

The New England Journal of Medicine

© Copyright, 1997, by the Massachusetts Medical Society

VOLUME 337

OCTOBER 23, 1997

NUMBER 17



EFFICACY OF THE RHESUS ROTAVIRUS-BASED QUADRIVALENT VACCINE IN INFANTS AND YOUNG CHILDREN IN VENEZUELA

IRENE PÉREZ-SCHAEL, M.S., MARÍA J. GUNTIÑAS, M.D., MIREYA PÉREZ, M.D., VITO PAGONE, M.S., ANA M. ROJAS, PH.D.,
ROSABEL GONZÁLEZ, B.S., WALTER CUNTO, PH.D., YASUTAKA HOSHINO, D.V.M., AND ALBERT Z. KAPIKIAN, M.D.

ABSTRACT

Background Rotaviruses are the principal known etiologic agents of severe diarrhea among infants and young children worldwide. Although a rhesus rotavirus-based quadrivalent vaccine is highly effective in preventing severe diarrhea in developed countries, in developing countries its efficacy has been less impressive. We thus conducted a catchment study in Venezuela to assess the efficacy of the vaccine against dehydrating diarrhea.

Methods In this randomized, double-blind, placebo-controlled trial, 2207 infants received three oral doses of the quadrivalent rotavirus vaccine (4×10^5 plaque-forming units per dose) or placebo at about two, three, and four months of age. During approximately 19 to 20 months of passive surveillance, episodes of gastroenteritis were evaluated at the hospital.

Results The vaccine was safe, although 15 percent of the vaccinated infants had febrile episodes (rectal temperature, $\geq 38.1^\circ\text{C}$) during the six days after the first dose, as compared with 7 percent of the controls ($P < 0.001$). However, the vaccine gave 88 percent protection against severe diarrhea caused by rotavirus and 75 percent protection against dehydration, and produced a 70 percent reduction in hospital admissions. Overall, the efficacy of the vaccine against a first episode of rotavirus diarrhea was 48 percent. Horizontal transmission of vaccine virus was demonstrated in 15 percent of the vaccine recipients and 13 percent of the placebo recipients with rotavirus-positive diarrhea.

Conclusions In this study in a developing country, the quadrivalent rhesus rotavirus-based vaccine induced a high level of protection against severe diarrheal illness caused by rotavirus. (N Engl J Med 1997;337:1181-7.)

©1997, Massachusetts Medical Society.

ROTAVIRUS diarrhea is a public health problem throughout the world, and it takes its greatest toll on infants and young children less than 24 months of age. Development of a rotavirus vaccine to prevent severe diarrhea is a high priority.^{1,2} Although rotavirus infections occur with nearly equal frequency in developed and developing countries, the consequences are quite different. More than 870,000 children under five years of age are estimated to die annually from rotavirus infections in developing countries, and only 75 to 150 in the United States.^{3,4} However, more than 100,000 infants and young children are hospitalized annually for rotavirus diarrhea in the United States.³

The most extensively evaluated candidate rotavirus vaccine consists of a quadrivalent formulation incorporating the VP7 neutralization specificity of each of the four clinically important serotypes with the attenuation phenotype of rhesus rotavirus.¹ This modified "Jennerian" approach combines the rhesus rotavirus strain VP7:3 with three reassortant rotaviruses in which the human rotavirus gene that encodes serotype VP7:1, 2, or 4 specificity was substituted for the rhesus rotavirus gene that encodes VP7 serotype specificity in a background of 10 rhesus rotavirus genes, after cocultivation and subsequent selection of the desired single-gene-substitution reassortant viruses.¹ This was possible because the rotavirus genome possesses 11 segments of double-stranded RNA that readily undergo reassortment.¹

From the Sección de Investigación de Enfermedades Entéricas, Instituto de Biomedicina/Universidad Central de Venezuela/Ministerio de Sanidad, Fuvesin, Caracas, Venezuela (I.P.-S., M.J.G., M.P., V.P., A.M.R., R.G.); the Instituto de Biomedicina de Venezuela, Caracas (W.C.); and the Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Md. (Y.H., A.Z.K.). Address reprint requests to Dr. Pérez-Schael at the Instituto de Biomedicina — Fuvesin, A.P. 4043, Carmelitas, Caracas 1010A, Venezuela.

Studies of this quadrivalent rotavirus vaccine in the United States and Finland have been encouraging, with marked reductions in the incidence of severe diarrhea.⁵⁻⁷ However, the results of studies in developing countries have been less promising.^{8,9} This report describes a study of this vaccine carried out in a poor population in Caracas, Venezuela. The goal of the clinical trial was to evaluate the efficacy of the vaccine against dehydration as the primary end point.

