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Safety and Efficacy of an Attenuated Vaccine against Severe Rotavirus Gastroenteritis

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ABSTRACT

BACKGROUND

The safety and efficacy of an attenuated G1P[8] human rotavirus (HRV) vaccine were tested in a randomized, double-blind, phase 3 trial.

METHODS

We studied 63,225 healthy infants from 11 Latin American countries and Finland who received two oral doses of either the HRV vaccine (31,673 infants) or placebo (31,552 infants) at approximately two months and four months of age. Severe gastroenteritis episodes were identified by active surveillance. The severity of disease was graded with the use of the 20-point Vesikari scale. Vaccine efficacy was evaluated in a subgroup of 20,169 infants (10,159 vaccinees and 10,010 placebo recipients).

RESULTS

The efficacy of the vaccine against severe rotavirus gastroenteritis and against rotavirus-associated hospitalization was 85 percent ($P < 0.001$ for the comparison with placebo) and reached 100 percent against more severe rotavirus gastroenteritis. Hospitalization for diarrhea of any cause was reduced by 42 percent (95 percent confidence interval, 29 to 53 percent; $P < 0.001$). During the 31-day window after each dose, six vaccine recipients and seven placebo recipients had definite intussusception (difference in risk, -0.32 per 10,000 infants; 95 percent confidence interval, -2.91 to 2.18 ; $P = 0.78$).

CONCLUSIONS

Two oral doses of the live attenuated G1P[8] HRV vaccine were highly efficacious in protecting infants against severe rotavirus gastroenteritis, significantly reduced the rate of severe gastroenteritis from any cause, and were not associated with an increased risk of intussusception. (ClinicalTrials.gov numbers, NCT00139347 and NCT00263666.)

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ROTAVIRUS IS THE LEADING RECOGNIZED cause of diarrhea-related illness and death among infants and young children.¹⁻⁵ Every year, rotavirus is associated with 25 million clinic visits, 2 million hospitalizations, and more than 600,000 deaths worldwide among children younger than five years of age.^{6,7} Development of a safe and effective rotavirus vaccine is therefore a high priority, particularly but not exclusively in developing countries, where the burden of disease is highest.^{8,9} Since the withdrawal from the market of the tetravalent rhesus–human reassortant vaccine (RotaShield, Wyeth Laboratories) because of an association with intussusception,^{10,11} ruling out such a risk has become critical for the licensure and universal use of any new rotavirus vaccine.

A live attenuated human rotavirus (HRV) vaccine containing the RIX4414 strain of G1P[8] specificity¹² has been developed from the parent vaccine strain 89-12.¹³⁻¹⁵ Clinical trials with the HRV vaccine in Finnish¹⁶ and Latin American¹⁷ (Brazilian, Mexican, and Venezuelan) infants showed that two doses were well tolerated and immunogenic. In phase 2 clinical trials, the efficacy of the vaccine against severe rotavirus gastroenteritis reached 90 to 100 percent.¹⁶⁻¹⁸ Protection started as early as the first dose, lasted until the subjects were up to two years of age, and was demonstrated against both G1P[8] and G9P[8] rotaviruses.¹⁶⁻¹⁸

Although the initial trials had included 6670 infants, a larger, multinational, randomized, double-blind, placebo-controlled, phase 3 trial was required to evaluate any potential risk of intussusception within 31 days after administration of each of two oral doses of the HRV vaccine, as well as any other serious adverse events. Other end points were assessed to confirm previously reported evidence that two oral doses of the HRV vaccine are efficacious against severe rotavirus gastroenteritis, to define the effect of vaccination on the burden of severe diarrhea of any cause, and to extend the observations of protection against different circulating strains in infants up to one year of age.

METHODS

STUDY DESIGN AND PARTICIPANTS

Investigators from Argentina, Brazil, Chile, Colombia, the Dominican Republic, Honduras, Mexico, Nicaragua, Panama, Peru, Venezuela, and Fin-

land recruited infants at public pediatric clinics or hospitals for this randomized, double-blind, placebo-controlled, phase 3 trial. The study protocol and informed-consent document were approved by ethics review committees at each center, and the study was conducted in accordance with the Declaration of Helsinki guidelines for good clinical practice.

After a parent or guardian had provided written informed consent, 6-to-13-week-old healthy infants were enrolled. The infants were randomly assigned to receive two oral doses of either the HRV vaccine or placebo — the first dose at visit 1 and the second at visit 2, one to two months later. After the administration of the second dose, the overall cohort was followed for a median duration of 100 days after the first dose for the assessment of any adverse events, including the occurrence of intussusception (the safety cohort, evaluated at visit 3), and a subgroup of infants was followed for 9 to 10 months for the assessment of efficacy (the efficacy cohort, evaluated at visit 4).

Cases of intussusception, severe gastroenteritis, and serious adverse events were the outcomes captured by an active-surveillance system implemented six months before initiation of the study in all medical facilities able to receive infants with these outcomes (as described in the Supplementary Appendix, available with the full text of this article at www.nejm.org). Outcomes were recaptured during the scheduled visits, if missed by the active-surveillance system.

VACCINE

The HRV vaccine (Rotarix, GlaxoSmithKline Biologicals) contained 10^{6.5} median cell-culture infective doses of the RIX4414 vaccine strain. The placebo had the same constituents as the active vaccine but without the vaccine virus. After the vaccine or placebo had been reconstituted with 1.3 ml of liquid calcium carbonate buffer, an oral dose was administered in a blinded manner to infants when they were approximately two months of age and again when they were four months of age. Infants received routine immunizations according to local regulations; oral poliovirus vaccination was provided at least two weeks before or after the administration of a dose of the HRV vaccine.

DEFINITION OF CASES

All possible cases of intussusception identified by active surveillance were analyzed by an independent

clinical-events committee. Using the case definition from the Brighton Collaboration Working Group on Intussusception¹⁹ and remaining blinded to study-group assignments, this committee categorized cases of intussusception as definite, probable, or possible, according to the certainty of the diagnosis. A case of definite intussusception required confirmation at surgery or autopsy or with the use of imaging techniques, such as imaging with gas- or liquid-contrast enema or abdominal ultrasonography (as described in the Supplementary Appendix).

