Safety, Immunogenicity, and Protective Efficacy of One and Three Doses of the Tetravalent Rhesus Rotavirus Vaccine in Infants in Lima, Peru

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An oral rhesus-human rotavirus tetravalent (RRV-TV) vaccine (10^4 pfu of rhesus rotavirus [type G3] and of 3 human-rhesus reassortants [G1, G2, and G4]) was evaluated in a field trial in Lima, Peru. At 2, 3, and 4 months of age, infants received either a dose of RRV-TV, an initial dose of vaccine followed by a dose of placebo at 3 and 4 months, or a dose of placebo. Rotavirus-specific IgA responses were detected by ELISA in 75% of the three-dose vaccine group, 59% of the one-dose vaccine group ($P = .05$), and 24% of the placebo group ($P < .001$): 64%, 48%, and 12% of each group, respectively, had a neutralizing antibody response to at least 1 serotype. Both one and three doses of vaccine failed to induce a significant level of protection against rotavirus diarrhea; however, they did provide some protection (range, 35%–66%) against more severe rotavirus diarrhea, especially for episodes caused by type G1.

Diarrheal disease caused by rotavirus represents an important public health problem, affecting infants and young children in both developed and developing countries [1]. It is estimated that an effective vaccine could prevent up to 30% of all deaths due to diarrhea in children 6–23 months of age [2].

The efficacy of monovalent rotavirus vaccines of animal origin (bovine rotavirus strains RIT 4237 and WC3 and rhesus rotavirus [RRV] strain MMU 18006) against rotavirus diarrhea has varied from 0% to >80% [3–16]. A possible explanation for this is that infants not primed by prior rotavirus infections have predominantly homotypic neutralizing antibody responses [9, 17–19]. Studies of natural rotavirus infection support a role for G serotype–specific immunity in protection from rotavirus diarrhea [20, 21], and field trials have suggested that a vaccine containing the 4 predominant rotavirus G serotypes (G1–4) provides better protection than single-serotype vaccines [22, 23].

Reassortant strains have been developed by incorporating the genetic segment that encodes the production of the VP7 antigen (determining the G serotype) for the human rotavirus belonging to type G1, G2, or G4 into the RRV strain (G3) [24, 25]. These reassortant vaccine strains have similar reactogenicity and immunogenicity to the parent RRV strain [26–28]. A tetravalent RRV (RRV-TV) vaccine was prepared to provide a single vaccine that could offer protection against all 4 G serotypes commonly associated with rotavirus diarrhea in children. It contains 10^4 pfu of each of the reassortant strains and of RRV. This formulation was safe and immunogenic in studies done in developed and developing countries [29–32].

A recent evaluation of these vaccine strains in Peru demonstrated that one 10^4-pfu dose of either the G1 or G2 reassortant vaccines did not give protection against rotavirus diarrhea [33]. Only the RRV vaccine gave marginal (29%) protection against rotavirus diarrhea, mostly episodes due to G1 and G2 strains. The lack of protection in this developing-country setting was associated with poor serotype-specific serologic responses, probably due to poor vaccine immunogenicity because of interference from preexisting antibodies or from other organisms present in the intestines of vaccine recipients. Two alternative strategies have been suggested to overcome this problem: an increase in the number of vaccine doses and an increase in the vaccine titer. We evaluated the safety, immunogenicity, and efficacy of RRV-TV with 10^4 pfu of each strain to determine if three doses provided better protection than one dose.

Methods

Subjects and study design. The study was conducted in Canto Grande, Lima, a densely populated periurban area of low socioeconomic status, where diarrheal diseases are highly endemic [34]. Rotavirus is present all year, with a slightly higher incidence from

Received 11 October 1995; revised 27 March 1996.

Presented in part: Conference on Vaccines for Enteric Diseases, Cambridge, UK, April 1992.

The study was approved by the Peruvian Ministry of Health, community leaders of the study area, and the human research committees of the Instituto de Investigación Nutricional and the Johns Hopkins School of Hygiene and Public Health. Informed consent was obtained from the parents of study children, and human experimentation guidelines of the Department of Health and Human Services were followed.

Financial support: Wyeth-Ayerst Research; United Kingdom Overseas Development Administration (for C.F.L. as a Visiting Research Fellow at London School of Hygiene and Tropical Medicine).

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The Journal of Infectious Diseases 1996; 174:268–75

0022–1899/96/7402–0004$01.00

March through May. Infants were randomized to receive, at ~2, 3, and 4 months of age, one of the following: a dose of vaccine, an initial dose of vaccine followed by a dose of placebo at 3 and 4 months, or a dose of placebo. Block-randomization was done by computer using the infant's sequential enrollment number. At each visit, children also received inactivated polio vaccine (IPV) combined with diphtheria–tetanus toxoid–pertussis (DTP) vaccine (TETRACOQ; BioMérieux Laboratories, Lyon, France). Other immunizations included bacille Calmette-Guérin at birth, measles-mumps-rubella at 9 months of age, and IPV booster after 12 months of age.