METHODS

Study Population

The clinical phase of the study was conducted at the Hospital Materno Infantil de Caricuao, an obstetrical and pediatric hospital located in an urban area of middle-to-low socioeconomic status southwest of Caracas. The hospital has 201 inpatient beds: 87 for maternity patients, 90 for pediatric patients, and 24 for emergency use. After hearing an explanation of the purpose, risks, and benefits of the study, at least one parent of each participating infant signed a consent form that had been approved by the ethics committee of the Instituto de Biomedicina, Caracas, and the Clinical Research Subpanel of the National Institute of Allergy and Infectious Diseases, Bethesda, Maryland. Only full-term, healthy infants weighing more than 2500 g at birth were enrolled in the study. Infants were excluded from the study if they were from a multiple pregnancy, were below the 10th percentile of weight-for-age scores according to World Health Organization curves, had major birth defects, or lived in the same dwelling with a pregnant woman. If an infant had fever, diarrhea, or vomiting, vaccination was delayed until the symptoms disappeared. Information about socioeconomic status was obtained at the time of enrollment by the modified Graffar method, which considers the professional activity of the father, educational level of the mother, income level of the family, and sanitary conditions in the home.¹⁰

Vaccination and Postvaccination Reactions

The quadrivalent rotavirus vaccine consisted of equal parts (1×10^5 plaque-forming units) of rhesus rotavirus with a VP7 of serotype 3 specificity now referred to as G3 (RRV) and of the following human-rhesus rotavirus reassortants: G1 (D×RRV), G2 (DS1×RRV), and G4 (ST3×RRV). The placebo consisted of uninfected tissue-culture fluid resembling the vaccine in appearance. Both were produced by Wyeth-Ayerst Research Laboratories, Philadelphia, and were stored at 2°C to 8°C at the hospital.

The study was a double-blind, placebo-controlled trial in which infants were randomly assigned to receive three doses of rotavirus vaccine or placebo at the ages of two, three, and four months, according to the schedule shown in Table 1. Randomization of infants was accomplished by a computerized algorithm that balanced the number of infants in the two groups for the numbers 1 to 500. Further codes were obtained as an extension of that basic sequence. Sequential code numbers were assigned at the time of the first dose of vaccine or placebo.

The routine immunizations were given according to the regulations of the Ministry of Health. To decrease the possibility of interference, oral poliovirus vaccine was intentionally given two to four weeks before or after rotavirus vaccine was given (Table 1). The second dose of diphtheria-pertussis-tetanus toxoid vaccine was administered concurrently with the second dose of rotavirus vaccine or placebo. Before the administration of rotavirus vaccine or placebo, the infants received 30 ml of buffered formula (Similac [Ross Laboratories, Columbus, Ohio] with 400 mg of bicarbonate added) to neutralize stomach acid. The vaccine or placebo was lyophilized and reconstituted with 3 ml of citrate-bicarbonate buffer just before 2.5 ml was administered orally. Food was withheld for one hour before and after vaccination.

TABLE 1. VACCINATION SCHEDULE.

AGE OF INFANT AT TIME OF VISIT ACCORDING TO STUDY PLAN (WK)*	SEQUENCE OF ADMINISTRATION OF VACCINE OR PLACEBO†	
	VACCINE GROUP	PLACEBO GROUP
0-2	OPV + BCG	OPV + BCG
6-8	OPV + DPT	OPV + DPT
8-10	RV dose 1	Placebo dose 1
12-14	RV dose 2 + DPT	Placebo dose 2 + DPT
16-18	RV dose 3	Placebo dose 3
20-24	OPV + DPT	OPV + DPT
24-26	OPV	OPV

*Visits to receive the vaccine or placebo were scheduled at four-week intervals, plus two more weeks to allow for unpredicted delays.

†OPV denotes oral poliovirus vaccine, BCG bacillus Calmette-Guérin vaccine, DPT diphtheria-pertussis-tetanus toxoid vaccine, and RV quadrivalent rotavirus vaccine.

The infants were evaluated passively for side effects for six days after each vaccination.

Surveillance for Efficacy

Gastroenteritis was defined as the presence of three or more liquid or semiliquid stools or a single stool with blood during a period of 24 hours, with or without vomiting. A period of 48 hours without gastroenteritis was required for a subsequent diarrheal illness to be considered a new episode.¹¹ Dehydration was defined according to the criteria of the World Health Organization.¹² The passive-surveillance period started 15 days after the third dose and lasted approximately 19 to 20 months, ending at 24 months of age. A home visit was made if a child had not been contacted for more than four months. The infants were under the care of the study pediatricians during the daytime and of hospital personnel during the night. The mothers were instructed in the use of oral rehydration solution at home.

Laboratory Studies

Stool specimens obtained during diarrheal episodes were tested for rotavirus by a preserum-postserum confirmatory enzyme-linked immunosorbent assay (ELISA), as described elsewhere.^{13,14} Rotavirus serotypes were identified by standard methods or by modifications of standard methods, such as ELISA with serotype-specific monoclonal antibodies, polymerase chain reaction (PCR), amplification in MA104 and African green monkey kidney-cell cultures, and polyacrylamide-gel electrophoresis¹⁵⁻²⁰ (and unpublished data). Parasites were detected by direct microscopical observation in saline and Lugol's solution. *Cryptosporidium parvum* was identified by modified Ziehl-Neelsen staining.²¹ *Campylobacter* species, shigella species, *Escherichia coli*, and *Vibrio cholerae* were detected by standard methods from two rectal swabs placed in Cary Blair transport medium.²² Rotavirus-positive specimens were tested for thermostable and thermolabile toxins of *E. coli* by a commercial immunoassay from Denka-Seiken (*E. coli* COLIST EIA Seiken and VET-RPLA Seiken, Tokyo, Japan), and enteroinvasive and enteroadherent *E. coli* (five colonies) were assayed by culture in HEP-2 cells.^{23,24} Enterohemorrhagic *E. coli* were detected by slide and test-tube agglutination methods with the corresponding antiserum (O157).²⁵