The clinical case definition of an episode of severe gastroenteritis was an episode of diarrhea (the passage of three or more loose or watery stools within a 24-hour period), with or without vomiting, that required overnight hospitalization or rehydration therapy equivalent to World Health Organization (WHO) plan B (oral rehydration therapy) or plan C (intravenous rehydration therapy) in a medical facility such as a hospital, clinic, or supervised rural health care center. To quantify the severity of gastroenteritis, the same scale used in the evaluation of the rhesus-human reassortant rotavirus vaccine²⁰ was implemented. It is a widely used scoring system²¹ referred to as the Vesikari scale, with scores ranging from 0 to 20 (where higher scores indicate greater severity). An episode of gastroenteritis with a score of 11 or greater was considered a severe episode.²¹

ASSESSMENT OF SAFETY

Serious adverse events were defined as any new health-related problems that resulted in death, were life-threatening, necessitated hospitalization or prolongation of existing hospitalization, or resulted in disability or incapacity. Thus defined, serious adverse events included intussusception. Investigators asked parents about the occurrence of serious adverse events at each follow-up visit and recorded this information. To standardize the reporting of serious adverse events, medical terms used by investigators were analyzed at two levels, according to the Medical Dictionary for Regulatory Activities (MedDRA)²²: one level was that of the unique “preferred term,” and the other that of the “system organ class,” which is a grouping of related preferred terms.

An independent data-monitoring committee of expert clinicians who were not blinded to the study-group assignments and an independent statistician were empowered to stop the trial. They

periodically reviewed all serious adverse events, including intussusception. A blinded safety-review committee independently reviewed all cases involving death to assign a primary cause of death and to determine associated secondary diagnoses and other underlying conditions.

LABORATORY ANALYSIS

Stool specimens from each infant with severe gastroenteritis were tested for rotavirus by means of enzyme-linked immunoassay (Rotaclone, Meridian Bioscience)²³⁻²⁵ at GlaxoSmithKline Biologicals (details are provided in the Supplementary Appendix). Rotavirus serotyping and identification of the vaccine virus were performed by reverse-transcriptase-polymerase-chain-reaction (RT-PCR) analysis followed by a reverse hybridization assay at Delft Diagnostic Laboratory.²⁶ Testing for other enteropathogens was not part of the study protocol and was left to the discretion of the individual investigators or sites.

END POINTS

The primary and secondary safety objectives were to assess the risk of definite intussusception within 31 days after the administration of each vaccine dose and to assess the occurrence of serious adverse events, including intussusception, during the entire study period. The primary efficacy end point was the prevention of severe rotavirus gastroenteritis, according to the case definition, from two weeks after the second dose (i.e., after completion of the full vaccination course) until one year of age. The secondary end points were efficacy against severe rotavirus gastroenteritis defined according to the Vesikari scale, efficacy against gastroenteritis associated with specific circulating rotavirus types, and efficacy against severe rotavirus gastroenteritis occurring after the first dose. Other end points were the prevention of hospitalization due to rotavirus gastroenteritis, of hospitalization for any reason, and of severe gastroenteritis from any cause.

STATISTICAL ANALYSIS

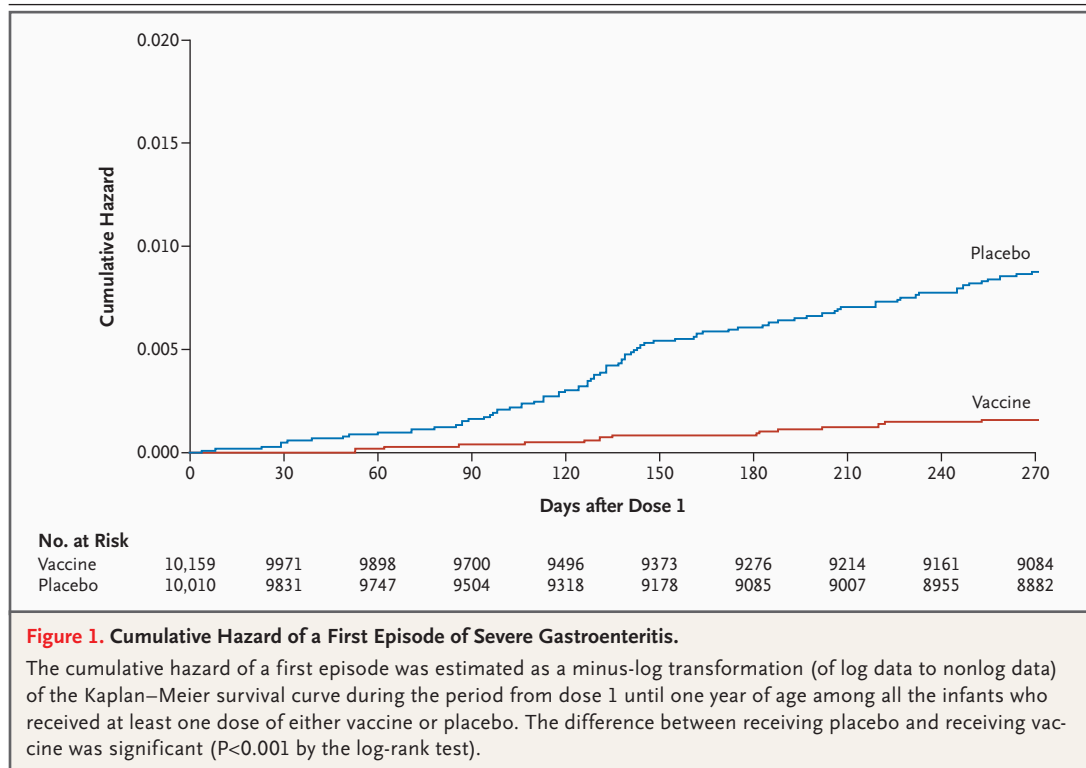
The numbers of infants with intussusception within 31 days after receipt of a dose of vaccine or placebo and during the entire safety-surveillance period were compared between the study groups. The asymptotic standardized 95 percent confidence interval for the difference in risk between the groups and for the relative risk in the vaccine

group as compared with the placebo group was calculated.²⁷ According to prespecified criteria, the primary safety objective would be met if the two-sided 95 percent confidence interval of the difference in the risk of intussusception within 31 days after vaccination was less than 6 per 10,000 and included zero (as described in the Supplementary Appendix). Serious adverse events, reasons for hospitalization, and primary causes of death were categorized according to the MedDRA classification system and compared between the groups with use of the two-sided asymptotic standardized 95 percent confidence interval for the difference in risk, without adjustment for multiple testing. A two-sided asymptotic score test for the null hypothesis of identical incidence in the two groups (alpha level, 0.05) was used to screen for potential differences between the two groups. All infants who had received at least one dose of the study vaccine or placebo were included in the safety analysis.

