ORIGINAL ARTICLE

Safety and Efficacy of a Pentavalent Human–Bovine (WC3) Reassortant Rotavirus Vaccine

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ABSTRACT

BACKGROUND

Rotavirus is a leading cause of childhood gastroenteritis and death worldwide.

METHODS

We studied healthy infants approximately 6 to 12 weeks old who were randomly assigned to receive three oral doses of live pentavalent human–bovine (WC3 strain) reassortant rotavirus vaccine containing human serotypes G1, G2, G3, G4, and P[8] or placebo at 4-to-10-week intervals in a blinded fashion. Active surveillance was used to identify subjects with serious adverse and other events.

RESULTS

The 34,035 infants in the vaccine group and 34,003 in the placebo group were monitored for serious adverse events. Intussusception occurred in 12 vaccine recipients and 15 placebo recipients within one year after the first dose including six vaccine recipients and five placebo recipients within 42 days after any dose (relative risk, 1.6; 95 percent confidence interval, 0.4 to 6.4). The vaccine reduced hospitalizations and emergency department visits related to G1–G4 rotavirus gastroenteritis occurring 14 or more days after the third dose by 94.5 percent (95 percent confidence interval, 91.2 to 96.6 percent). In a nested substudy, efficacy against any G1–G4 rotavirus gastroenteritis through the first full rotavirus season after vaccination was 74.0 percent (95 percent confidence interval, 66.8 to 79.9 percent); efficacy against severe gastroenteritis was 98.0 percent (95 percent confidence interval, 88.3 to 100 percent). The vaccine reduced clinic visits for G1–G4 rotavirus gastroenteritis by 86.0 percent (95 percent confidence interval, 73.9 to 92.5 percent).

CONCLUSIONS

This vaccine was efficacious in preventing rotavirus gastroenteritis, decreasing severe disease and health care contacts. The risk of intussusception was similar in vaccine and placebo recipients. (ClinicalTrials.gov number, NCT00090233.)

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Rotavirus is the leading cause of hospitalization and death from acute gastroenteritis among infants and young children worldwide. More than 2 million hospitalizations and nearly half a million deaths are attributed to this infection annually.[1,2] The strategy of preventing rotavirus through vaccination derives from studies demonstrating that wild-type rotavirus infection induces immunity against subsequent rotavirus gastroenteritis.[3-6] Primary rotavirus infection provides substantial protection against gastroenteritis caused by the same serotype and against severe disease regardless of serotype. The four most prevalent serotypes, which account for more than 80 percent of cases of human rotavirus disease worldwide, are G1P[8], G2P[4], G3P[8], and G4P[8].[7,8]

In 1998, a tetravalent rhesus–human reassortant rotavirus vaccine (RRV-TV; RotaShield, Wyeth Laboratories) was licensed and recommended for routine immunization of infants in the United States.[9] Shortly thereafter, an association between the use of the vaccine and intestinal intussusception — an uncommon illness with a background incidence of 18 to 56 cases per 100,000 infant-years during the first year of life — was recognized.[10-13] The risk was greatest during the 3-to-14-day period after the first dose and the 3-to-7-day period after the second dose. Experts estimated that the population attributable risk of RRV-TV–associated intussusception was approximately 1 per 10,000 recipients.[14] RRV-TV was also associated with fever, vomiting, diarrhea, abdominal pain, and bloody stools.[15-18] The vaccine was voluntarily withdrawn from the market in October 1999.[19]

Development of a human–bovine reassortant rotavirus vaccine was continued because of the need for a safe and effective rotavirus vaccine and the importance of such a vaccine to public health.[19-21] In phase 2 clinical trials, various formulations of the human–bovine reassortant vaccine prevented approximately 70 percent of episodes of rotavirus gastroenteritis of any severity and 100 percent of episodes of severe disease.[22,23] In contrast to the findings with RRV-TV, the incidence of fever and gastrointestinal symptoms was generally similar in the vaccine and placebo groups. Further development of the human–bovine reassortant vaccine was also supported by the absence of an apparent association between intussusception and wild-type human rotavirus disease,[24,25] indicating that intussusception was not necessarily associated with all rotaviruses.

We report the results of the Rotavirus Efficacy and Safety Trial (REST), a randomized, placebo-controlled clinical trial of an oral, live pentavalent (G1, G2, G3, G4, and P[8]) human–bovine (WC3) reassortant rotavirus vaccine (RotaTeq, Merck). The trial included an evaluation of the safety of the vaccine with regard to intussusception and other adverse events and its efficacy in preventing rotavirus gastroenteritis and the associated use of health care resources.

METHODS

STUDY DESIGN

The study was a double-blind (with sponsor blinding), placebo-controlled, randomized trial conducted from 2001 to 2004 in 11 countries (as detailed in Part I of the Supplementary Appendix, available with the full text of this article at www.nejm.org). The protocol was approved by the ethics review committees of participating sites, and written informed consent was obtained from each participant's parent or guardian before enrollment. Healthy infants between 6 and 12 weeks of age were eligible. Infants were excluded if oral poliovirus vaccine had been given during the 42-day period preceding the planned first dose or if it was anticipated that oral poliovirus vaccine would be administered during the study. Concomitant administration of other licensed vaccines and breast-feeding were not restricted.

This study was designed to evaluate safety with respect to intussusception. The large sample size also provided us the opportunity to evaluate the efficacy of the vaccine in reducing the need for hospitalization or emergency department care for rotavirus gastroenteritis. Substudies nested within the large-scale study were designed to evaluate safety with respect to all adverse events (the detailed safety substudy) as well as immunogenicity and efficacy against rotavirus gastroenteritis of any severity (the clinical-efficacy substudy) (Fig. 1A of the Supplementary Appendix). Sites for each substudy were prospectively identified.

The trial (Merck protocol V260-006) was designed, managed, and analyzed by the sponsor in conjunction with the external investigators and members of the data and safety monitoring board and safety end-point adjudication committee (listed in Part I of the Supplementary Appendix). The investigators had access to all study data. This report was drafted primarily by Drs. Vesikari, Dallas, DiNubile, and Heaton and was reviewed