Sample-size calculations were based on rotavirus infection rates observed in a previous study done in the same area [8], on an 80% power to detect ≥75% vaccine efficacy (VE) against severe rotavirus diarrhea or G1 or G2 rotavirus diarrhea in cases in which no other enteropathogen was isolated in the stool during 24 months of observation, and on an α error of 5% using a one-tailed test. Allowing for expected loss to follow-up during surveillance, the study required 200 children/study group. Seven hundred infants received their first dose of vaccine or placebo between October 1988 and August 1989.

Vaccine and vaccine administration. Lyophilized RRV-TV was supplied in single-dose vials containing 10^4 pfu of each of its four components: RRV, the G1 reassortant (D × RRV), the G2 reassortant (DS-1 × RRV), and the G4 reassortant (ST3 × RRV) strains [10, 24, 25]. The vaccine and placebo (derived from uninfected cell cultures) were provided by Wyeth-Ayerst Research Laboratories in identically appearing vials. The sequentially coded vials were transported with cold packs to Lima, where they were stored at 2–8°C.

On each vaccination day, the necessary number of vials were transported with cold packs to the field clinic, where they were reconstituted with 1 mL of sterile water and given orally with a 1-mL tuberculin syringe without the needle. Immediately before vaccination, all subjects were fed 30 mL of reconstituted evaporated milk (Gloria, Arequipa, Peru) that lacked rotavirus-neutralizing activity and contained 0.4 g of $NaHCO_3$. Breast-feeding was withheld for 1 h before and after vaccination. Infants were vaccinated if they were free from reported fever for 48 h and diarrhea for 72 h before the vaccination. The vaccine titer in a sample of coded vials returned from Lima was validated by Wyeth-Ayerst Research. The vaccine or placebo code was kept at Wyeth-Ayerst Research and was not broken until the study was completed.

For 6 days after each dose, trained field workers made twice daily home visits, to observe for possible side effects. Information collected included rectal temperature (taken twice per day) and daily information on total number of stools and number of liquid or semi-liquid stools, blood in stools, abdominal pain, vomiting, reported fever, cough, nasal secretions, general irritability, ear pain, and other illnesses. A stool sample or rectal swab was taken on day 3 or 4 after immunization to look for vaccine virus excretion.

Diarrhea surveillance. Trained field workers visited each child at home two times per week to collect information on diarrheal episodes and to collect stool specimens or rectal swabs. Diarrhea was defined as ≥3 liquid or semi-liquid stools passed in 24 h; the episode was considered terminated on the last day of diarrhea followed by 48 h free of diarrhea symptoms [35]. A maximum recall period of 7 days was allowed to obtain information from the mother. For each diarrheal episode, field workers obtained daily clinical information. World Health Organization criteria were used for the classification of dehydration [36]. Since no children with severe dehydration and only a few with moderate dehydration were identified, the analysis was done for children with any clinically detectable dehydration. Mothers were instructed in the use of oral rehydration solution provided by the study and were told to bring the children to the study field clinic or any health facility if dehydration or severe illness occurred. Specific antibiotics were provided by the study pediatricians for dysenteric illness or culture-proven shigellosis and for clinically diagnosed pneumonia. Children were dropped from surveillance after completing 24 months of follow-up after the last rotavirus immunization (i.e., at 29 months of age). The study ended in October 1991.

Laboratory procedures. The first available prevaccination, post–dose 1, and post–dose 3 serum samples from 307 subjects were tested by rotavirus-specific IgA ELISA, using RRV as antigen. The characteristics of these subjects did not differ from those of the full study groups from which they were selected. Sera from the first 25 recipients in the placebo group (regardless of IgA antibody response) and the first 25 with an IgA antibody rise in the one- and three-dose RRV-TV groups were tested by plaque reduction neutralization assay (PRNA) to human rotavirus strains Wa, DS-1, P, and ST3 (G1–4, respectively). The ELISA and PRNA were done as previously described [37, 38], starting with a serum dilution of 1:50 for ELISA and 1:40 for PRNA. Pre- and postvaccination sera were tested simultaneously, and a significant antibody response was defined as a ≥4-fold rise in titer between any 2 of 3 serum specimens (prevaccination vs. post–dose 1 or 3, and post–dose 1 vs. post–dose 3). The first 25 subjects from the three-dose RRV-TV group whose 4- to 6-day postvaccination stool or rectal swab specimens became available were studied for vaccine virus shedding. Their stool samples were inoculated into MA104 cells with one subsequent blind passage and tested for the presence of rotavirus antigen by ELISA [39].