Assuming an attack rate of 1.5 percent for severe rotavirus gastroenteritis in the placebo group,⁶ a true vaccine efficacy of 70 percent,¹¹ and a 10 percent withdrawal rate, we calculated that a sample of 20,000 infants would provide at least 80 percent power to detect a lower limit of the 95

percent confidence interval for vaccine efficacy of greater than 50 percent. The number of children enrolled varied among countries and centers because enrollment above a minimum target sample size was competitive.

In the efficacy study, an “according-to-protocol” cohort was used to calculate the efficacy of the vaccine and included participants who completed the full two-dose vaccination course and for whom compliance with the protocol was complete (as shown in Fig. 1 of the Supplementary Appendix). The overall efficacy cohort was used to calculate efficacy beginning at time of administration of the first dose and included all infants who received at least one dose of either vaccine or placebo. For each efficacy end point, the percentages of infants for whom at least one episode was reported were compared between the groups and expressed in terms of the relative risk. Vaccine efficacy was calculated with its 95 percent confidence interval with use of the following formula: $(1 - \text{relative risk}) \times 100$. The 95 percent confidence intervals for vaccine efficacy were derived from the exact confidence interval for the Poisson rate ratio.²⁸ In the analysis of vaccine efficacy according to each G serotype of the virus, infants were counted in each G-type category when more than



one G type was isolated for a given episode. The cumulative hazard of a first episode of severe rotavirus gastroenteritis between comparative groups was estimated as a minus-log transformation of the Kaplan–Meier survival curve.

The P value for the cumulative-hazard curve was calculated with the use of the log-rank test. All other reported P values are two-sided for the null hypothesis of equivalence of the two treatment groups. Data analysis was performed with the use of SAS software (version 8.2) and Proc-StatXact 5 with Windows NT 4.0. GlaxoSmithKline Biologicals held the data and performed the analyses, with continuous feedback from the academic authors. The manuscript was written jointly by the company authors and the academic authors, who vouch for the accuracy and completeness of the reported data.

RESULTS

STUDY GROUPS

A total of 63,225 infants were enrolled and were to receive two doses of the HRV vaccine or placebo between August 5, 2003, and March 12, 2004. The entire cohort (the safety cohort) was followed for safety until July 23, 2004, when the last subject completed visit 3. From this cohort, the first 20,169 infants were enrolled in the evaluation of efficacy and were followed until they were one year of age (efficacy cohort) (Fig. 1 of the Supplementary Appendix). In both cohorts, the vaccine and pla-

cebo groups were similar in terms of sex distribution and distribution of race or ethnic background and in terms of mean age at the time of each vaccination and at the end of the safety and efficacy follow-up periods (Table 1). The proportions of infants who were withdrawn from the study and the reasons for withdrawal were similar between the groups (Fig. 1 of the Supplementary Appendix).

INTUSSUSCEPTION

In the safety cohort of 63,225 children, 26 cases of intussusception were detected by capture–recapture methods during hospital surveillance or by active follow-up; 25 cases were determined to be definite intussusception.¹⁹ Thirteen cases of definite intussusception were diagnosed within 31 days after administration of either dose, six in the vaccine group and seven in the placebo group (respective incidence rates, 1.89 and 2.21 per 10,000 infants; difference in risk, -0.32 per 10,000 infants; 95 percent confidence interval, -2.91 to 2.18 ; relative risk in the vaccine group, 0.85 ; $P=0.78$) (Table 2). Twelve cases of intussusception, three in the vaccine group and nine in the placebo group, were reported after the 31-day window (difference in risk, -1.91 per 10,000 infants; 95 percent confidence interval, -4.58 to 0.29 ; $P=0.08$). Thus, during the entire safety-surveillance period (median duration, 100 days after dose 1), intussusception occurred in 9 vaccine recipients and 16 placebo recipients (incidence

Table 1. Characteristics of the Study Populations, According to Study Group.*

Characteristic	Safety Study		Efficacy Study	
	HRV Vaccine	Placebo	HRV Vaccine	Placebo
Infants — no.	31,673	31,552	10,159	10,010
Male sex — no. (%)	16,105 (50.8)	16,150 (51.2)	5100 (50.2)	5160 (51.5)
Age — wk				
At dose 1	8.2±2.39	8.2±2.39	8.4±2.39	8.4±2.38
At dose 2	15.8±3.75	15.8±3.79	16.3±3.74	16.3±3.78
At end of safety or efficacy follow-up	22.7±5.3	22.7±5.3	50.8±10.4	50.5±10.6
Race or ethnic background — no. (%)†				
Hispanic	25,729 (81.2)	25,648 (81.3)	8776 (86.4)	8651 (86.4)
White	3,488 (11.0)	3,434 (10.9)	780 (7.7)	738 (7.4)
Other‡	2,456 (7.8)	2,470 (7.8)	603 (5.9)	621 (6.2)

* Plus–minus values are means ±SD. HRV denotes human rotavirus.

† Race or ethnic group was determined by the investigators.

‡ Other races and ethnic backgrounds included African, South Asian, Arabic or North African, Aborigine, Afro-Caribbean, Caribbean, mixed race, and Indian.

rates, 2.84 and 5.07 per 10,000 infants; difference in risk, -2.23 per 10,000 infants; 95 percent confidence interval, -5.70 to 0.94 ; $P=0.16$) (Table 2).