Serologic Response

Blood samples were obtained from a randomly selected subgroup of the study infants and children. Serum specimens were

collected before the first dose and four weeks after the first, second, or third dose. Three hundred one pairs of serum samples were tested by IgA ELISA and 60 percent plaque-reduction neutralization assay for serologic response against the Wa (G1), DS1 (G2), P (G3), VA70 (G4), and RRV (G3) strains.^{26,27}

Statistical Analysis

All the infants were included in the analysis of postvaccination reactions. Infants who received three doses of vaccine or placebo were followed during surveillance from the 15th day after the third dose up to 24 months of age and were included in the efficacy analysis. Because there was no difference in the dropout rate between the group receiving vaccine and the group receiving placebo during surveillance, every fully immunized infant was included in the efficacy analyses. Only the first rotavirus-related diarrheal episode detected during the surveillance period (irrespective of other pathogens) was included in the efficacy analysis. A 20-point severity-scoring system that has been described previously was used to analyze the efficacy of the vaccine.²⁸ The calculation of sample size was based on a rate of rotavirus illness (15 percent) that would detect an efficacy of 70 percent with a power of 0.8 at a one-tailed significance level of less than 0.05.²⁹ The efficacy of the vaccine was determined with the following formula: (rate of gastrointestinal illness in the placebo group - rate in the vaccinated group ÷ rate in the placebo group) × 100. The significance was determined by 95 percent confidence intervals and a two-tailed Fisher's exact test.³⁰

RESULTS

Study Groups

The study began in March 1992 and ended in October 1995. Of the 2480 infants enrolled, 2207 received all three doses of vaccine or placebo, and 2037 completed 19 or 20 months of surveillance. The study groups were similar with regard to the characteristics shown in Table 2.

Safety

Fever (rectal temperature, $\geq 38.1^{\circ}\text{C}$) occurred significantly more often in vaccinated infants than in placebo recipients on day 3 after the first dose of vaccine or placebo (5 percent vs. 1 percent, $P=0.01$) and overall during the six days after the first dose (15 percent vs. 7 percent, $P<0.001$) (Fig. 1). There were no other significant differences between the two groups in the occurrence of diarrhea, vomiting, coughing, runny nose, rash, or other clinical symptoms in the six days after any dose of vaccine or placebo. The high frequency of fever in both groups after the second dose undoubtedly reflected a response mostly to diphtheria-pertussis-tetanus vaccine, which was given concurrently.

Serologic Response to the Quadrivalent Rotavirus Vaccine

Significantly more vaccinated infants than controls had fourfold or greater increases in antibody titers after each dose according to at least one assay (Table 3). The IgA ELISA and the neutralization test for rhesus rotavirus were the most efficient in detecting a response (80 percent in the vaccine group after the second or third dose). Neutralizing-antibody responses to the human rotavirus serotypes

TABLE 2. CHARACTERISTICS OF THE SUBJECTS ACCORDING TO STUDY GROUP.

CHARACTERISTIC*	VACCINE GROUP	PLACEBO GROUP
Infants enrolled†	1247	1233
Age at each dose (wk)		
Dose 1		
Median	9	9
Range	8-11	8-11
Dose 2		
Median	13	13
Range	12-16	12-16
Dose 3		
Median	18	18
Range	16-20	16-20
No. of fully immunized infants under surveillance	1112	1095
No. of infants under surveillance for 19 to 20 months	1027	1010
Child-years of observation	1738	1713
Sex (M/F)	568/544	540/555
Socioeconomic status‡		
1-2 (high and high-middle class)	31	39
3 (middle to low class)	351	368
4-5 (low and marginal class)	721	675
Diet at dose 1/2/3‡		
Breast-fed	196/180/151	187/174/175
Breast- and bottle-fed	627/658/662	629/670/637
Not breast-fed	64/119/195	61/99/182

*No significant differences were found with regard to sex, socioeconomic status, dropout rate, median age at time of vaccination, or type of feeding at time of vaccination between the vaccine and placebo groups (two-tailed $P>0.05$ by Fisher's exact test).

†Nineteen infants were excluded because of coding irregularities.

‡Numbers do not add to total number of subjects because of missing data.

after three doses ranged from only 10 percent to 45 percent. The administration of a third dose, as compared with a single dose, significantly increased the likelihood of a response to each of the human rotavirus serotypes (Table 3). It should be noted, however, that the number of responses after the third dose of vaccine was not significantly different from that after two doses.

Efficacy

Stool specimens from 1537 of the 1550 reported diarrheal episodes that occurred during surveillance were tested for rotavirus. Of these, 742 episodes occurred in vaccinated infants and 808 in controls. Rotavirus was identified in 219 (14 percent) of all the episodes tested; 205 of the 219 rotavirus strains were from the initial episode of rotavirus diarrhea that was detected. The relative protective efficacy of three doses of rotavirus vaccine against the first rotavirus diarrheal episode of any severity was 48 percent (Table 4). However, the vaccine was more effective in preventing severe rotavirus diarrheal illness, achieving a protective efficacy of 75 percent against dehydrating rotavirus diarrheal illness, the primary end point. Consequently, it was highly effective in