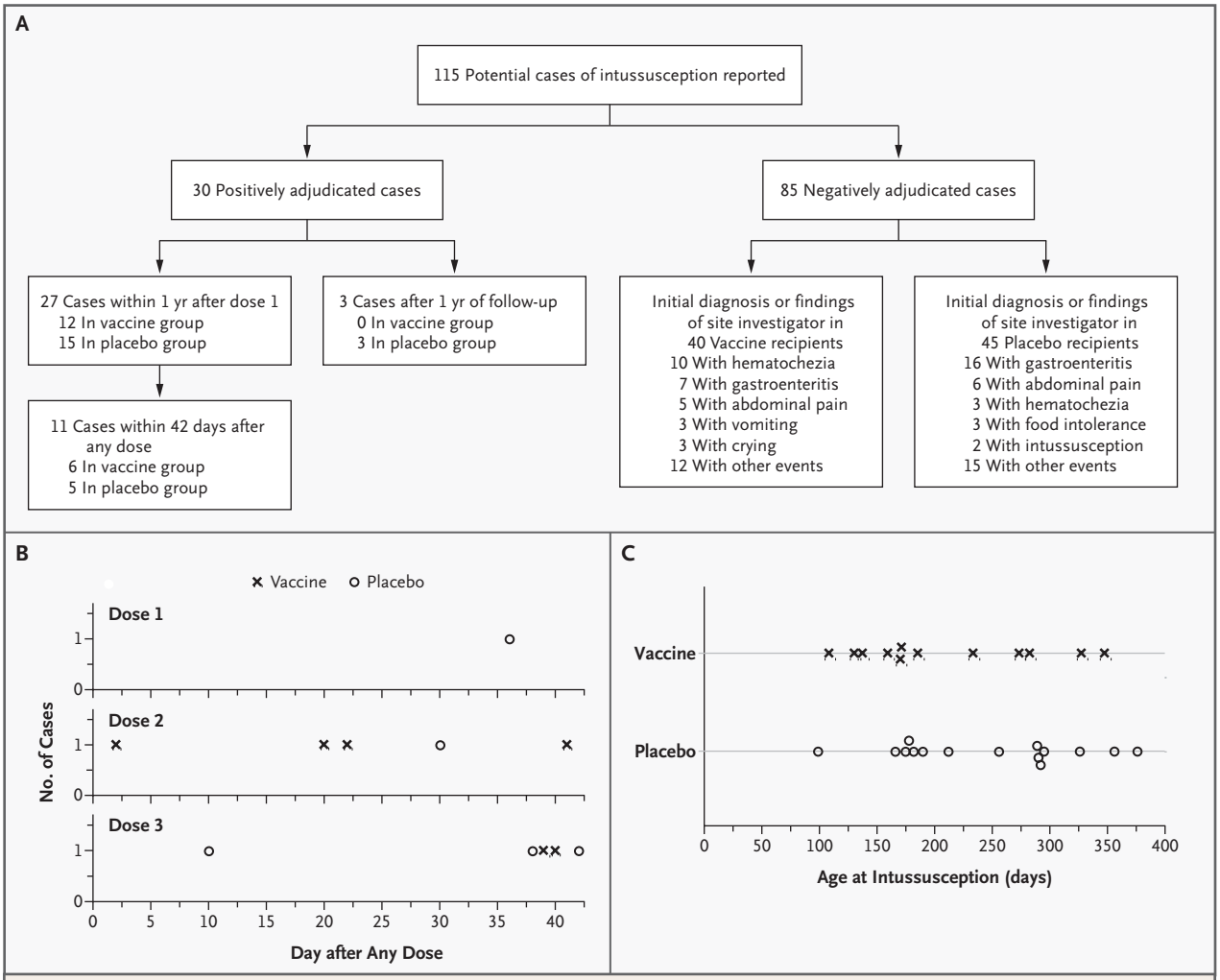

Figure 1. Cases of Intussusception.

Panel A shows potential cases of intussusception reported to the safety end-point adjudication committee before the recommendation of the data safety and monitoring board to discontinue enrollment, and the results of adjudication. One potential case of intussusception that was reported could not be adjudicated because radiographs were not available (owing to equipment malfunction). This case occurred in a placebo recipient 55 days after the second dose. The initial diagnosis or findings of the site investigator are shown for the negatively adjudicated cases (those not confirmed to be cases of intussusception). Panel B shows confirmed cases of intussusception that were reported within 42 days after vaccination, according to dose number and study group (vaccine or placebo). Panel C shows the subjects' ages at the time of the diagnosis of intussusception, according to study group (vaccine or placebo). Subjects already enrolled in the study at the time that enrollment was stopped, as recommended by the data safety and monitoring board, were followed until the end of the study. A single additional case of intussusception, in an eight-month-old boy, occurred 116 days after the third dose of vaccine. The child recovered fully.

and approved by each coauthor. The Indian Health Service approved the protocol but was otherwise uninvolved in the study.

VACCINE

The live pentavalent rotavirus vaccine contained five human–bovine reassortant rotaviruses, each consisting of the WC3 bovine strain with viral surface proteins corresponding to human rotavirus serotypes G1, G2, G3, G4, and P[8].[26] Reassortants were suspended in a liquid sodium citrate and phosphate buffer at an aggregate viral titer of approximately 6.7×10^7 to 12.4×10^7 infectious units per dose. Infants were randomly assigned, in a 1:1 ratio, to receive three 2-ml oral doses of vaccine or visibly indistinguishable placebo, 4 to

10 weeks apart. Doses were administered year round.

EVALUATION OF INTUSSUSCEPTION AND OTHER ADVERSE EVENTS

All subjects were monitored for at least 42 days after each dose for serious adverse events, including intussusception. Vaccine-related serious adverse events, deaths, and instances of intussusception were reported until the end of the study. Active surveillance was used to obtain safety data; parents or legal guardians were contacted on days 7, 14, and 42 after each dose and every six weeks thereafter for one year after the first dose with respect to intussusception and serious adverse events. Safety follow-up was completed for subjects for whom vaccinations were discontinued early. When available, stool specimens from infants with intussusception were tested for rotavirus antigen by enzyme immunoassay.[27] In the detailed safety substudy, parents or guardians were also asked to record their infants' temperature and the number of episodes of vomiting and diarrhea daily for 7 days after each dose and all adverse events for 42 days after each dose. Potential fecal shedding of vaccine strains between four and six days after each dose was monitored in a subgroup of subjects at prespecified sites, regardless of symptoms, by viral culture with use of a plaque assay and RNA electropherotyping.[28]

ADJUDICATION OF CASES OF INTUSSUSCEPTION AND ROLE OF THE DATA AND SAFETY MONITORING BOARD

All suspected cases of intussusception were reported to an independent, blinded adjudication committee, which included a pediatric surgeon, a pediatric radiologist, and a pediatrician with extensive experience in emergency medicine. The committee adjudicated potential cases of intussusception according to a prespecified case definition that required confirmation of the diagnosis by radiography or at surgery or autopsy.

As they were reported, positively adjudicated cases of intussusception were unblinded according to treatment group by the data and safety monitoring board to allow decisions to be made about the continuation of the study. The board's guidelines called for early stopping of the study if a significantly higher risk of intussusception among vaccine recipients than among placebo recipients (lower bound of the 95 percent confidence interval, >1.0) was detected during interim monitoring for the 7-day or 42-day period after any dose.

The data and safety monitoring board also made recommendations regarding completion of overall enrollment according to whether the criteria associated with the primary safety hypothesis that the vaccine would not increase the risk of intussusception within 42 days after any dose had been satisfied. The study used a group-sequential design,[29] with a minimum enrollment of 60,000 subjects and sequential enrollment of groups of 10,000 subjects if statistical criteria for the primary safety hypothesis were not met, to a maximum of 100,000 subjects.

CASE DEFINITION OF ROTAVIRUS GASTROENTERITIS

A case of rotavirus gastroenteritis was defined as the production of three or more watery or looser-than-normal stools within a 24-hour period or forceful vomiting, along with the detection of rotavirus by enzyme immunoassay in a stool specimen obtained within 14 days after the onset of symptoms. G serotypes were identified by one-step reverse-transcriptase–polymerase-chain-reaction analysis followed by sequencing.[30] All rotavirus-positive stools were to be evaluated for vaccine strains by viral culture with the use of a plaque assay and RNA electropherotyping.

EVALUATION OF EFFICACY IN TERMS OF HOSPITALIZATION AND EMERGENCY DEPARTMENT CARE FOR ROTAVIRUS GASTROENTERITIS

All subjects in the study were followed with respect to hospitalizations and emergency department visits for acute gastroenteritis. Parents or guardians were questioned about health care contacts for gastroenteritis at the same time that they were asked about intussusception and other adverse events. Lost work time was assessed for parents or guardians of subjects with confirmed rotavirus gastroenteritis.

EVALUATION OF CLINICAL EFFICACY AGAINST ROTAVIRUS GASTROENTERITIS

The clinical-efficacy substudy enrolled subjects from Finland and the United States (including subjects from the Navajo Nation and the White Mountain Apache Tribe). Parents or guardians were asked to report any episodes of acute gastroenteritis in their infants after the first dose. Active surveillance for all episodes of gastroenteritis, in-

cluding office visits to a physician for gastroenteritis, was conducted by contacting parents or guardians every two weeks. The rotavirus season was prospectively determined from historical epidemiologic data.[31-33] Most subjects were followed for one full rotavirus season after vaccination; however, some subjects were enrolled early enough to allow follow-up through a second full season. For subjects enrolled during a rotavirus season, surveillance was continued for the remainder of that season and through the next full rotavirus season.

To determine whether an episode of acute gastroenteritis satisfied the definition of a case of rotavirus gastroenteritis and to assess its clinical severity, parents or guardians were asked to complete diary cards and record symptoms daily until the illness resolved. An established clinical scoring system based on the intensity and duration of fever, vomiting, diarrhea, and changes in behavior was used to categorize episodes of rotavirus gastroenteritis on a 24-point severity scale; scores greater than 16 were considered to indicate severe disease (details are provided in Part II of the Supplementary Appendix).[22,34]

EVALUATION OF IMMUNOGENICITY

Immune responses to vaccination were assessed in a subgroup of subjects in the clinical-efficacy substudy. Serum samples were collected before the first dose and approximately 14 days after the third dose for measurement of antirotavirus IgA titers[35] and neutralizing antibodies against the G1, G2, G3, G4, and P[8] serotypes.[35] Seroconversion was defined as an increase in the antibody titer by a factor of 3 or more from baseline.

