were higher in children in Vietnam than in children in Bangladesh. Furthermore, IgA geometric mean titres measured in infants in Vietnam were similar to those in infants in Latin America,[8] but IgA antibody concentrations measured in infants in Bangladesh were similar to those of impoverished populations in Africa.[21] This difference might be because of dissimilar epidemiological and socioeconomic circumstances for infants in Bangladesh and Vietnam.

Geometric mean titres of serum neutralising antibodies to the human rotavirus serotypes contained in pentavalent rotavirus vaccine varied across a wide range (table 4), and were similar to those reported in children in Latin America[8] (which ranged from 21·2 for serotype G3 to 125·8 for serotype G1). However, we noted a lower serum neutralising antibody seroresponse than has been reported previously.[8] This low seroresponse was probably attributable to high concentrations of serum neutralising antibodies before the first dose of vaccine or placebo, because of high concentrations of residual maternal (transplacental or breast milk) antibodies. However, results from specific antibody tests (ie, IgA or serum neutralising antibody) should not be overinterpreted.

Several factors affect the immune response to live oral vaccines, including the concentration of transplacentally acquired maternal antibody, immune and non-immune components of breast milk, the amount of gastric acid in the digestive tract, micronutrient malnutrition, interfering gut flora, and diarrhoeal and immune system diseases.[22] Additionally, oral poliovirus vaccine reduces the immunogenicity of live oral rotavirus vaccines when given concomitantly,[8,23,24] and, unlike in previous efficacy trials of pentavalent rotavirus vaccine, around 90% of infants in

	Pentavalent rotavirus vaccine (n=1017)*	Placebo (n=1018)
Infants with one or more serious adverse events	25 (2·5%)	20 (2·0%)
Gastrointestinal disorders	4 (0·4%)	4 (0·4%)
Diarrhoea	4 (0·4%)	4 (0·4%)
General disorders	1 (0·1%)	0
Pyrexia	1 (0·1%)	0
Infections	19 (2·0%)	17 (1·7%)
Bronchiolitis	3 (0·3%)	0
Bronchitis	3 (0·3%)	0
Cytomegalovirus	1 (0·1%)	0
Dysentery	1 (0·1%)	0
Gastroenteritis	0	1 (0·1%)
Pneumonia	12 (1·2%)	15 (1·5%)
Sepsis	1 (0·1%)	0
Upper respiratory tract infection	0	1 (0·1%)
Other†	4 (0·4%)	1 (0·1%)

Data are n (%). *One participant left the study immediately after dose 1 and contributed no follow-up time for safety analysis. †Anaemia, cardiopulmonary failure, hepatic failure, omphalitis, or head injury. Every patient is counted once for every applicable specific adverse event. Participants with multiple adverse events within a system organ class were counted once for that system organ class.

Table 5: **Serious adverse events 1–14 days after any dose of pentavalent rotavirus vaccine or placebo**

this study received both vaccines concomitantly. Infants in Bangladesh might, in fact, have lower immune responses to pentavalent rotavirus vaccine than do infants in Vietnam, which puts them closer to an unknown minimum threshold for protection. Understanding of the contribution of these factors to low immune responses and efficacy in specific populations might allow investigators to design immunisation programmes and vaccines that will be more effective for prevention of severe rotavirus gastroenteritis than those we have at present.

Two rotavirus vaccines, RotaTeq and Rotarix (GlaxoSmithKline, Rixensart, Belgium), have been approved for use by national regulatory authorities in many countries worldwide. However, until efficacy results of trials from representative regional populations were available, neither vaccine could be incorporated into the routine public health immunisation programmes in developing countries in Asia and Africa that were eligible to receive GAVI Alliance co-funding. In November 2007, WHO convened an ad-hoc group of experts who concluded that efficacy data could be extrapolated to populations that are in equivalent child-mortality strata,[25] thus helping to alleviate the need for regional data. In 2009, results were presented to WHO's SAGE from an efficacy trial[26] in Malawi and South Africa that showed that Rotarix was efficacious against severe rotavirus gastroenteritis in the first year of life. These results, along with postmarketing effectiveness data for RotaTeq from Nicaragua[27] and the USA,[28] and data for Rotarix from El Salvador[29], led the WHO group to expand recommendations for rotavirus vaccination to all regions of the world.[30]