Of the 25 cases of definite intussusception, 10 occurred after dose 1 (in three vaccinees and seven placebo recipients) and 15 occurred after dose 2 (in six vaccinees and nine placebo recipients). There was no temporal cluster of intussusception cases after either dose. Most of the cases (15 of 25) occurred at four to five months of age. The intussusception was reduced by enema in 6 infants (2 vaccinees and 4 placebo recipients) and by surgery in 19 infants (7 vaccinees and 12 placebo recipients). After hospitalization (mean duration, five days), all the infants had a complete recovery (as described in the Supplementary Appendix).

SERIOUS ADVERSE EVENTS

In the safety cohort, significantly fewer serious adverse events were reported in the vaccine group than in the placebo group (293.0 vs. 331.8 events per 10,000 infants, $P=0.005$) (Table 2). Serious adverse events related to gastroenteritis, such as

diarrhea, vomiting, dehydration, and hypovolemic shock, were reported in fewer vaccinees than placebo recipients. The hospitalization rate was also lower in the vaccine group than in the placebo group (279.7 vs. 317.9 hospitalizations per 10,000 infants, $P=0.005$) (Table 2). A post hoc exploratory analysis revealed a reduction of 42 percent (95 percent confidence interval, 28.6 to 53.1 percent) in the vaccine group in the need for hospitalization for gastroenteritis or diarrhea of any cause during the 100-day observation period (100 hospitalizations, vs. 179 hospitalizations in the placebo group; $P<0.001$).

Overall mortality did not differ significantly between the vaccine recipients and the placebo recipients. Fifty-six deaths occurred in the vaccine group, and 43 in the placebo group ($P=0.20$) (Table 2); 4 and 2, respectively, were related to diarrhea ($P=0.41$). The causes of diarrhea in those cases were not determined, because stool samples were not available.

Further analysis of the deaths stratified at the level of MedDRA preferred terms suggested that there was a potential imbalance of deaths due to

Table 2. Risk of Definite Intussusception and Other Serious Adverse Events among Infants Receiving Vaccine or Placebo.*

Adverse Event	HRV Vaccine (N=31,673)		Placebo (N=31,552)		Difference in Risk per 10,000 Infants (95% CI) [†]	Relative Risk (95% CI) [‡]	P Value [§]
	No. of Events	Incidence Rate [¶]	No. of Events	Incidence Rate [¶]			
Definite intussusception							
≤31 Days after either dose	6	1.89	7	2.21	-0.32 (-2.91 to 2.18)	0.85 (0.30 to 2.42)	0.78
≤31 Days after dose 1	1	0.31	2	0.63	-0.32 (-2.03 to 1.20)	0.50 (0.07 to 3.80)	0.56
≤31 Days after dose 2	5 ^{**}	1.57	5 ^{††}	1.58	-0.01 (-2.48 to 2.45)	0.99 (0.31 to 3.21)	0.99
Between dose 1 and visit 3 ^{‡‡}	9	2.84	16	5.07	-2.23 (-5.70 to 0.94)	0.56 (0.25 to 1.24)	0.16
Serious adverse event between dose 1 and visit 3							
Overall ^{§§}	928	290.99	1047	331.83	-38.84 (-66.02 to -11.73)	0.88 (0.81 to 0.96)	0.005
Hospitalization	886	279.73	1003	317.89	-38.15 (-64.76 to -11.62)	0.88 (0.81 to 0.96)	0.005
Death	56	17.68	43	13.63	4.05 (-2.15 to 10.40)	1.30 (0.87 to 1.93)	0.20

* HRV denotes human rotavirus, and CI confidence interval.

[†] The difference in risk is the incidence rate in the HRV-vaccine group minus that in the placebo group.

[‡] The relative risk is the risk in the HRV-vaccine group as compared with that in the placebo group.

[§] P values are the results of a comparison between the groups by a two-sided asymptotic score test for the null hypothesis of identical incidence in the groups (alpha level, 0.05).

[¶] The incidence rate is the number of infants with the specified serious adverse event per 10,000 infants.

^{||} The 31-day postvaccination window included the day of vaccination and the 30-day period after the dose.

^{**} Data were available for 29,616 infants.

^{††} Data were available for 29,465 infants.

^{‡‡} Visit 3 took place 30 to 90 days after dose 2.

^{§§} Overall serious adverse events were any untoward medical occurrence that resulted in death, was life-threatening, required hospitalization or prolonging of existing hospitalization, or resulted in disability or incapacity. These events were not necessarily mutually exclusive.

pneumonia among infants receiving the HRV vaccine. This potential imbalance was a further investigated. In 16 vaccine recipients and 6 placebo recipients, the primary cause of death was related to pneumonia ($P=0.05$). However, the distribution of pneumonia-related deaths within the first 31 days after vaccination did not differ statistically between the two groups (seven cases in the vaccine group and three in the placebo group). An additional analysis showed that there was no difference between the two study groups in terms of the number of serious adverse events related to pneumonia (280 in the vaccine group and 276 in the placebo group), overall pneumonia-related hospitalizations (277 and 273, respectively), or pneumonia-related hospitalizations within 31 days after the first dose (99 and 94), within 31 days after the second dose (49 and 56), or at any other time point. Data on retention within the efficacy cohort are provided in the Supplementary Appendix.

EFFICACY OF THE VACCINE AGAINST SEVERE ROTAVIRUS GASTROENTERITIS

There were 12 children in the vaccine group and 77 in the placebo group with severe rotavirus gastroenteritis according to the clinical definition (2.0 vs. 13.3 children with at least one episode per 1000 infant-years, $P<0.001$), resulting in a vaccine efficacy of 84.7 percent ($P<0.001$) against severe rotavirus gastroenteritis from two weeks after dose 2 until one year of age (Table 3). Similar results were obtained with the overall cohort of infants who received at least one dose of vaccine or placebo (vaccine efficacy from dose 1 until one year of age, 81.1 percent; 95 percent confidence interval, 68.4 to 95.3 percent; $P<0.001$). Hospitalization for at least one night was required for 9 children in the vaccine group and 59 in the placebo group (1.5 vs. 10.2 hospitalizations per 1000 infant-years), for a vaccine efficacy against hospitalization for severe rotavirus gastroenteritis of 85.0 percent ($P<0.001$) (Table 3).