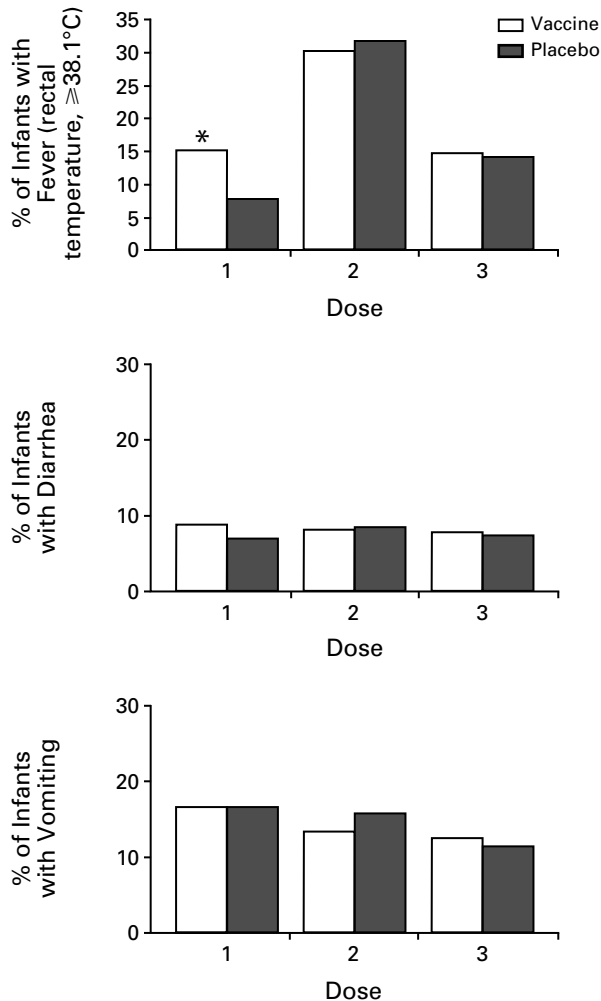


Figure 1. Occurrence of Fever (Rectal Temperature of 38.1°C or More), Diarrhea, and Vomiting during the Six Days after the Administration of Each Dose of Vaccine or Placebo. Asterisk denotes $P < 0.001$ for the comparison with the placebo group.

protecting against illness with a severity score of more than 14 (88 percent), of more than four days' duration (71 percent), and involving hospital admissions for rotavirus diarrhea (70 percent). The vaccine was also associated with a 49 percent reduction of fever (95 percent confidence interval, 24 to 66 percent; $P < 0.001$) and a 54 percent reduction in vomiting (95 percent confidence interval, 36 to 67 percent; $P < 0.001$).

Other enteric pathogens were found simultaneously with rotavirus in 47 percent of the episodes in vaccinated infants (33 of 70) and in 53 percent of the episodes in controls (71 of 135) ($P = 0.47$). Enteroadherent *E. coli* was the most frequently detected copathogen (79 percent in both the vaccine and the placebo recipients), followed by campylobacter

species (7 cases in the vaccine group and 17 in the placebo group) and shigella species (4 cases in the vaccine group and 3 in the placebo group). Enteroadherent *E. coli* with a localized, diffuse, or aggregated pattern was found in 10, 7, and 9 episodes in vaccinated infants, respectively, and in 17, 11, and 28 episodes in the placebo group. No parasites were found in 109 of the rotavirus-positive specimens tested. The efficacy of the vaccine was 54 percent against mixed infection (95 percent confidence interval, 31 to 69 percent; $P < 0.001$) and 43 percent against pure rotavirus infection (95 percent confidence interval, 15 to 62 percent; $P = 0.005$). The efficacy of the vaccine against mixed infections and against all rotavirus infections with the same severity score was very similar (Table 4). The efficacy of the vaccine against nondehydrating rotavirus diarrhea was 43 percent (95 percent confidence interval, 24 to 58 percent; $P < 0.001$). The vaccine was not effective against nonrotavirus diarrhea. We also analyzed the efficacy of the vaccine according to the child's age at the time of onset of the rotavirus diarrheal episode. The relative efficacy against all rotavirus diarrhea was 61 percent (95 percent confidence interval, 36 to 75 percent; $P < 0.001$) in infants more than 12 months old and was 41 percent in those 4 to 12 months of age (95 percent confidence interval, 18 to 58 percent; $P = 0.001$).

Rotavirus Serotypes and Transmission of Vaccine Virus

One hundred ninety-nine of the 205 rotavirus-positive specimens obtained from the first diarrheal episodes of infants who received vaccine or placebo were serotyped: 162 were G1 alone; 7 were G2 alone; 2 were G1 and G2; 1 was G1 and wild-type G3; and 27 were G1 and a vaccine strain. In addition, two children shed a G1 strain and a vaccine strain during a second episode of rotavirus diarrhea.