With a WHO recommendation for rotavirus vaccines now in place,[31] governments of developing countries in Africa and Asia are deciding how to prioritise introduction of rotavirus vaccine in their public health agendas. Our trial shows that a live oral rotavirus vaccine has the potential to halve the incidence of severe rotavirus gastroenteritis in developing populations in Asia. Alongside efficacy results for this vaccine in Africa,[21] our study supports WHO's strong recommendation for expansion of rotavirus vaccine use to the poorest nations in Africa and Asia. Rotavirus vaccines have the potential to protect the lives of nearly 2 million children in the next decade alone.[32]

Contributors

KZ, DDA, JCV, SS, MY, MJD, VDT, SPL, MLC, DAS, FS, ADS, KMN, and MC contributed to the study design. KZ, DDA, SS, MY, GP, VDT, LPM, and LHT contributed to the implementation of the study and supervision at the sites. JCV, MJD, KL, SBR, FS, ADS, KMN, and MC contributed to planning of protocol-stated analyses and post-hoc analyses. MJD designed and did the statistical analysis and verified its accuracy. MJD, SBR, and MC contributed to compiling of the official clinical study report. KZ, DDA, JCV, SS, MY, MJD, KL, ADS, KMN, and MC took part in a 2 day meeting to discuss and interpret the data. KZ was the principal investigator for Bangladesh; DDA was the principal investigator for Vietnam. KN led the clinical team at PATH; MC led the clinical team at Merck. All authors had full access to the data. KZ, DDA, JCV, SS, MY, SPL, MLC, KL, SBR, DAS, FS, ADS, KMN, and MC helped draft this report or critically revise the draft. All authors reviewed and approved the final version of the report.

Conflicts of interest

MJD, MLC, SBR, FS, and MC are employees of Merck and own shares in the company. DAS was Director of ICDDR,B at the time of initiation of the study; after his departure from ICDDR,B, he received consultancy fees as part of his ongoing participation in the site's conduct of the study. Consultancy fees were part of the ICDDR,B budget, which was funded by PATH. ICDDR,B, NIHE, and IVI were funded by PATH's Rotavirus Vaccine Program through a GAVI Alliance Grant to PATH to conduct this trial. All other authors declare that they have no conflicts of interest.

Acknowledgments

The study, with protocol V260-015, was designed, managed, undertaken, and analysed by the co-sponsors in collaboration with the site investigators and under the supervision and advice of the data and safety monitoring board. Investigators and their institutions were funded by PATH's Rotavirus Vaccine Program, with a grant from the GAVI Alliance. We thank the volunteers and their families; the scientific advisers Shams El Arifeen and Tasnim Azim (International Centre for Diarrhoeal Disease Research, Bangladesh); participating investigators Abu Syed Golam Faruque, Al Fazal Khan, and Ilias Hossain (International Centre for Diarrhoeal Disease Research, Bangladesh); all other staff from the International Centre for Diarrhoeal Disease Research, Bangladesh; the scientific advisers John Clemens and Paul Kilgore (International Vaccine Institute in Korea) and Truong Tan Minh (Khanh Hoa Health Service), and participating investigators Nguyen Hien Anh (National Institute of Hygiene and Epidemiology) and Phu Quoc Viet (Khanh Hoa Health Service); staff from the Department of Pediatrics, Khanh Hoa General Hospital, from the Pasteur Institute in Nha Trang, and from the 16 participating Commune Health Centres in Nha Trang; the members of the data and safety monitoring board (King Holmes [Chairman], Wasif Ali Khan, Edward Tsiri Agbenyega, Grace Irimu, Mamadou Marouf Keita, Dinh Sy Hien, Nik Zarifah, Nik Hussain Reed, and Janet Wittes); Penny M Heaton and Michelle G Goveia for their contribution to the design of the study; Bradley Raybold for contributions to the initiation and implementation of the study; Fay DiCandilo and Margaret Nelson for contributing to careful review of the data; Donna Hyatt for data management; Laura Mallette and Vladimir Liska for laboratory data coordination; Richard Ward and Monica McNeal for overseeing laboratory assays; Tracy Burke for ensuring adequate vaccine and placebo supplies; Family Health International and PharmaLink, especially Carolyn Enloe, Vivian Bragg, Laura Niver, Jen Auerbach, Linda McNeil, and all the Family Health International field monitors and safety-reporting staff; Vu Minh Huong (PATH) for monitoring assistance; Joyce Erickson (PATH) for contracting and financial analysis; and Carolien Bakker and David Oxley (PATH) for administrative assistance.

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