The cumulative hazard of severe rotavirus gastroenteritis was significantly lower in the vaccine group than in the placebo group, both in the according-to-protocol analysis (data not shown) and in the intention-to-treat analysis ($P<0.001$ by the log-rank test) (Fig. 1). The difference increased with time and led to an approximately sevenfold risk of severe rotavirus gastroenteritis in the placebo group as compared with the vaccine group at one year of age.

Eleven of 12 children with episodes of severe gastroenteritis in the vaccine group and 71 of 77 in the placebo group had a Vesikari score of 11 or greater, yielding a vaccine efficacy of 84.8 percent ($P<0.001$). For increasing disease severity with scores between 11 and 20, the efficacy of the vaccine was increasingly higher, reaching 100 percent against more severe rotavirus gastroenteritis, defined as gastroenteritis with a Vesikari score of 19 or 20 (Table 4). Sixteen episodes of severe rotavirus gastroenteritis with a Vesikari score of 11 or greater were reported from dose 1 until dose 2 (6 in the vaccine group and 10 in the placebo group).

The type-specific²⁹ efficacy of the vaccine against wild-type strains is shown in Table 3. Its efficacy against severe rotavirus episodes with a Vesikari score of 11 or greater caused by type G1P[8] strains, homologous to the vaccine strain, was 90.8 percent ($P<0.001$). The efficacy of the vaccine against strains sharing only the P[8] antigen (G3P[8], G4P[8], and G9P[8]) was 87.3 percent ($P<0.001$). The type G2P[4] rotavirus, which does not share either the G or the P antigen with the vaccine strain, was detected in specimens from five infants in the vaccine group and nine in the placebo group, for an efficacy of 41.0 percent ($P=0.30$).

EFFECT OF THE VACCINE ON THE BURDEN OF DIARRHEAL ILLNESS

The incidence rate of severe gastroenteritis of any cause that required rehydration according to WHO plan B or C was 30.9 per 1000 infant-years in the vaccine group, as compared with 51.7 per 1000 infant-years in the placebo group, for an overall rate reduction of 40.0 percent among vaccine recipients ($P<0.001$). Likewise, the rate of hospitalization for diarrhea of any cause was significantly reduced, by 42.0 percent, in the vaccine group ($P<0.001$) (Table 3).

DISCUSSION

In this large, multinational trial conducted in 20,169 infants for efficacy and 63,225 infants for safety, the live attenuated RIX4414 G1P[8] HRV vaccine was highly protective against severe rotavirus gastroenteritis and related hospitalizations. This rotavirus vaccine also proved to be safe with respect to the risk of intussusception.

Within this trial setting, the vaccine was not

Table 3. Efficacy of the HRV Vaccine against Gastroenteritis during the Period from Two Weeks after Dose 2 until One Year of Age.*

Type of Gastroenteritis	HRV Vaccine (N=9009)		Placebo (N=8858)		Relative Risk†	Vaccine Efficacy (95% CI)
	No. of Infants with ≥1 Episode	1000 Infant-Yr Ratio‡	No. of Infants with ≥1 Episode	1000 Infant-Yr Ratio‡		
Severe, according to clinical case definition§						
Rotavirus gastroenteritis						
Severe	12	2.0	77	13.3	0.153	84.7 (71.7 to 92.4)
Hospitalization	9	1.5	59	10.2	0.150	85.0 (69.6 to 93.5)
Gastroenteritis from any cause						
Severe	183	30.9	300	51.7	0.600	40.0 (27.7 to 50.4)
Hospitalization	145	24.5	246	42.4	0.580	42.0 (28.6 to 53.1)
Serotype-specific gastroenteritis						
G1P[8]¶	3	0.5	36**	6.2	0.082	91.8 (74.1 to 98.4)
G3P[8], G4P[8], G9P[8]	4††	0.66	31‡‡	5.3	0.126	87.3 (64.1 to 96.7)
G2P[4]	6	1.0	10§§	1.7	0.590	41.0 (−79.2 to 82.4)
Serotype-specific severe rotavirus gastroenteritis with a score of ≥11 on the Vesikari scale¶¶						
G1P[8]¶	3	0.5	32	5.5	0.092	90.8 (70.5 to 98.2)
G3P[8], G4P[8], G9P[8]	4	0.7	30	5.2	0.130	86.9 (62.8 to 96.6)
G2P[4]	5	0.8	9	1.5	0.546	45.4 (−81.5 to 85.6)

* Infants with episodes involving more than one isolated G type were counted in each of the detected type categories. One isolate from the placebo group could not be serotyped because the quantity of the sample was insufficient; one isolate from the placebo group was negative on reverse-transcriptase–polymerase-chain-reaction analysis; and one isolate from the placebo group could not be typed, but the vaccine strain was ruled out. HRV denotes human rotavirus, and CI confidence interval.

† The relative risk is the ratio of the incidence rate among infants in the vaccine group with at least one episode to the incidence rate among infants in the placebo group with at least one episode.

‡ The 1000 infant-year ratio is the number of infants presenting with ≥1 specified episode per 1000 infants per year.

§ The clinical definition of a case, according to the study protocol, was an episode of diarrhea (passage of three or more loose or watery stools within one day) with or without vomiting that required overnight hospitalization or rehydration therapy equivalent to World Health Organization plan B (oral rehydration therapy) or plan C (intravenous rehydration therapy) in a medical facility such as a hospital, clinic, or supervised rural health care center.

¶ All G1 types isolated were wild-type rotavirus.

|| G1P[8] type alone was isolated from two infants; G1P[8] and G9P[8] types were isolated from one infant.

** G1P[8] type alone was isolated from 34 infants; G1P[8] and G9P[8] types were isolated from 1 infant; and G1, G2, and G9 types were isolated from 1 infant.

†† G3P[8] type alone was isolated from one infant, G4P[8] alone from one infant, and G9P[8] alone from one infant; both G1P[8] and G9P[8] were isolated from one infant.

‡‡ G3P[8] type alone was isolated from 8 infants, G4P[8] alone from 2 infants, and G9P[8] alone from 19 infants; G1P[8] and G9P[8] were isolated from 1 infant and G1P[8], G2P[4], and G9P[8] from 1 infant.