STATISTICAL ANALYSIS

Intussusception and Other Adverse Events

All subjects receiving at least one dose who had follow-up evaluations were included in the safety analyses according to the treatment actually received. The primary safety hypothesis was that the vaccine, relative to placebo, would not increase the risk of intussusception within 42 days after any dose. To satisfy this hypothesis, two criteria were prespecified, as follows. First, during the study, there could not be a significantly increased risk of intussusception among vaccine recipients as compared with placebo recipients within 7 days and 42 days after any dose. Second, at the end of the study, the upper bound of the 95 percent confidence interval for the relative risk of intussusception within 42 days after any dose had to be 10 or less, representing vaccine-to-placebo case ratios for intussusception of 2 or less based on the total number of expected cases, with such ratios considered to indicate a clinically acceptable relative risk of an uncommon event. This hypothesis was tested with the use of an exact binomial procedure based on the proportion of subjects with intussusception who received vaccine. The P value, point estimate, and confidence limits were appropriately adjusted for the group-sequential design of the study.[29] The relative risk was also assessed for the 7-day, 14-day, and 60-day periods after any dose and for the 365-day period after the first dose.

The power to detect an increased risk of intussusception during the study and to satisfy the primary safety hypothesis at the end of the study was estimated with the use of Monte Carlo simulation, with the assumption that cases of intussusception would accrue at a rate of 50 per 100,000 infant-years. If the risk of intussusception after vaccination was not increased, the probability that the primary safety hypothesis would be satisfied was approximately 94 percent. If the risk was similar to that reported for the RRV-TV,[14] the probability that the study would be stopped early was 85 to 91 percent.

Use of Health Care Resources

Use of health care resources because of rotavirus gastroenteritis occurring 14 or more days after the completion of the three-dose series for up to 2 years was assessed in the per-protocol population, which consisted of subjects for whom there was no protocol violation. Poisson regression with generalized estimating equations was used to estimate the reduction in the rate of use of health care resources and lost days of parents' or guardians' work in the vaccine group as compared with the placebo group.

Clinical Efficacy

The primary efficacy hypothesis specified that the vaccine would be efficacious in preventing wild-type G1–G4 rotavirus gastroenteritis occurring 14 or more days after completion of the three-dose series through the first full rotavirus season after vaccination. Subjects with multiple episodes meeting the case definition were counted only once. The statistical analysis was based on the total

number of subjects with rotavirus gastroenteritis from both groups, such that the number of subjects with rotavirus gastroenteritis in the vaccine group followed a binomial distribution. Exact inference was used. To permit the conclusion that the vaccine was efficacious, the lower bound of the two-sided 95 percent confidence interval had to be greater than 35 percent.

The primary efficacy analysis was based on the per-protocol population from the clinical-efficacy substudy, with use of the protocol case definition for G1–G4 rotavirus gastroenteritis occurring 14 or more days after the third dose. In secondary analyses of the per-protocol population, efficacy against severe G1–G4 rotavirus gastroenteritis and efficacy through a second rotavirus season after vaccination were examined. Another efficacy analysis was based on an intention-to-treat population, which consisted of all subjects (regardless of protocol violations) who received at least one dose and in which all cases of G1–G4 rotavirus meeting the protocol case definition and occurring at any time after the first dose were counted.

RESULTS

SUBJECTS

After 60,000 subjects had been monitored for 42 days after their last dose, the data and safety monitoring board reviewed the intussusception data with respect to treatment assignment and recommended the enrollment of 10,000 additional subjects because the criteria for stopping enrollment associated with the primary safety hypothesis had not yet been satisfied. After the additional subjects had been enrolled and followed, the board advised stopping enrollment because the prespecified criteria had been met. The analyses in this report are based on data available when the board members made their recommendation to stop enrollment.

In total, 70,301 subjects were enrolled, and data for 69,274 randomly assigned subjects were available in the clinical database. Overall, 68,038 subjects (98.2 percent) received at least one dose of vaccine or placebo; 59,210 (85.5 percent) received three doses and were followed for safety for 42 days after the third dose; and 56,310 (81.3 percent) were followed for 1 year after the first dose (Fig. 1B of the Supplementary Appendix). Among the subjects who received at least one dose, 67,756 (99.6 percent) were followed for 42 days after their last dose. The demographic characteristics of the subjects in the vaccine and placebo groups were generally similar (Table 1). The median age of the subjects at the time of entry was 10 weeks.

INTUSSUSCEPTION

A confirmed case of intussusception occurred within one year after the first dose in 12 vaccine recipients and 15 placebo recipients (relative risk, 0.8; 95 percent confidence interval, 0.3 to 1.8) (Fig. 1A). A confirmed case of intussusception occurred within the 42-day period after any dose in six vaccine recipients and five placebo recipients (multiplicity-adjusted relative risk, 1.6; 95 percent confidence interval, 0.4 to 6.4) — a result that satisfied the primary safety hypothesis (Fig. 1B). In no case did intussusception occur in a vaccine recipient within 42 days after the first dose.

Of the 27 confirmed cases of intussusception occurring within one year after the first dose, 16 (59 percent) involved boys. At the time of intussusception, the vaccine recipients were not younger than the placebo recipients (Fig. 1C). One death from postoperative sepsis occurred in a vaccine recipient in whom intussusception had been diagnosed 98 days after dose 3. Five stool specimens available from subjects with a confirmed case of intussusception at the time of diagnosis tested negative for rotavirus antigen.

OTHER ADVERSE EVENTS

Serious adverse events were reported in 803 of 34,035 vaccine recipients (2.4 percent) and 859 of 34,003 placebo recipients (2.5 percent). Overall, 44 deaths occurred during the study, 24 among vaccine recipients (<0.1 percent) and 20 among placebo recipients (<0.1 percent). The most common cause of death in both groups was sudden infant death syndrome, which occurred in seven vaccine recipients and eight placebo recipients. No deaths were attributed to vaccination by investigators blinded to treatment assignment.

Among the 9605 subjects in the detailed safety substudy (4806 in the vaccine group and 4799 in the placebo group), the rates of fever, vomiting, and diarrhea within 42 days after any dose were similar among vaccine recipients and placebo recipients (Fig. 2). The overall incidence of hematochezia within 42 days after any dose was 0.6 percent in each group. Among those with nega-

Table 1. Baseline Demographic Characteristics of the Subjects.*

Variable	Large-Scale Study		Detailed Safety Substudy		Clinical-Efficacy Substudy	
	Vaccine Group	Placebo Group	Vaccine Group	Placebo Group	Vaccine Group	Placebo Group
Randomly assigned to study group — no.	34,644	34,630	4826	4821	2841	2845
Sex — no. (%)						
Male	17,586 (50.8)	17,529 (50.6)	2482 (51.4)	2491 (51.7)	1462 (51.5)	1467 (51.6)
Female	17,058 (49.2)	17,101 (49.4)	2344 (48.6)	2330 (48.3)	1379 (48.5)	1378 (48.4)
Age at entry — wk						
Mean	9.8±1.4	9.8±1.4	9.7±1.4	9.7±1.4	9.7±1.6	9.7±1.5
Median	10	10	10	10	10	10
Range	3–13	1–16	3–13	4–13	3–13	4–13
Race or ethnic group — no. (%)†						
White	23,772 (68.6)	23,788 (68.7)	3052 (63.2)	3031 (62.9)	1854 (65.3)	1,885 (66.3)
Hispanic	4,963 (14.3)	4,911 (14.2)	499 (10.3)	486 (10.1)	282 (9.9)	251 (8.8)
Black	2,908 (8.4)	2,941 (8.5)	209 (4.3)	237 (4.9)	49 (1.7)	58 (2.0)
Multiracial	1,815 (5.2)	1,817 (5.2)	305 (6.3)	304 (6.3)	126 (4.4)	143 (5.0)
Asian	536 (1.5)	552 (1.6)	221 (4.6)	237 (4.9)	18 (0.6)	12 (0.4)
Native American	531 (1.5)	514 (1.5)	512 (10.6)	493 (10.2)	510 (18.0)	492 (17.3)
Other	119 (0.3)	107 (0.3)	28 (0.6)	33 (0.7)	2 (0.1)	4 (0.1)

* Plus–minus values are means ±SD.
† Race or ethnic group was determined by the investigator according to prespecified categories.

tively adjudicated cases of intussusception, hematochezia occurred more frequently in the vaccine group (10 subjects) than in the placebo group (3 subjects). With the exception of dermatitis (which was more common among vaccine recipients than among placebo recipients), adverse events were reported with similar frequency in the two groups.