The detection of a vaccine strain in 29 (14 percent) of the 213 rotavirus-positive stools (199 from the first diarrheal episode, 13 from the second, and 1 from the third) examined by PCR, tissue culture, or electrophoresis always occurred in conjunction with that of a second rotavirus strain, which when possible was examined further and shown to be a wild-type rotavirus. Twenty-four of the vaccine strains detected were RRV, one was ST3×RRV, and four were RRV and ST3×RRV. Eighteen of the 139 episodes of rotavirus diarrhea in the placebo group (13 percent) and 11 of the 74 episodes in the vaccine group (15 percent) yielded one or more vaccine strains. Among episodes of dehydrating rotavirus diarrhea, 5 of 23 tested in the placebo group (22 percent) and none of 6 in the vaccine group were positive for vaccine strains. The vaccine strains were shed in low titer, ranging from 10^1 to 10^3 plaque-forming units per 0.5 ml in 19 of the 29 ten-percent stool suspensions that could be evaluated (9 had a

TABLE 3. SEROLOGIC RESPONSES AFTER ONE, TWO, OR THREE DOSES OF VACCINE OR PLACEBO.*

GROUP AND DOSE	IgA ELISA	NEUTRALIZATION TEST VS. INDICATED STRAIN (SEROTYPE)					
		Wa (G1)	DS1 (G2)	P (G3)	VA70 (G4)	RRV (G3)	ANY TEST
no. with response/no. tested (%)							
Vaccine group							
Dose 1	34/58 (59)	8/63 (13)	1/62 (2)	4/61 (7)	0/60 (0)	33/62 (53)	43/63 (68)
Dose 2	32/42 (76)†	17/42 (40)††	7/41 (17)†‡§	6/42 (14)	5/43 (12)§	38/43 (88)†¶	41/43 (95)†§
Dose 3	32/38 (84)†§	18/40 (45)†¶	13/39 (33)†¶	11/39 (28)†§	4/39 (10)§	30/39 (77)†§	35/40 (88)†§
Placebo group							
Dose 1	2/48 (4)	3/49 (6)	1/49 (2)	2/48 (4)	0/48 (0)	1/49 (2)	7/49 (14)
Dose 2	8/53 (15)	4/54 (7)	2/54 (4)	4/54 (7)	4/53 (8)	1/54 (2)	8/55 (15)
Dose 3	11/50 (22)	11/51 (22)	1/51 (2)	4/51 (8)	5/51 (10)	1/51 (2)	15/51 (29)

*A fourfold or greater increase in titer between tests of serum pairs was considered a significant serologic response.

†P<0.001 for the comparison with the same dose of placebo by Fisher's exact test.

††P<0.05 for the comparison with the first dose of vaccine by Fisher's exact test (two-tailed).

§P<0.05 for the comparison with the same dose of placebo by Fisher's exact test.

¶P<0.001 for the comparison with the first dose of vaccine by Fisher's exact test (two-tailed).

||P<0.05 for the comparison with the third dose of placebo by Fisher's exact test (two-tailed).

titer of 10^1 plaque-forming units, 8 had 10^2 plaque-forming units, and 2 had 10^3 plaque-forming units). Vaccine viruses were not detected in stools by ELISA or polyacrylamide-gel electrophoresis.

DISCUSSION

The rhesus rotavirus-based quadrivalent vaccine has been very successful in preventing severe rotavirus diarrhea in two trials in the United States and in one in Finland, with protection rates of 80 to 91 percent.⁵⁻⁷ However, it is perplexing that its efficacy has been inconsistent in developing countries, where the toll from diarrheal illness is greatest. In Peru and Brazil this vaccine induced only limited protection against moderate-to-severe diarrhea (30 to 46 percent efficacy).^{8,9} We carried out a large study in a poor area of a developing country to evaluate the efficacy of the vaccine against severe illness, as determined by passive surveillance.

In this trial, three doses of the quadrivalent vaccine proved to be very efficacious. It significantly reduced the incidence of severe diarrhea by 88 percent, dehydration by 75 percent, hospital admission by 70 percent, and illnesses that lasted more than four days by 71 percent. These results are similar to those of the U.S. and Finnish studies.⁵⁻⁷ In addition, the results are similar to those observed after natural rotavirus infections, which confer better protection against severe disease than against mild illness.^{31,32} The lesser efficacy observed in the Peruvian and Brazilian studies may have been due to the lower dose of vaccine used in those trials (4×10^4 plaque-forming units), the paucity of severe diarrhea because of the frequent interventions in these longitudinal, ac-

tive-surveillance studies, or the greater number of diarrheal episodes per child-year (8 and 6 in Peru and Brazil, respectively, as compared with only 2.2 in Venezuela).^{8,9,33} Our study was not designed for an intention-to-treat analysis. However, the distribution of the 273 participants who dropped out of the study after receiving the first dose (76 in the vaccine group and 78 in the placebo group), the second dose (56 vaccine, 58 placebo), or the third dose (3 vaccine, 2 placebo) was similar in the vaccine and placebo groups. In addition, the reasons for dropping out were similarly distributed between the vaccine and placebo recipients. Thus, with the large number of study participants, it is unlikely that these events unduly influenced the analysis of efficacy.

Rotavirus vaccine induced similar protection against all episodes or against episodes of mixed infection, irrespective of the severity of the illness. This observation may be associated with the age of the study population, because the pathogenicity of localized and aggregative *E. coli* is age-dependent.³⁴ Enteroadherent *E. coli* has been shown to be pathogenic in infants less than three months of age, but not in older infants.³⁴ Similar results were found for campylobacter species (unpublished data).

The efficacy of the vaccine was greater in children more than 12 months of age (61 percent) than in infants 4 to 12 months of age (41 percent). This finding is consistent with what is observed after natural infections.^{31,32} Velázquez et al.³² showed that the cumulative probability of rotavirus infection increased with age, and that two or more rotavirus infections, whether symptomatic or asymptomatic, resulted in complete protection against moderate-

TABLE 4. PROTECTIVE EFFICACY OF THREE DOSES OF ROTAVIRUS VACCINE AGAINST THE FIRST EPISODE OF ROTAVIRUS DIARRHEA DETECTED.