§§ G2P[4] alone was isolated from nine infants, and G1P[8], G2P[4], and G9P[8] were isolated from one infant.

¶¶ Scores on the Vesikari scale range from 0 to 20, with higher scores indicating more severe cases. An episode with a score of 11 or greater was considered severe.

associated with an increased risk of intussusception during a 31-day period after administration of either of the two doses, as compared with placebo. Because the second dose was administered toward the peak age incidence of intussusception in the Latin American population,³⁰⁻³⁴ the risk of intussusception associated with the

HRV vaccine would have been most apparent. However, cases of intussusception after dose 2 were evenly distributed between the HRV group and the placebo group. Not only is the observed risk estimate of −0.32 per 10,000 infants below the initial risk increase of 4 per 10,000^{35,36} that led to the withdrawal of tetravalent rhesus–human

Table 4. Efficacy of the HRV Vaccine against Severe Rotavirus Gastroenteritis with a Vesikari Score between 11 and 20 during the Period from Two Weeks after Dose 2 until One Year of Age.*

Vesikari Score	HRV Vaccine (N=9009)		Placebo (N=8858)		Relative Risk†	Vaccine Efficacy (95% CI)
	No. of Infants with ≥1 Episode	1000 Infant-Yr Ratio‡	No. of Infants with ≥1 Episode	1000 Infant-Yr Ratio‡		
≥11	11	1.9	71	12.2	0.152	84.8 (71.1–92.7)
≥15	7	1.2	54	9.3	0.127	87.3 (71.9–95.1)
≥19	0	0	16	2.8	0	100.0 (74.5–100.0)

* Scores on the Vesikari scale range from 0 to 20, with higher scores indicating more severe cases. An episode with a score of 11 or greater was considered severe. CI denotes confidence interval.

† The relative risk is the ratio of the incidence rate among infants in the vaccine group with at least one episode to the incidence rate among infants in the placebo group with at least one episode.

‡ The 1000 infant-year ratio is the number of infants presenting with ≥1 specified episode per 1000 infants per year.

reassortant vaccine; it also is below the subsequent consensus risk estimate of 1 per 10,000^{37,38} for that vaccine. By meeting the predefined criteria of a 95 percent confidence interval for a difference in risk that included zero and was below 6 per 10,000 infants, this trial shows that the HRV vaccine, given according to a two-dose vaccination schedule at two and four months of age, is safe with respect to the risk of intussusception. The incidence rate of intussusception observed in the placebo group (51 per 100,000 infants) is consistent with rates previously reported in Latin America.^{30–34} In addition, the age at intussusception was similar in infants who had received vaccine and those who had received placebo — in contrast to the post-marketing observation with the tetravalent rhesus–human reassortant vaccine that vaccinees with intussusception tended to be younger than unvaccinated infants.³⁵ These data suggest that the intussusception problem encountered with the tetravalent rhesus–human reassortant vaccine may have resulted from the use of a rhesus strain, rather than from the oral administration of live rotavirus in general.^{35,39} These observations are in agreement with a recent report that wild-type rotavirus infection is not associated with intussusception.⁴⁰

The overall profile of serious adverse events was in favor of the HRV vaccine: fewer vaccinated infants than infants who received placebo had serious adverse events or required hospitalization because of gastrointestinal events. Numerous comparisons of serious adverse events grouped according to MedDRA preferred term (without adjustment for multiplicity) found no risk imbalance attributable to the vaccine that could be sup-

ported by clinical review and no plausible temporal or biologic causality. There was no significant difference in overall mortality between the groups. An observed potential imbalance in the number of pneumonia-related deaths among the vaccine recipients was not supported by observation of other pneumonia-related serious adverse events.

The vaccine proved to be highly protective against episodes of rotavirus gastroenteritis measured by a clinical definition for case capture that focused on hospitalization and rehydration, as well as by the validated Vesikari scale,²¹ which includes quantifiable outcomes related to diarrhea, vomiting, fever, dehydration, and hospitalization. The efficacies observed with two doses of this HRV vaccine — 85 percent against severe episodes of rotavirus gastroenteritis and 100 percent against more severe episodes — are similar to those found in a previous HRV vaccine study conducted in Brazil, Mexico, and Venezuela^{17,18} and to those of a three-dose study of tetravalent rhesus–human reassortant vaccine in Venezuela.⁴¹ The results are also in agreement with data indicating that two wild-type rotavirus infections are fully protective against subsequent episodes of severe disease.⁴²

The live attenuated vaccine protected against common serotypes circulating in Latin America and the Caribbean. A high level of protection (vaccine efficacy, 91 percent) was demonstrated against homologous G1P[8] rotaviruses, which have two outer capsid proteins (VP4 and VP7) and one inner capsid protein (VP6) antigenically similar to those in the HRV vaccine.²⁹ It also protected well against strains sharing only the P[8] genotype (the VP4 antigen) and the VP6 antigen (vaccine effica-

cy, 87 percent). Protection against rotavirus strains not having any of the outer or inner capsid antigens of the HRV vaccine seemed to be lower (vaccine efficacy, 45 percent). However, in a meta-analysis including the results of this study and the two phase 2 studies from Finland¹⁶ and Latin America¹⁷ (all based on identical methods and efficacy criteria), the efficacy of the vaccine against the G2P[4] type was 67 percent (95 percent confidence interval, 15 to 87 percent),⁴³ indicating that the vaccine can also protect, though to a lesser extent, against strains that do not share G or P epitopes with the vaccine strain.