During the four-to-six-day period after the administration of the first dose, fecal shedding of vaccine strains was detected in 17 of 134 vaccine recipients (12.7 percent). None of 109 vaccine recipients shed vaccine strains from four to six days after dose 2 and none of 99 did so after dose 3.

USE OF HEALTH CARE RESOURCES FOR ROTAVIRUS GASTROENTERITIS

In the large-scale study, 28,646 and 28,488 subjects in the vaccine and placebo groups, respectively, were included in the per-protocol analysis of the efficacy of the vaccine in reducing the need for hospitalization or emergency department care for rotavirus gastroenteritis (Fig. 1C of the Supplementary Appendix). Overall, 204 subjects (13 in the vaccine group and 191 in the placebo group) visited emergency departments and 144 subjects (6 in the vaccine group and 138 in the placebo group) were hospitalized for G1–G4 rotavirus gastroenteritis. The vaccine reduced the combined incidence of hospitalization or emergency department care for G1–G4 rotavirus gastroenteritis by 94.5 percent (95 percent confidence interval, 91.2 to 96.6 percent), with a 95.8 percent reduction in the rate of hospitalization (95 percent confidence interval, 90.5 to 98.2 percent) and a 93.7 percent reduction in the rate of emergency department visits (95 percent confidence interval, 88.8 to 96.5 percent). The numbers of hospitalizations and emergency department visits are shown according to serotype in Table 2.

The efficacy of the vaccine against all gastroenteritis-related hospitalizations after the first dose was 58.9 percent (95 percent confidence interval, 51.7 to 65.0 percent). There also was an 86.6 percent reduction (95 percent confidence interval, 78.0 to 91.9 percent) in the number of lost workdays associated with G1–G4 rotavirus gas-

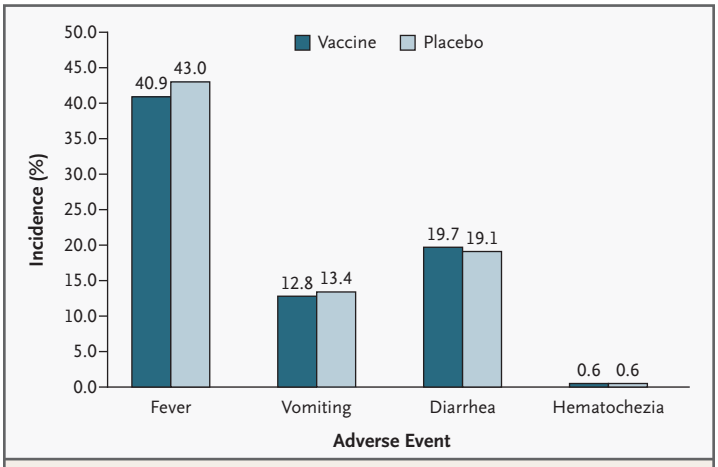

Figure 2. Percentage of Subjects in the Detailed Safety Substudy with Fever, Vomiting, Diarrhea, or Hematochezia within 42 Days after Any Dose, According to Study Group.

Fever refers to all reported episodes of fever.

Table 2. Reduction in the Numbers of Hospitalizations and Emergency Department Visits in the Per-Protocol Population of the Large-Scale Study, According to G Serotype Identified in the Subject's Stool.*

Serotype	No. of Cases of Rotavirus Gastroenteritis		Percent Rate Reduction (95% CI)
	Vaccine Group (N = 34,035)	Placebo Group (N = 34,003)	
G1	16	328	95.1 (91.6–97.1)
G2	1	8	87.6 (<0–98.5)
G3	1	15	93.4 (49.4–99.1)
G4	2	18	89.1 (52.0–97.5)
G9	0	13	100.0 (67.4–100.0)
G12	0	1	100.0 (<0–100.0)

* The number of subjects in each group is the number who received at least one dose. Some subjects had more than one event. CI denotes confidence interval.

troenteritis: the parents or guardians of vaccine recipients lost 65 workdays, whereas the parents or guardians of placebo recipients lost 487 workdays. In the clinical-efficacy substudy, the vaccine reduced office or clinic visits for G1–G4 rotavirus gastroenteritis by 86.0 percent (95 percent confidence interval, 73.9 to 92.5 percent).

CLINICAL EFFICACY AGAINST ROTAVIRUS GASTROENTERITIS, ACCORDING TO SEVERITY

The clinical-efficacy substudy included 5673 vaccinated subjects (Fig. 1D of the Supplementary Appendix). Among 4512 subjects (2207 in the vaccine group and 2305 in the placebo group) whose data could be evaluated in the per-protocol efficacy analysis, 397 cases of rotavirus gastroenteritis (82 and 315, respectively) caused by G1–G4 serotypes (G1 in 358, G2 in 23, G3 in 7, and G4 in 9) occurred 14 or more days after the third dose during the first full rotavirus season. The efficacy of the vaccine against G1–G4 rotavirus gastroenteritis of any severity was 74.0 percent (95 percent confidence interval, 66.8 to 79.9 percent) and that against severe G1–G4 rotavirus gastroenteritis was 98.0 percent (95 percent confidence interval, 88.3 to 100 percent). Only one case of severe rotavirus gastroenteritis occurred among vaccine recipients during the first full rotavirus season after vaccination. The mean severity score for cases in vaccine recipients was 9.1 (range, 1 to 17), as compared with 12.9 (range, 2 to 21) for cases in placebo recipients. Serotype-specific results are presented in Table 3. In a modified intention-to-treat analysis that included all subjects who received at least one dose and in which per-protocol cases occurring anytime during the first full rotavirus season after the first dose were counted, the efficacy of the vaccine was 60.0 percent (95 percent confidence interval, 51.5 to 67.1 percent) against G1–G4 rotavirus gastroenteritis of any severity.

During the second rotavirus season after vaccination, there were 36 G1–G4 cases among 813 vaccine recipients with data that could be evaluated and 88 G1–G4 cases among 756 placebo recipients that could be evaluated. Second-season efficacy against G1–G4 rotavirus gastroenteritis of any severity was 62.6 percent (95 percent confidence interval, 44.3 to 75.4 percent) and that against severe disease (which occurred in 2 vaccine recipients and 17 placebo recipients) was 88.0 percent (95 percent confidence interval, 49.4 to 98.7 percent).

IMMUNOGENICITY

Antibody responses were measured in a subgroup of subjects from whom serum samples had been obtained, according to a predetermined schedule, before the first dose and approximately two weeks after the third dose (Fig. 3). Seroconversion rates for serum neutralizing antibody to each human rotavirus serotype in the vaccine were significantly higher in the vaccine group than in the placebo group. A higher proportion of vaccine recipients whose data could be evaluated had seroconversion

to G1, G4, and P[8] than to G2 or G3. Seroconversion rates for serum antirotavirus IgA were 95.2 percent (95 percent confidence interval, 91.2 to 97.8 percent) among 189 vaccine recipients whose data could be evaluated, as compared with 14.3 percent (95 percent confidence interval, 9.3 to 20.7 percent) among 161 placebo recipients that could be evaluated.