FEATURE OF THE EPISODE	VACCINE GROUP (N = 1112)	PLACEBO GROUP (N = 1095)	PROTECTIVE EFFICACY (95% CONFIDENCE INTERVAL)
	no. of infants (%)		
All episodes	70 (6)	135 (12)	48 (33 to 61)*
Severity scores†			
All episodes			
1-8	35 (3)	50 (5)	31 (-5 to 55)‡
9-14	32 (3)	60 (5)	47 (20 to 66)§
15-20	3 (0.3)	25 (2)	88 (61 to 96)*
Episodes of mixed infection			
1-8	14 (1)	24 (2)	43 (-10 to 70)‡
9-14	17 (2)	32 (3)	48 (6 to 71)§
15-20	2 (0.2)	15 (1)	87 (43 to 97)§
Dehydration	6 (0.5)	24 (2)	75 (40 to 90)*
Duration >4 days	14 (1)	47 (4)	71 (47 to 84)*
Hospital admission¶	10 (0.9)	33 (3)	70 (40 to 85)*

*P<0.001 for the comparison between groups by Fisher's exact test (two-tailed).

†A 20-point scoring system was used to grade the severity of illness on the basis of the duration of diarrhea or vomiting, maximal number of diarrheal stools and episodes of vomiting in 24 hours, dehydration, fever, and hospitalization.²⁸ Scores of 1 to 8, 9 to 14, and 15 to 20 were consistent with mild, moderate, and severe illness, respectively.

‡P not significant for the comparison between groups by Fisher's exact test (two-tailed).

§P<0.05 for the comparison between groups by Fisher's exact test (two-tailed).

¶Nosocomial episodes of diarrhea were not included in numbers of hospital admissions.

to-severe disease. Moreover, studies of the immunogenicity of natural or vaccine-induced rotavirus infection have shown predominantly homotypic responses after the initial infection and a broadening of the responses heterotypically after subsequent infections.^{35,36} Thus, protection may increase with age because of multiple contacts with rotavirus after a primary immunization (natural or artificial). This is more likely to occur in developing countries, where rotavirus is present year-round.^{32,35}

We could not determine the efficacy of the vaccine against specific serotypes, because more than 90 percent of the strains were VP7 serotype 1 (G1). However, it remains important to have coverage against each of the four epidemiologically important serotypes, since it is not possible to predict or anticipate the predominant serotype in any season. It does appear from other studies that serotype-specific protection may indeed be a feature of this quadrivalent vaccine.^{5,6}

The vaccine was safe. As in most previous studies,^{5,6,8,9,37-39} however, during the six days after the first dose there were self-limited febrile responses; these occurred in 15 percent of vaccinated infants

and 7 percent of placebo recipients in our study. On day 3 after vaccination, 5 percent of the vaccinated infants and 1 percent of the placebo recipients had temperatures of 38.1°C or more.

The neutralizing-antibody responses to human serotypes were consistent with those in previous trials,^{5,6,38,39} ranging from 10 to 45 percent after three doses of vaccine. The response to rhesus rotavirus was greatest, reaching over 80 percent after two doses. This might be explained by the VP4 of rhesus rotavirus, which is a distinct subtype of the VP4 serotype (P)5 not shared by human rotaviruses, and which is overrepresented in the vaccine because it is in each of the four vaccine components.⁴⁰ Overall, the vaccine was quite antigenic, since over 85 percent of the vaccinated infants had a serologic response in one or more assays each. Three doses of vaccine, as compared with a single dose, significantly increased the number of responses.

An unanticipated finding was the horizontal transmission of vaccine strains in 14 percent of the children with rotavirus-positive illness. This distribution was quite similar in those receiving vaccine (13 percent) and those receiving placebo (15 percent). The vaccine strain was shed in low titer and in conjunction with a second rotavirus strain, which, when possible, was further examined and shown to be a wild-type virus. Among the 29 patients tested who had dehydration, none of the 6 vaccine recipients and 5 of the 23 placebo recipients shed the vaccine strain along with a second rotavirus strain. The vaccine strain was not detected longer than 3 months and 10 days after the administration of the third dose of vaccine, suggesting that dissemination of the vaccine strain in the community needs further evaluation with respect to herd protection. The transmission of vaccine virus would represent reinfection of vaccine recipients and infection or reinfection of placebo recipients, both of which could be viewed as beneficial events if they resulted in induction of antibody responses. If antibody responses were stimulated, they could have masked the true efficacy of the vaccine by lowering the rate of illness in the placebo group.

The results observed in Venezuela, the United States, and Finland suggest that this vaccine may reduce considerably the morbidity and mortality from rotavirus diarrhea when incorporated into routine immunization programs in developed and developing countries.

Supported by grants from the Agency for International Development Vaccine Program through an Agency for International Development-Public Health Service Participating Agency Service Agreement (PASA BST-5947-P-HI-4265) with the National Institute of Allergy and Infectious Diseases (87-I-113), the World Health Organization (C6/181/341), and Wyeth-Ayerst Research (0587B-309-VE) and in part by Wyeth-Ayerst Laboratories through a Cooperative Research and Development Agreement with the National Institute of Allergy and Infectious Diseases and the Public Health Service National Vaccine Program Office.