Of public health importance is the finding that the HRV vaccine conferred 42 percent protection against hospitalization for gastroenteritis of any cause. This represents a significant reduction in the overall burden of gastroenteritis. With rotaviruses having been detected only in 26 percent of the cases of severe gastroenteritis in the control group, the observed 42 percent protection was greater than expected. The additional protection may be explained in part by rotavirus infections undetected by enzyme-linked immunoassay.⁴² Therefore, it appears that the burden of disease caused by rotavirus is much greater than that reflected by the incidence of rotavirus-associated hospitalizations calculated by antigen detection in the stool. This discordance between specific and nonspecific results may be further explored by means of RT-PCR, a more sensitive method, to detect rotavirus in stool specimens²⁶ or by probe studies, as has been reported for other vaccines, such as those against *Haemophilus influenzae* type b and *Streptococcus pneumoniae*.⁴⁴

The observed reduction in the rate of severe gastroenteritis of any cause and the strong protection against severe gastroenteritis due to G1P[8]

and non-G1P[8] rotaviruses indicate the potential public health value of the HRV vaccine. Efforts should now be focused on bringing this vaccine to infants as part of routine immunization programs, especially in areas where rotavirus is associated with an important proportion of the burden of illness and childhood death. Wide use of this vaccine will require parallel implementation of post-marketing surveillance, including follow-up investigations of deaths among HRV vaccine recipients, to answer a number of remaining questions. Because it has been shown that initiating the administration of tetravalent rhesus-human reassortant vaccine to infants after the age of 90 days considerably increases the rate of intussusception,⁴⁵ one of the relevant issues that will need to be monitored in the future includes intussusception-related safety when the vaccine is used in older children. Another important remaining question is efficacy against possible other new emerging serotypes that do or do not share any capsid antigen with the vaccine virus.⁴⁶

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Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Ruiz-Palacios GM, Pérez-Schael I, Velázquez FR, et al. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. *N Engl J Med* 2006;354:11-22.

Web supplement : Precisions related to merged manuscript 05-2431/05-2434 entitled ‘The new attenuated human rotavirus vaccine is safe and highly protective against severe rotavirus gastroenteritis: a randomized, double-blind, placebo-controlled multinational trial’, G Ruiz-Palacios et al

Surveillance for intussusception (IS), severe gastroenteritis and hospitalizations

An active surveillance system was established at hospital and medical facilities in the study areas to capture IS and severe gastroenteritis episodes. Parents were informed about the signs and symptoms of IS. They were instructed to seek medical care at the nearest hospital or medical facility in case IS was suspected or if their child had severe gastroenteritis, and to contact the investigator. The study team visited or contacted the hospitals and medical facilities at least twice per week to identify study participants with IS, severe gastroenteritis, or any other event. The surveillance system was tested and validated six months in advance of the initiation of the vaccine trial.

In addition, follow-up visits were scheduled at 30 to 90 days after each dose for IS and severe gastroenteritis surveillance, and at one year of age for surveillance of severe gastroenteritis. Investigators requested information on IS symptoms and any severe gastroenteritis episode using a standardized data collection form in order to recapture every event and to identify any missed event. Parents who missed a follow-up visit were contacted by the study team.

Brighton Collaboration Intussusception Working Group case definition

Level 1 of Evidence (Definite)

Surgical criteria

The demonstration of invagination of the intestine at surgery

AND/OR

Radiological criteria

The demonstration of invagination of the intestine by either gas or liquid contrast enema

Or

The demonstration of an intra-abdominal mass by abdominal ultrasound with specific characteristic features¹ that is proven to be reduced by hydrostatic enema on post-reduction ultrasound

AND/OR

Autopsy criteria

The demonstration of invagination of the intestine.

Level 2 of Evidence (Probable)

Clinical criteria

Using specific definitions listed in below

2 major criteria

OR

1 major criterion² and 3 minor criteria

Level 3 of Evidence (Possible)

Clinical criteria

4 or more minor criteria

1 target sign or doughnut sign on transverse section and a pseudo-kidney or sandwich sign on longitudinal section.

2 If 1 major criterion was rectal bleeding in the form of bloody diarrhea then consideration was given to infectious causes, such as E.coli, shigella or amoebiasis. In such cases 2 major criteria were to be met.

Definitions of Terminology used in the Clinical Case Definition

Major Criteria

1. Evidence of intestinal obstruction

- I. History of bile-stained vomiting
AND EITHER
- II. Examination findings of abdominal distension and abnormal or absent bowel sounds,
OR
- III. Plain abdominal radiograph showing fluid levels AND dilated bowel loops

2. Features of intestinal invagination

One or more of the following:

- I. Abdominal mass
- II. rectal mass
- III. rectal prolapse
- IV. plain abdominal radiograph showing a visible intussusceptum or soft tissue mass
- V. abdominal ultrasound showing a visible intussusceptum or soft tissue mass
- VI. abdominal CT scan showing a visible intussusceptum or soft tissue mass

3. Evidence of intestinal vascular compromise or venous congestion

- I. rectal bleeding, or
- II. “red currant jelly” stool, or
- III. blood on rectal examination

Laboratory analysis : stool specimens included in the efficacy analysis

Stool results were only included in the study analysis if the specimen was collected during the gastroenteritis episode or within the next seven days. In case of multiple gastroenteritis episodes, a sample had to be taken at the latest one day before the next gastroenteritis episode.

Statistical analysis : details about the definition of the primary safety objective

According to pre-specified criteria, the primary safety objective would be met if the two-sided 95% confidence interval of the risk difference for IS within 31 days after vaccination was below 6/10,000 and included zero. The 6/10,000 level corresponds to an observed increase of approximately 2.5 cases per 10,000 infants, well below the initial risk estimate of 4/10,000 that prompted withdrawal of

RotaShield™. Considering an expected incidence of 3 to 5 cases of IS per 10,000 infants within 31 days after placebo, the planned sample size of 30,000 participants per group provided >86% power to meet the primary safety objective if the risk difference was truly zero.