DISCUSSION

The results of our study provide a high level of confidence in the safety of the pentavalent human–bovine (WC3) reassortant rotavirus vaccine and demonstrate its potential benefit in preventing rotavirus gastroenteritis and the associated use of health care resources. Active surveillance did not detect a significantly increased risk of intussusception in vaccine recipients at any time during the study, and the primary safety hypothesis was satisfied at the end of the study. The relative risk of intussusception among vaccine recipients, as compared with placebo recipients, was 1.6 (95 percent confidence interval, 0.4 to 6.4) during the 42-day period after any dose — a result that met prespecified criteria for an acceptable safety profile. Cases of intussusception occurred sporadically, without evidence of increased risk among vaccine recipients during the 7-day and 14-day periods after each dose — the periods of greatest risk with RRV-TV.[10-14] In contrast to observations with RRV-TV,[15-18] the rates of fever, vomiting, diarrhea, and hematochezia were similar among vaccine and placebo recipients in our study. These data are consistent with the results of the early-phase clinical trials of the human–bovine (WC3) reassortant vaccine, in which only a single case of intussusception was reported (in a seven-month-old boy) among the 2470 vaccine recipients.[22,23]

The pentavalent rotavirus vaccine was highly efficacious against severe rotavirus gastroenteritis and provided substantial protection against rotavirus gastroenteritis of any severity. Its efficacy, especially against severe rotavirus disease, persisted through a second rotavirus season. These data are consistent with the considerable protection induced by wild-type rotavirus infection against mild-to-moderate rotavirus gastroenteritis and the virtually complete immunity induced by wild-type infection against severe disease.[3,5] Our study confirms the results of phase 2 trials of the pentavalent vaccine and its predecessors, in which efficacy was 68 percent to 75 percent against rotavirus gastroenteritis of any severity and 100 percent against severe disease.[22,23]

The large sample size in our trial provided an opportunity to quantify the effect of vaccination on health care outcomes related to rotavirus gastroenteritis in a prelicensure setting. The vaccine significantly reduced the need for hospitalization, emergency department visits, and office visits associated with rotavirus gastroenteritis, underscoring the potential public health benefit of a universal vaccination program if the efficacy

Table 3. Clinical Efficacy against Rotavirus Gastroenteritis of Any Severity in the Per-Protocol Population of the Clinical-Efficacy Substudy, According to G Serotype Identified in the Subject's Stool.*

Serotype	No. of Cases of Rotavirus Gastroenteritis		Percent Efficacy (95% CI)
	Vaccine Group (N=2834)	Placebo Group (N=2839)	
G1	72	286	74.9 (67.3–80.9)
G2	6	17	63.4 (2.6–88.2)
G3	1	6	82.7 (<0–99.6)
G4	3	6	48.1 (<0–91.6)
G9	1	3	65.4 (<0–99.3)

* The number of subjects in each group is the number who received at least one dose. CI denotes confidence interval.

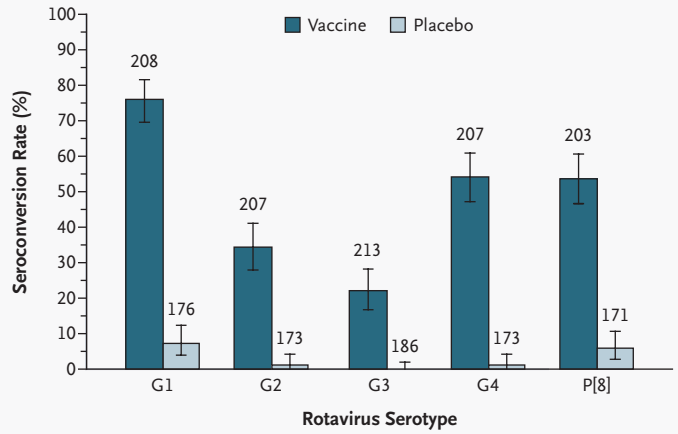

Figure 3. Seroconversion Rates for Serum Neutralizing Antibodies against Human Serotypes Included in the Vaccine.

Seroconversion was defined as an increase by a factor of 3 or more in the serum titer of neutralizing antibodies against the specified rotavirus serotype between baseline and approximately 14 days after the third dose. The number of subjects tested in each group is given above the corresponding bar. I bars represent the 95 percent confidence interval for the point estimates.

observed in our trial is reproduced in clinical practice. Vaccination could also have indirect benefits to society by reducing lost workdays for parents or guardians of young children.

The immunologic mechanism by which rotavirus vaccines protect against rotavirus gastroenteritis is unclear.[35,36] Primary wild-type rotavirus infection induces immunity that is predominantly serotype-specific.[4,5,37] Serotype-specific efficacy could be assessed in the present study only for the strains circulating during the study period. The efficacy of the vaccine could be demonstrated against serotypes G1 through G4 and in a small number of G9 cases, as evidenced by reductions in the incidence of gastroenteritis or the rate of use of health care resources, or both, associated with these serotypes.

For the most part, we enrolled healthy infants between 6 and 12 weeks of age who were from developed countries. Given that the vaccine is administered orally, additional studies to confirm efficacy in children who are malnourished or infected with multiple enteric pathogens are warranted. Along with the apparent absence of an association between intussusception and wild-type rotavirus disease,[24,25] the results of this large trial are reassuring in indicating that not all rotavirus vaccines are associated with intussusception. Because intussusception is an uncommon event, continued monitoring is appropriate. Our results also confirmed the efficacy of the vaccine against rotavirus gastroenteritis through two rotavirus seasons after vaccination. The vaccine markedly decreased the rotavirus-associated use of health care resources. Widespread administration of a safe and effective vaccine could substantially reduce the morbidity and mortality associated with this global childhood disease.

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The views expressed in this article are those of the authors and do not necessarily reflect those of the Indian Health Service.

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Supplementary Appendix

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

Supplement to: Vesikari T, Matson DO, Dennehy P, et al. Safety and efficacy of a pentavalent human–bovine (WC3) reassortant rotavirus vaccine. N Engl J Med 2006;354:23-33.

SUPPLEMENTARY APPENDIX

Part I. Study Sites and Boards.

A. Clinical Study Sites (listed in alphabetical order by country and by principal investigator).

Belgium

P. Aerssens, Kinderartsenpraktijk, Hasselt, Belgium; N. Balduck, Zaventem, Belgium; H. Buydens, Ninove, Belgium; L. Callewaert, Sint-Niklaas, Belgium; M. Claeys, Sint Vincentius Hospital, Antwerp, Belgium; A. De Cocker, Asse, Belgium; B. Delmotte, Heilig Hart Ziekenhuis, Roeselaere, Belgium; E. DeLoof, Heilig Hart Ziekenhuis, Leuven, Belgium; E. Devos, Heilig Hart Ziekenhuis, Consultation Pediatrics, Roeselare, Belgium; D. Dhondt, Gent, Belgium; J. Franckx, Mollem – Asse, Belgium; D. Haentjens, O.L.V. Middelares, Deume, Belgium; J-L Hennecker, Clinique Notre Dame de Grâce, Gosselies, Belgium; R-A Lemahieu, Tervuren, Belgium; K. Logghe, Heilig Hart Ziekenhuis, Consultation Pediatrics, Roeselare, Belgium; J.M.Ariel de Selys Longchamps, Cabinet de Pediatrie, Bruxelles, Belgium; M. Michel, Clinique Saint-Pierre, Ottignies, Belgium; S. Peeters, ASZ – Afdeling Pediatrie, Aalst, Belgium; F. Roelens, Heilig Hart Ziekenhuis, Consultation Pediatrics, Roeselare, Belgium; E. Rummens, A.S.Z. – Afdeling Pediatrie, Aalst, Belgium; C. Saintes, Cabinet de Pédiatrie, Bruxelles, Belgium; B. Schoenmakers, Katholieke Universiteit Leuven, Leuven, Belgium; E. Sokal, Cliniques Universitaires Saint-Luc, Brussels, Belgium; S. Traen, Maldegem, Belgium; J. Vanclaire, Cabinet de Pédiatrie, Kraainem, Belgium; E. Vandenbussche, Kinderarts, Heist-Op-Den-Berg, Belgium; C. Vandermeulen, Katholieke Universiteit Leuven, Leuven, Belgium; B. Vanheule, Heilig Hart Ziekenhuis, Roeselare, Belgium; M. Verboven, Brussels, Belgium; T. Vercruysse, Gent, Belgium; C. Verelst, Hasselt, Belgium; A. Vertruyen, Schoten, Belgium; A. Wilikens, Pediater – Kinderarts, Meise, Belgium; Marie Van der Wielen, University of Antwerp, Antwerp, Belgium