We are indebted to the Review and Advisory Committee (Drs. Nathaniel Pierce, Claudio Lanata, and John Clemens) for advice in study design and data analysis; the Public Health Service National Vaccine Program Office (Dr. Kimi Lin) for organizational and administrative support; the Safety Monitoring Board (Drs. Robert Black, Genevieve Losonsky, Nalini Singh, and William Blackwelder); analyst-programmer Zoraida Contreras; pediatricians Rosa Fernandez, David Inaty, Mariana Marino, Edgar Alfonso, and Ilvir Crespo; the laboratory and field staff (Yordi Boher, Jorge Vazquez, Delia Pequenez, Mariam Watson, Dr. Xiao-ye Yan, Melinda Jones, Ernest Williams, and Joseph Jackson); the authorities and personnel of the Hospital Materno Infantil de Caricuao who made it possible to carry out this study; the mothers and children who participated with patience and commitment in this trial; Drs. Luis Raul Pericchi and Maria Eglee Perez of CESMA-Universidad Simon Bolivar for statistical assistance; Dr. Robert Chanock for his support and review of the manuscript; and Dr. Jorge Flores for contributions to the planning and implementation of the study.

REFERENCES

- Kapikian AZ, Hoshino Y, Chanock RM, Perez-Schael I. Efficacy of a quadrivalent rhesus rotavirus-based human rotavirus vaccine aimed at preventing severe rotavirus diarrhea in infants and young children. *J Infect Dis* 1996;174:Suppl 1:S65-S72.
- Glass RI, Gentsch JR, Smith JC. Rotavirus vaccines: success by reassortment? *Science* 1994;265:1389-91.
- Prospects for immunizing against rotavirus. In: Institute of Medicine. New vaccine development: establishing priorities. Vol. 1. Diseases of importance in the United States. Washington, D.C.: National Academy Press, 1985:410-23.
- The prospects for immunizing against rotavirus. In: Institute of Medicine. New vaccine development: establishing priorities. Vol. 2. Diseases of importance in developing countries. Washington, D.C.: National Academy Press, 1986:308-18.
- Bernstein DI, Glass RI, Rodgers G, Davidson BL, Sack DA. Evaluation of rhesus rotavirus monovalent and tetravalent reassortant vaccines in US children. *JAMA* 1995;273:1191-6.
- Rennels MB, Glass RI, Dennehy PH, et al. Safety and efficacy of high-dose rhesus-human reassortant rotavirus vaccines — report of the National Multicenter Trial. *Pediatrics* 1996;97:7-13.
- Vesikari T. Clinical experience with rotavirus vaccine in Finland. Presented at the 2nd Satellite Symposium of the 14th Annual Meeting of the European Society for Paediatric Infectious Diseases (ESPID), Elsinore, Denmark, 18–21 June 1996.
- Lanata CE, Midthun K, Black RE, et al. Safety, immunogenicity, and protective efficacy of one and three doses of the tetravalent rhesus rotavirus vaccine in infants in Lima, Peru. *J Infect Dis* 1996;174:268-75.
- Linhares AC, Gabbay YB, Mascarenhas JDP, et al. Immunogenicity, safety and efficacy of tetravalent rhesus-human, reassortant rotavirus vaccine in Belem, Brazil. *Bull World Health Organ* 1996;74:491-500.
- Mendez-Castellano H, De Mendez MC. Estratificación social y biología humana: Metodo Graffar modificado. *Arch Venez Pueric Pediatr* 1986;49:93-104.
- Morris SS, Cousens SN, Lanata CE, Kirkwood BR. Diarrhoea — defining the episode. *Int J Epidemiol* 1994;23:617-23.
- A manual for the treatment of diarrhoea: for use by physicians and other senior health workers. Geneva: World Health Organization, 1990.
- Kapikian AZ, Yolken RH, Greenberg HB, et al. Gastroenteritis viruses. In: Lennette EH, Schmidt NJ, eds. Diagnostic procedures for viral, rickettsial and chlamydial infections. 5th ed. Washington, D.C.: American Public Health Association, 1979:927-95.
- Brandt CD, Kim HW, Rodriguez WJ, et al. Comparison of direct electron microscopy, immune electron microscopy, and rotavirus enzyme-linked immunosorbent assay for detection of gastroenteritis viruses in children. *J Clin Microbiol* 1981;13:976-81.
- Taniguchi K, Urasawa T, Morita Y, Greenberg HB, Urasawa S. Direct serotyping of human rotavirus in stools by an enzyme-linked immunosorbent assay using serotype 1-, 2-, 3-, and 4-specific monoclonal antibodies to VP7. *J Infect Dis* 1987;155:1159-66.
- Shaw RD, Stoner-Ma DL, Estes MK, Greenberg HB. Specific enzyme-linked immunoassay for rotavirus serotypes 1 and 3. *J Clin Microbiol* 1985;22:286-91.
- Gouvea V, Glass RI, Woods P, et al. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. *J Clin Microbiol* 1990;28:276-82.
- Das BK, Gentsch JR, Cicirello HG, et al. Characterization of rotavirus strains from newborns in New Delhi, India. *J Clin Microbiol* 1994;32:1820-2.