Stool specimens or rectal swabs were collected during episodes of diarrhea and placed in a calcium-containing TRIS buffer, in Cary-Blair transport media with and without Skirrow's antibiotic supplement (10 mg/L vancomycin, 2500 IU/L polymyxin B, and 5 mg/L trimethoprim) for the detection of *Campylobacter* species, and in MIF (merthiolate, iodine, and formalin) solution for parasitologic examination. Stool samples were transported on cold packs to the laboratory, where they were processed on the same day as collection. Samples were tested daily with a rotavirus ELISA kit (Dakopatts, Copenhagen) [40]. Rotavirus-positive stool specimens were stored at −20°C until they were transported on dry ice to Johns Hopkins University. Because of the small specimen size, samples were amplified by two to four passages in African green monkey kidney cells and then serotyped by ELISA using the following G serotype–specific monoclonal antibodies (MAbs): KU4 (G1), S2-2G10 and 2F1 (G2), 954/159 (G3), ST-2G7 (G4), and 6A1-1C8 (G9) [41–43]. The MAbs were used as capture antibodies, and rabbit hyperimmune serum to rotavirus (Dakopatts) was used as detector antibody, followed by goat anti-rabbit IgG conjugated to alkaline phosphatase (Boehringer Mannheim, Mannheim, Germany). The methods used were similar to those described by Taniguchi et al. [41]. Rotavirus-positive stool samples that could not be serotyped by ELISA were serotyped by solid-phase immune

electron microscopy, using MAbs against rotavirus serotypes G1–4 and G9 [44].

Salmonella, Shigella, and *Campylobacter* species were sought in all stool samples, using standard techniques [45]. Five coliform colonies on MacConkey agar were picked and inoculated onto nutrient agar slants and stored at room temperature for further testing. Enterotoxigenic *Escherichia coli* (ETEC), enteroadherent *E. coli* (EAEC), *Vibrio* species, and *Cryptosporidium* species were tested only in rotavirus-positive stool samples. ETEC were identified by colony DNA hybridization (DuPont, Boston), with the genetic sequence corresponding to the heat-stable and heat-labile enterotoxin [46]. EAEC were identified by the HEp-2 cell adhesion assay, in which all three types of adhesion patterns (localized, diffuse, and aggregative) were considered [47]. *Cryptosporidium* species were identified in stool smears stained with the Kinyoun modification of the Ziehl-Neelsen stain [48].

Data analysis. Of the 700 children who received the first dose of vaccine or placebo, 640 completed the three-dose vaccination schedule: 219 were in the placebo group, 212 in the one-dose vaccine group, and 209 in the three-dose vaccine group. There was no difference in the dropout rate by group. The reasons for not completing the immunization schedule were refusing subsequent blood samples (35 subjects) and migration from the study area (25 subjects). From the 640 children who completed the immunization schedule, 638 were under surveillance from 10 days after the third vaccine dose and were included in the analysis; 580 of the children (83%) who started the study (194 in the placebo group, 196 and 190 in the one- and three-dose vaccine groups, respectively) completed 24 months of surveillance. Reasons for withdrawal from surveillance included death not related to the vaccine (6 subjects), refusal to continue (6), and migration from the study area (46). The 638 children under surveillance generated a total of 1144 child-years of observation, which were distributed similarly among the 3 groups (382, 376, and 386 in the one-dose, three-dose, and placebo groups, respectively). At each vaccination, the 3 groups were comparable for age, proportion of boys (49%–55%), and proportion with birth weight <2500 g (4% in all groups).

Rates of rotavirus diarrhea were calculated using any rotavirus-positive diarrheal episodes and using rotavirus only–postive diarrheal episodes (i.e., only those episodes in which *Salmonella, Shigella, Campylobacter, Vibrio,* and *Cryptosporidium* species, ETEC, and EAEC were not identified in the stool sample). Rotavirus diarrheal episodes were analyzed by severity using several clinical indicators or a combined severity score (Kapikian's severity score, a modification of that used by Flores et al. [10], with 20 points as the maximum). Results are presented for a severity score of ≥9 because relatively few children had a higher score and because no greater efficacy was seen with higher scores. VE and 95% confidence intervals (CIs) were calculated [49].

Data analysis was done by use of SPSS software (SPSS, Chicago). The χ^2 and Fisher's exact tests were used when indicated.

Results

Reactions to vaccination. In all 3 groups, fever most frequently occurred in the first 24 h after vaccination (day 0) and 1 day after each vaccination: ~30%–44% of subjects reported fever and 15%–22% had documented fever (rectal temperatures ≥38.1°C). There were no significant differences between placebo and RRV-TV vaccine recipients, and the febrile reactions were probably related to the DTP-IPV vaccine, which was given concurrently with placebo and vaccine. There was a significant difference in the percentage of placebo and RRV-TV vaccine recipients with fever 3 days after the first immunization: A subjective report of fever was obtained for 4.8% of the placebo group, 9.8% of the one-dose vaccine group, and 10.3% of the three-dose vaccine group ($P = .05$ or $< .05$, respectively, for placebo vs. vaccine groups). A rectal temperature ≥38.1°C was also documented more frequently in both vaccine groups at this time point, although the difference was significant only between the one-dose vaccine and placebo groups (4.6% vs. 0.9%, $P < .05$). The prevalences of diarrhea, vomiting, and fever at any time in the week after vaccination were similar in the 3 groups (table 1).