Costa Rica

J.U. Bogantes, Clinica de Especialidades, Medicas Santa Teresita, San José, Costa Rica; L.M.A. Cruz, Clínica Santa Catalina, San José, Costa Rica; J.E.M-M Diaz, Clinica San Agustin, San Jose, Costa Rica; M.C. Hernandez, Centro Médico la Guaria, San José, Costa Rica; J.V. Saenz-Castro – Centro de Especialidades Medicas, Heredia, Costa Rica; J.P.A. Sancho, San Jose, Costa Rica; R.A.G. Varela, Clinica San Agustin, San Jose, Costa Rica; E.E. Vargas, Hosptial Cristiano Jerusalem, San Jose, Costa Rica; J.C. Vargas, Clinica San Agustin, San José, Costa Rica

Finland

Timo Vesikari, Principal Investigator; Aino Karvonen, Physician Coordinator, University of Tampere, Vaccine Research Center with the following clinical trial sites: T.S.M Korhonen, Tampere Clinic, Tampere, Finland; N. Lindblad, M.H. Grönroos, J.P. Maunu, Turku Clinic, Turku, Finland; ; P.E. Riikonen, Pori Clinic, Pori, Finland; T. Karppa, J. Majuri, Lahti Clinic, Lahti, Finland; H. Siljander, C.J. Anttila-Bondestam, M.E. Koskikallio, M.T. Lumia, Helsinki West Clinic, Helsinki East Clinic and Helsinki South Clinic, Helsinki, Finland; H. Siljander, M. Espo, S. Sairanen, E.A. Pere, Vantaa East Clinic and Vantaa West Clinic, Vantaa, Finland; H. Siljander, M. Aaltonen, Espoo Clinic, Espoo, Finland; S.M. Parry, Jyväskylä Clinic, Jyväskylä, Finland, A. Kotaniemi-Syrjänen, Kuopio Clinic, Kuopio, Finland; H.A-M Laurent, Lappeenranta Clinic, Lappeenranta, Finland; S. Ylitalo, Oulu Clinic, Oulu, Finland; O. Nyblom, Vaasa Clinic, Vaasa, Finland; J. H. Khan, H. M. Khary, P. Laitinen, Järvenpää Clinic, Järvenpää, Finland; L-K. Kujala, Nurmijärvi Clinic, Klaukkala, Finland; V. Vähäsarja, Seinäjoki Clinic, Seinäjoki, Finland; K.J. A. Lönnberg, Porvoo Clinic, Porvoo, Finland; M. Espo, Kotka Clinic, Kotka, Finland; M. Cederberg, Rauma Clinic, Rauma, Finland; C. Häggqvist, Kokkola Clinic, Kokkola, Finland; A. Benyamin, Kouvola Clinic, Kouvola, Finland

Germany

H. Althen, Beckum, Germany; R. Bahr, Eckernförde, Germany; C. Bakowski, Herrsching, Germany; C.W. Baukhage, Munich, Germany; B. Becker (principal investigator), T. Beck, Bochum, Germany; T. Becker, Alsfeld, Germany; U. Behre, Kehl, Germany; V. Bekelaer, Zwiesel, Germany; J. Berger, Linz, Germany; B. Bittmann, Tutzing, Germany; M. Bölich, Jena, Germany; J. Bonanati, Aalen, Germany; G. Börzsonyi, Freising, Germany; H. Boss, Minden, Germany; S-H Braun, Viersen, Germany; H. Brück,

Korschenbroich, Germany; F. Bundscherer, Geretstried, Germany; M. Burk, Crailsheim, Germany; A. Busse, Tegernsee, Germany; H-J Büttner, Gau-Odernheim, Germany; S. Caspers-Hazay, Hamburg, Germany; R. Clementsen, Neumünster, Germany; E. Danners, Steinfurt, Germany; J. Dawoud, Ingolstadt, Germany; R. Dierschke, Oppenheim, Germany; E. Dietmair, Bobingen, Germany; D. Distel, Neustadt/Aisch, Germany; D. Drexler, Wolfenbüttel, Germany; F-P Drobnitzky, Gütersloh, Germany; D. Faul, Stuttgart, Germany; T. Fendel, Munich, Germany; C. Fiedler, Eisenberg, Germany; R. Freund, Berlin, Germany; T. Fröhlich, Bammental, Germany; R. Gall, Bernau, Germany; U. Goering, Pegnitz, Germany; D. Grunert, Nördlingen, Germany; P. Habermehl, Universitatskinderklink, Mainz, Germany; T. Hangen, Landshut, Germany; K. Helm, Detmold, Germany; E. Herm, Bad Oeynhausen, Germany; I. Hiller, Furth, Germany; M.R. Holtorf, Brunsbüttel, Germany; J. Hornivius, Mönchengladbach, Germany; H. Hornstein, Oberhausen, Germany; W. Hultzsch, Munich, Germany; H. Husgen, Willich, Germany; U. Jakob, Mainz, Germany; U. Janssen, Hochberg, Germany; P-J Kaas, Wesseling, Germany; J. Kandzora, Neumünster, Germany; R. Kemmerich, Weinstadt, Germany; S. Kiran, Heilbronn, Germany; B. Klaassen, Oppenheim, Germany; G. Knapp, Schwieberdigen, Germany; N. Kniess, Ingolstadt, Germany; R. Köllges, Mönchengladbach, Germany; K-H Krause, Goch, Germany; W.H. Kustermann, Veitshöchheim, Germany; D. Lasius, Berlin, Germany; O.F. Laub, Rosenheim, Germany; N. Lüttringhaus, Oberhausen, Germany; U. Macholdt, Neuhaus am Rennweg, Germany; S. Mahdi, Lubeck, Germany; A. Maurer, Frankenthal, Germany; N. Meister, Bayreuth, Germany; H-J Merkel, Ludwigshafen, Germany; W. Mollmann, Wuerzburg, Germany; K. Müller, Bamberg, Germany; S. Noll, Porta Westfalica, Germany; H. Pabel, Herford, Germany; H. Pankow-Culot, Heiligenhaus, Germany; C. Pauli (principal investigator), H. Preidel, Olching, Germany; A. Paulus-Koschik, Erkrath, Germany; U. Pfletschinger, Stuttgart, Germany; A. Pizzulli, Koeln, Germany; W. Pösentrup, Beckum, Germany; V. Reschke, Kaugbeuren, Germany; H-H Rohé, Hille, Germany; F. Ruland, Ahrweiler, Germany; H. Sauter, Echterdingen, Germany; G. Schacker, Bielefeld, Germany; M. Schaefer, Mainz, Germany; H. Scheele, Niedernhausen, Germany; F. Scheffer, Bochum, Germany; H. Schirmer, Marktredwitz, Germany; R. Schleupner, Würzbur, Germany; S. Schloesser, Kirchlengern, Germany; A. Schmetzer, Aalen, Germany; E. Schmitz-Hauss, Willich, Germany; H. Schöpfer, Espelkamp, Germany; D. Schweingel, Bayreuth, Germany; H-C Sengespeik, Munich, Germany; C. Sievers, Worms, Germany; S. Simmet, Schweigen-Rechtenbach, Germany; U. Sträubler, Berlin, Germany; K-J Taube, Berlin, Germany; P. Tcherepnine, Roding, Germany; I. Tichmann-Schumann, München, Germany; A. Timnik, Neusäss, Germany; K. Vogel, Bodenheim, Germany; G. Voigt, Melle, Germany; M. Vomstein, Schwäbisch Hall, Germany; P.F. von Feldegg, Münster, Germany; K.T. Weber, Berlin, Germany; J. Weidinger (principal investigator), W. Wolf, Nabburg, Germany; K-W Weigel, Karlstadt, Germany; O. Weise (principal investigator), E. Clapier, Landsberg am Lech, Germany; M. Wendeborn, Munich, Germany; T. Wenzel, Ulm, Germany; C. Wittermann, Weilheim, Germany; C. Wolff, Hagen, Germany; H. Wolschner, Minden, Germany; M. Zinke, Hamburg, Germany; W. Zöllner, Wittmund, Germany

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Wynn, Utah Valley Pediatrics, Orem, UT; A.O. Yap, Yap Medical Clinic, Los Angeles, CA; R.J. Yetman, University of Texas-Houston Medical Center, Houston, TX; C.W. Yut, The Woodlands, TX; N.R. Zosa, Clinical Health Research Institute for the Sciences, Pico Rivera, CA.