- Kalica AR, Sereno MM, Wyatt RG, Mebus CA, Chanock RM, Kapikian AZ. Comparison of human and animal rotavirus strains by gel electrophoresis of viral RNA. *Virology* 1978;87:247-55.
- Flores J, Greenberg HB, Myslinski J, et al. Use of transcription probes for genotyping rotavirus reassortants. *Virology* 1982;121:288-95.
- Henriksen SA, Pohlenz JF. Staining of cryptosporidia by a modified Ziehl-Neelsen technique. *Acta Vet Scand* 1981;22:594-6.
- Kelly MT, Brenner DJ, Farmer JJ III. Enterobacteriaceae. In: Lennette EH, Balows A, Hausler WJ Jr, Shadomy HJ, eds. Manual of clinical microbiology. 4th ed. Washington, D.C.: American Society for Microbiology, 1985:263-76.
- Day NP, Scotland SM, Rowe B. Comparison of an HEp-2 tissue culture test with the Sereny test for detection of enteroinvasiveness in *Shigella* spp. and *Escherichia coli*. *J Clin Microbiol* 1981;13:596-7.
- Cravioto A, Gross RJ, Scotland SM, Rowe B. An adhesive factor found in strains of *Escherichia coli* belonging to the traditional enteropathogenic serotypes. *Curr Microbiol* 1979;3:95-9.
- Orskov F, Orskov I. Serotyping of *Escherichia coli*. In: Bergen E, ed. Methods in microbiology. London: Academic Press, 1984:43-112.
- Losonsky GA, Rennels MB, Lim Y, Krall G, Kapikian AZ, Levine MM. Systemic and mucosal immune responses to rhesus rotavirus vaccine MMU 18006. *Pediatr Infect Dis J* 1988;7:388-93.
- Hoshino Y, Wyatt RG, Greenberg HB, Flores J, Kapikian AZ. Serotypic similarity and diversity of rotaviruses of mammalian and avian origin as studied by plaque-reduction neutralization. *J Infect Dis* 1984;149:694-702.
- Ruuska T, Vesikari T. Rotavirus disease in Finnish children: use of numerical scores for clinical severity of diarrhoeal episodes. *Scand J Infect Dis* 1990;22:259-67.
- Perez-Schael I, Garcia D, Gonzalez M, et al. Prospective study of diarrheal diseases in Venezuelan children to evaluate the efficacy of rhesus rotavirus vaccine. *J Med Virol* 1990;30:219-29.
- Orenstein WA, Bernier RH, Dondero TJ, et al. Field evaluation of vaccine efficacy. *Bull World Health Organ* 1985;63:1055-68.
- Bishop RF, Barnes GL, Cipriani E, Lund JS. Clinical immunity after neonatal rotavirus infection: a prospective longitudinal study in young children. *N Engl J Med* 1983;309:72-6.
- Velázquez FR, Matson DO, Calva JJ, et al. Rotavirus infection in infants as protection against subsequent infections. *N Engl J Med* 1996;335:1022-8.
- Perez-Schael I. The impact of rotavirus disease in Venezuela. *J Infect Dis* 1996;174:Suppl 1:S19-S21.
- Gonzalez R, Diaz C, Marino M, Cloralt R, Pequenez M, Perez-Schael I. Age-specific prevalence of *Escherichia coli* with localized and aggregative adherence in Venezuelan infants with acute diarrhea. *J Clin Microbiol* 1997;35:1103-7.
- Rojas AM, Boher Y, Guntinas MJ, Perez-Schael I. Homotypic immune response to primary infection with rotavirus serotype G1. *J Med Virol* 1995;47:404-9.
- Green KY, Taniguchi K, Mackow ER, Kapikian AZ. Homotypic and heterotypic epitope-specific antibody responses in adult and infant rotavirus vaccinees: implications for vaccine development. *J Infect Dis* 1990;161:667-79.
- Perez-Schael I, Blanco M, Vilar M, et al. Clinical studies of a quadrivalent rotavirus vaccine in Venezuelan infants. *J Clin Microbiol* 1990;28:553-8.
- Flores J, Perez-Schael I, Blanco M, et al. Reactogenicity and immunogenicity of a high-titer rhesus rotavirus-based quadrivalent rotavirus vaccine. *J Clin Microbiol* 1993;31:2439-45.
- Perez-Schael I, Blanco M, Garcia D, et al. Evaluation of the antigenicity and reactogenicity of varying formulations of the rhesus rotavirus-based quadrivalent and the M37 rotavirus vaccine candidates. *J Med Virol* 1994;42:330-7.
- Hoshino Y, Kapikian AZ. Classification of rotavirus VP4 and VP7 serotypes. *Arch Virol Suppl* 1996;12:99-111.

CORRECTION

Efficacy of the Rhesus Rotavirus–Based Quadrivalent Vaccine in Infants and Young Children in Venezuela

Efficacy of the Rhesus Rotavirus–Based Quadrivalent Vaccine in Infants and Young Children in Venezuela . On page 1185, the footnotes to Table 3 should have read as follows:

Also in Table 3, in the third column, second row, the footnote markers should have been †§, not †‡, as printed. In the eighth column, second row, the footnote markers should have been †¶, not †§, as printed.

In addition, Dr. Cunto's affiliation should have been IBM de Venezuela.

Corrected Table 3 footnotes.

†‡P<0.001 for the comparison with the same dose of placebo by Fisher's exact test (two-tailed).

‡§P<0.05 for the comparison with the same dose of placebo by Fisher's exact test (two-tailed).

§¶P<0.05 for the comparison with the first dose of vaccine by Fisher's exact test (two-tailed).