Relevant characteristics of definite intussusception episodes occurring during the safety surveillance period

HRV vaccine recipients					Placebo recipients				
Gender	Age in months at IS	Diagnosis day	Treatment	Days in hospital	Gender	Age in months at IS	Diagnosis day	Treatment	Days in hospital
During the 31 days-window after each vaccine dose									
After dose 1									
Male	3	18	Hydrostatic enema	2	Female	3	17	Surgery	6
					Male	2	22	Hydrostatic enema	3
After dose 2									
Female	2	4	Surgery	9	Female	4	8	Surgery	3
Female	5	6	Surgery	11	Female	3	11	Surgery	5

Male	5	17	Surgery	3	Female	4	18	Hydrostatic enema	4
Female	4	18	Surgery	7	Male	5	26	Surgery	7
Male	5	26	Hydrostatic enema	2	Female	5	30	Surgery	7

During the 31 days-window after each vaccine dose

After dose 1

Male	2	31	Surgery	18	Female	3	41	Hydrostatic enema	3
Male	4	54	Surgery	4	Female	4	53	Surgery	7
					Male	5	69	Surgery	5
					Male	4	75	Surgery	4

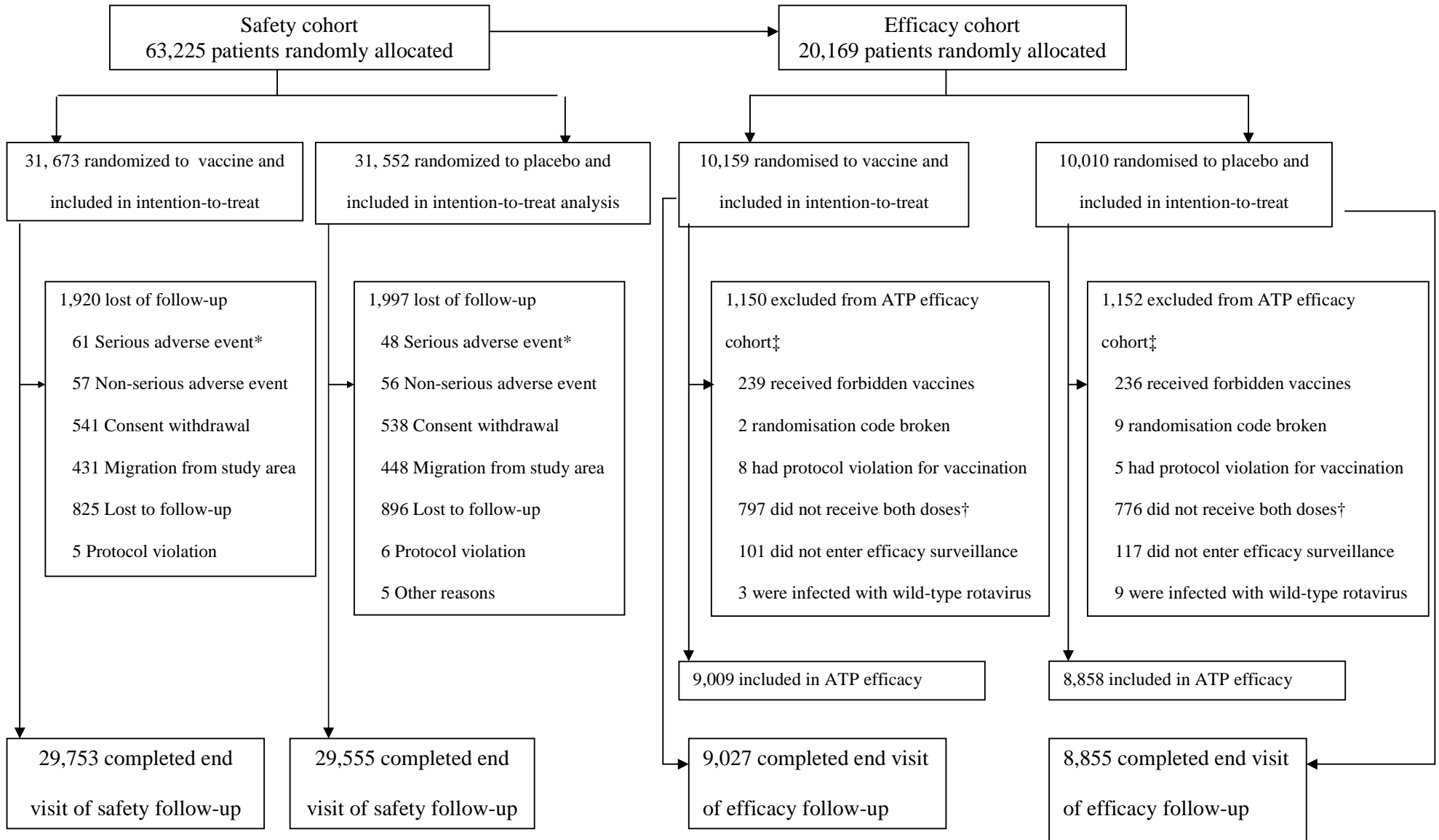
				Male	5	83	Hydrostatic enema	15	
After dose 2									
Female	7	145	Surgery	8	Female	4	35	Surgery	6
					Female	5	46	Surgery	9
					Female	4	53	Surgery	12
					Male	6	107	Surgery	5

Efficacy cohort retention

A total of 17,867 (89 percent) infants (9,009 vaccine/8,858 placebo) from the efficacy cohort of 20,169 completed follow-up from two weeks post-dose 2 until one year of age (mean duration: eight months). The numbers of infants who withdrew and the reasons for withdrawal were similar between the groups (Figure 1). From the 20,169 infants, a total of 621 subjects (233 (38 percent) of the 10,159 vaccinees and 388 (62 percent) of the 10,010 placebo recipients) reported 669 severe gastroenteritis episodes, Stool analysis results were available for 573/669 (86 percent) of these episodes. Unavailable results were due to insufficient quantity of stool samples or samples not collected and were equally distributed between both groups. Overall, rotavirus was detected in 112/621 (18 percent) infants, mainly among placebo recipients (94/112, 84 percent).

During the period starting at two weeks post-dose 2 up to one year of age (according-to-protocol cohort, mean duration of follow-up: eight months), 483 subjects (183 (38 percent) of the 9,009 vaccinees and 300 (62 percent) of the 8,858 placebo recipients) reported 516 severe gastroenteritis episodes, resulting in an overall severe gastroenteritis incidence of 51.7/1,000 child-years in the placebo group (Table 3). Stool analysis results were available for 449/516 (87 percent) episodes. Rotavirus was detected in 89/483 (18 percent) children. There were no children with more than one severe episode of rotavirus associated gastroenteritis.

Figure 1 : Trial profile



* One child in each group dropped out due to intussusception.

‡ Infants who met more than one criterion for exclusion from according-to-protocol analysis were only counted once according to the lowest ranking criterion

† Main reasons for failing dose 2 were consent withdrawal, migrations from study area or lost to follow-up