B. Data and Safety Monitoring Board

The members of the Data and Safety Monitoring Board were as follows: K. Holmes (Chairman), Professor of Medicine, Director, Center for AIDS and STDs, University of Washington, Seattle, WA; E. Ledbetter, National Network on Immunization Information, Technical Reviewer, Adjunct Professor of Pediatrics, University of Texas Medical Branch, San Antonio, TX; M. Levin (Consultant), Professor of Pediatrics and Medicine, University of Colorado, Denver, Colorado; G. Peter, Professor of Pediatrics, Brown University, Providence, RI; D.P. Torres, Professor of Pediatrics, Universidad de Costa Rica, San José, Costa Rica; M. Uhari, Professor of Pediatrics, University of Oulu, Oulu, Finland; J. Wittes, Biostatistician, Statistics Collaborative, Inc., Washington, DC; D. Yost, Medical Director of Indian Health Services, Clinical Director's Office, Whiteriver Indian Health Service Hospital, Whiteriver, AZ.

C. Safety Endpoint Adjudication Committee

The members of the Safety Endpoint Adjudication Committee were as follows: J. Steinberg (Chairman), Children's Medical Center, Dallas, TX; G. Bates, Children's Hospital, Columbus, OH; D. Doody, Harvard Medical School, Boston, MA.

Part II. Clinical Scoring System[22,34] for Acute Rotavirus Gastroenteritis.

Point value*	1	2	3
Diarrhea			
Number of stools/day†	2 to 4	5 to 7	≥8
Duration in days‡	1 to 4	5 to 7	≥8
Vomiting			
Number of emeses/day§	1 to 3	4 to 6	≥7
Duration in days‡	2	3 to 5	≥6
Rectal Temperature			
Degrees in Celsius‖	38.1 to 38.2	38.3 to 38.7	≥38.8
Duration in days‡	1 to 2	3 to 4	≥5
Behavioral Symptoms/Signs			
Description¶	Irritable/Less Playful	Lethargic/Listless	Seizure
Duration in days‡	1 to 2	3 to 4	≥5

* Final score represented the sum of the individual components. Scores >8 were considered moderate-and-severe and scores >16 were considered severe disease.
† Maximum number of watery or looser-than-normal stools/day on any given day over the course of the episode.
‡ Number of days in which the subject had relevant symptom(s) or sign(s). Total days did not need to be consecutive.
§ Maximum number of emeses on any given day over the course of the episode.
‖ Highest rectal temperature recorded during the episode (only counted when the equivalent of a rectal temperature exceeded 38°C). Reported temperatures were converted to rectal equivalents by adding 1°F to otic and oral temperatures and 2°F to axillary temperatures.
¶ When a subject had ≥2 behavioral symptoms/signs, only the highest score was counted.

Part III. Figure Legend

Figure 1. REST organization and subject accounting.

Panel A. Relationship of the prospectively identified sub-studies to the large-scale study.

Panel B. Subject accounting in the large-scale study and detailed safety sub-study.

Panel C. Subject accounting in the large-scale study followed for efficacy against hospitalizations and emergency department visits due to rotavirus gastroenteritis.

Panel D. Subject accounting in the clinical efficacy sub-study.

Figure 1A.

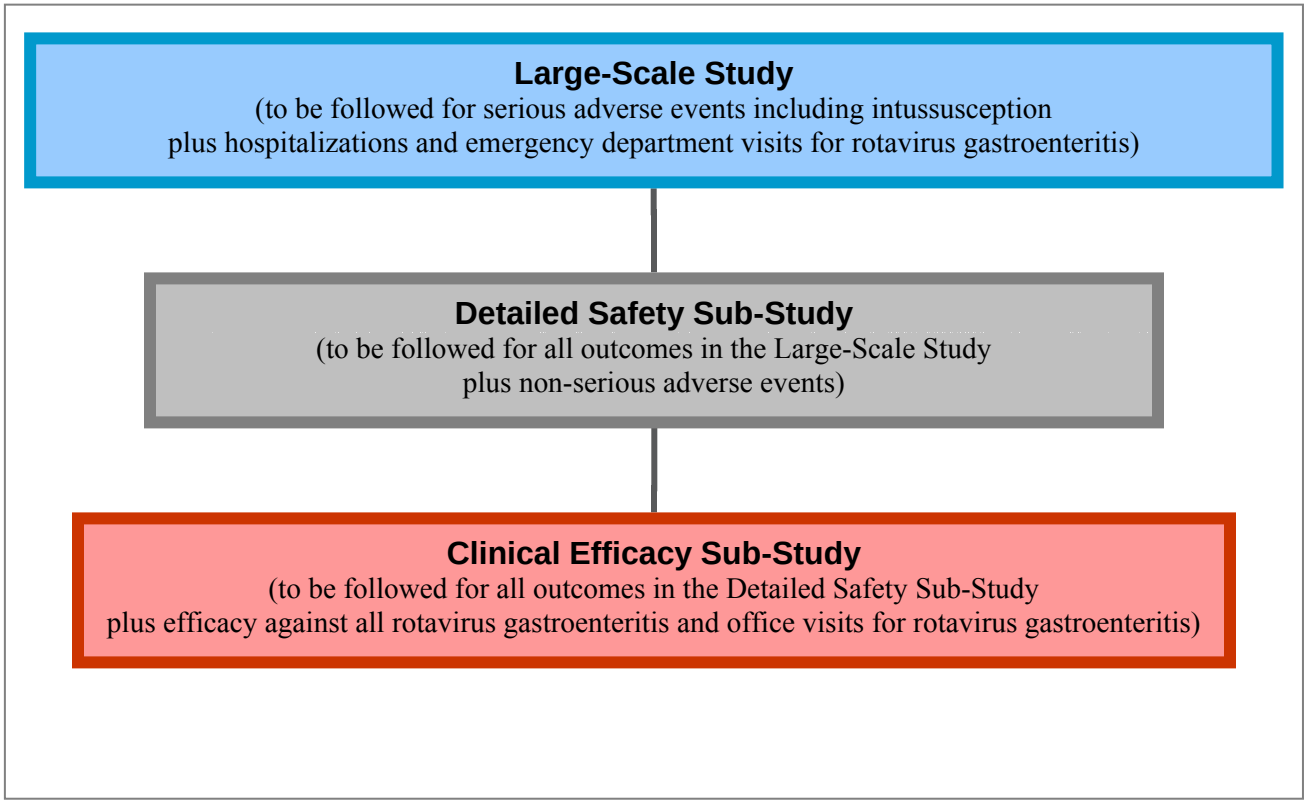

Large-Scale Study
(to be followed for serious adverse events including intussusception
plus hospitalizations and emergency department visits for rotavirus gastroenteritis)
Detailed Safety Sub-Study
(to be followed for all outcomes in the Large-Scale Study
plus non-serious adverse events)
Clinical Efficacy Sub-Study
(to be followed for all outcomes in the Detailed Safety Sub-Study
plus efficacy against all rotavirus gastroenteritis and office visits for rotavirus gastroenteritis)

Figure 1B.

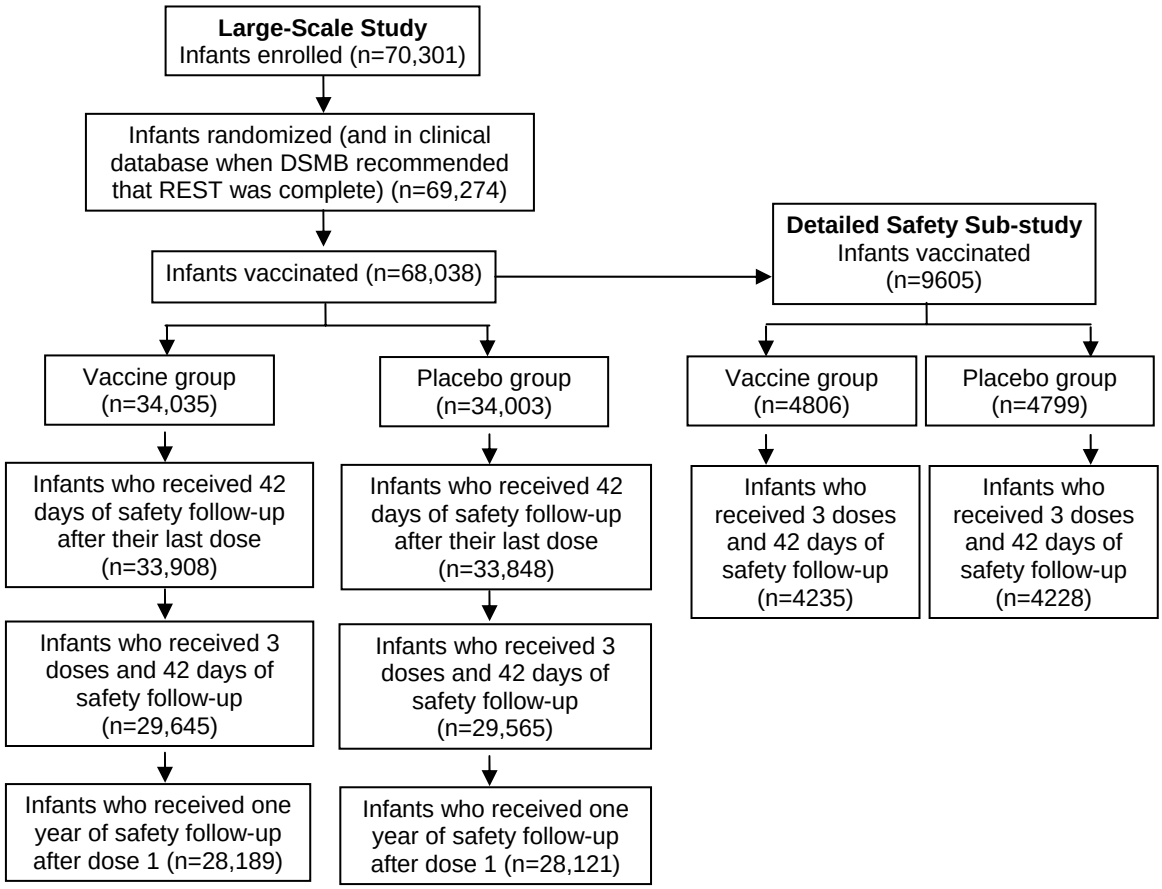
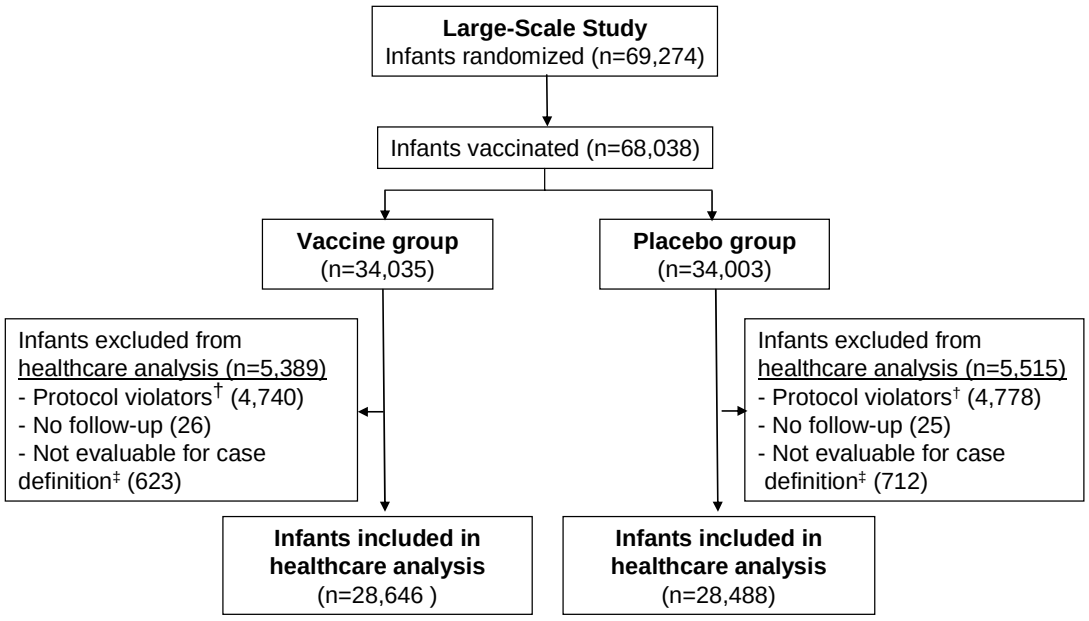
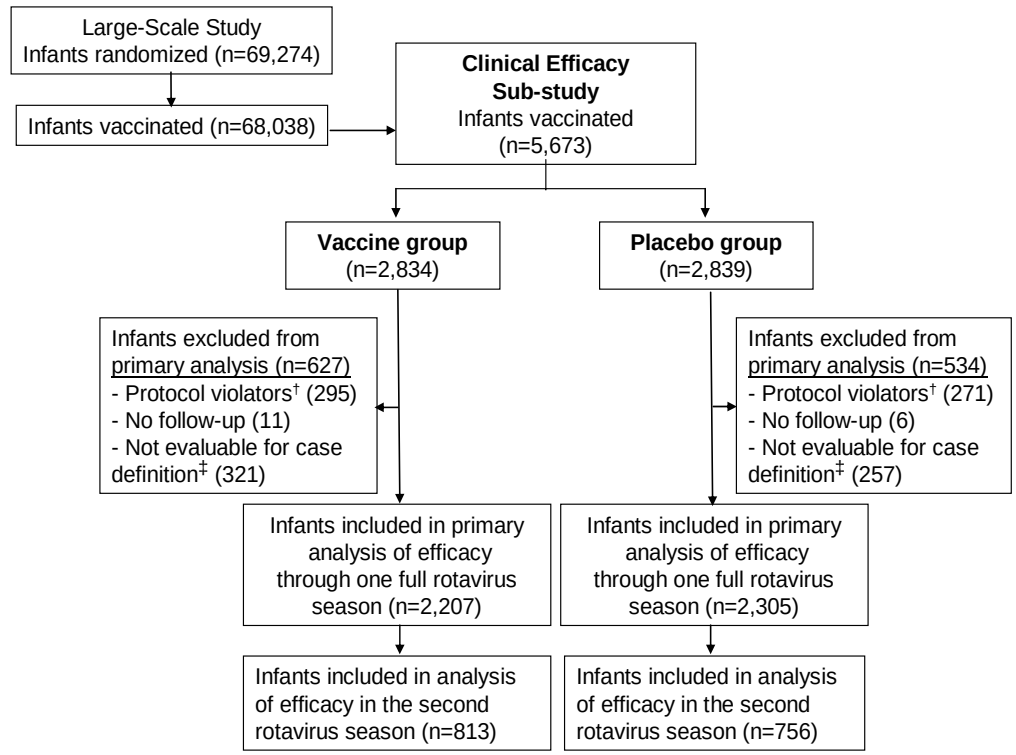

Large-Scale Study
Infants enrolled (n=70,301)
Infants randomized (and in clinical database when DSMB recommended that REST was complete) (n=69,274)
Infants vaccinated (n=68,038)
Detailed Safety Sub-study
Infants vaccinated
(n=9605)
Vaccine group
(n=34,035)
Placebo group
(n=34,003)
Vaccine group
(n=4806)
Placebo group
(n=4799)
Infants who received 42 days of safety follow-up after their last dose (n=33,908)
Infants who received 42 days of safety follow-up after their last dose (n=33,848)
Infants who received 3 doses and 42 days of safety follow-up (n=4235)
Infants who received 3 doses and 42 days of safety follow-up (n=4228)
Infants who received 3 doses and 42 days of safety follow-up (n=29,645)
Infants who received 3 doses and 42 days of safety follow-up (n=29,565)
Infants who received one year of safety follow-up after dose 1 (n=28,189)
Infants who received one year of safety follow-up after dose 1 (n=28,121)

Figure 1C.



†The large majority (>80%) of protocol violators did not receive all 3 doses.
‡Includes infants with incomplete clinical or laboratory data, wild-type rotavirus EIA-positive stool before the third dose, or stool samples collected >14 days after symptom onset.

Figure 1D.



†The large majority (>90%) of protocol violators did not receive all 3 doses.
‡Includes infants with incomplete clinical or laboratory data, wild-type rotavirus EIA-positive stool before the third dose, or stool samples collected >14 days after symptom onset.