Table 1. Number (%) of children with diarrhea, vomiting, or temperature ≥38.1°C on days 0–6 after vaccination with placebo or RRV-TV vaccine.

Study group	No. tested	Diarrhea	Vomiting	Fever
1st immunization				
Placebo	232	93 (40)	59 (25)	97 (42)
RRV-TV, 1 dose	234	92 (39)	55 (24)	96 (41)
RRV-TV, 3 doses	233	96 (41)	46 (20)	91 (39)
2nd immunization				
Placebo	220	53 (24)	47 (21)	72 (33)
RRV-TV, 1 dose	217	54 (25)	43 (20)	81 (37)
RRV-TV, 3 doses	210	47 (22)	38 (18)	73 (35)
3rd immunization				
Placebo	218	51 (23)	47 (22)	52 (24)
RRV-TV, 1 dose	212	47 (22)	42 (20)	59 (28)
RRV-TV, 3 doses	207	49 (24)	32 (15)	55 (27)

Responses to the RRV-TV vaccine. Prior to the first vaccination, 35% of participants had rotavirus-specific IgA responses by ELISA. After one dose, 50% of the three-dose and 51% of the one-dose vaccine recipients had a response compared with 7% of the placebo recipients ($P < .001$; table 2). After three doses, 75% of three-dose and 59% of one-dose vaccine recipients ($P = .05$) and 24% of placebo recipients ($P < .001$) had serologic responses (table 2). After the second and third doses of vaccine, only 18% of first-dose responders in the three-dose vaccine group had serologic responses compared with 50% of subjects who did not have a serologic response after the first dose (9/51 vs. 26/52, $P < .001$). Serologic responses to the first dose occurred in 61% of vaccinees without preexisting rotavirus-specific IgA (titer <1:50) and in 32% of vaccinees with detectable IgA (titer ≥1:50) (68/111 vs. 25/75, $P < .001$).

Antibody rises were also detected in 11 (58%) of 19 infants in whom nonspecific reactivity at low serum dilution precluded

Table 2. Serologic responses and geometric mean titers (GMTs), as determined by rotavirus-specific IgA ELISA, of infants after receipt of placebo or RRV-TV vaccine at 2, 3, and 4 months of age.

Study group; no. tested	No. (%) with ≥4-fold antibody rise between indicated serum pairs			GMTs of sera		
	Before dose 1–after dose 1	After dose 1–after dose 3	Any rise*	Before dose 1	After dose 1	After dose 3
Placebo; 102	7 (7)†	16 (16)‡	24 (24)§	48	51‖	73¶
RRV-TV, 1 dose; 102**	52 (51)	10 (10)	60 (59)	53	175	136
RRV-TV, 3 doses; 103	51 (50)	34 (33)	77 (75)	51	173	237

* Includes all subjects with rise before dose 1–after dose 1, after dose 1–after dose 3, or before dose 1–after dose 3.
† $P < .00001$, placebo vs. 1 or 3 doses RRV-TV.
‡ $P < .30$ and .0001, placebo vs. 1 and 3 doses RRV-TV, respectively.
§ $P < .0001$, placebo vs. 1 or 3 doses RRV-TV; $P < .05$, 1 vs. 3 doses RRV-TV.
‖ $P < .0001$, placebo vs. 1 or 3 doses RRV-TV.
¶ $P < .01$ and .0001, placebo vs. 1 and 3 doses RRV-TV, respectively; $P < .05$, 1 vs. 3 doses RRV-TV.
** 2nd and 3rd doses were placebo.

determination of an exact prevaccination titer. The prevaccination titer in these cases was known to be <1:100, but the exact titer could not be determined because of nonspecific reactivity of the serum sample to the control wells. The geometric mean titers (GMTs) of rotavirus-specific IgA in the 3 study groups mirrored the serologic responses in the groups (table 2). After the first dose, the one- and three-dose vaccine groups had almost identical GMTs (175 and 173, respectively), which were significantly higher than the GMT (51, $P < .001$) in the placebo group. After all doses, the three-dose vaccine group had a GMT significantly higher than that of the one-dose vaccine and placebo groups (237 vs. 136 and 73; $P = .008$ and $P < .001$, respectively).

In the subset of 25 subjects from each study group who were tested by PRNA, neutralizing antibody rises to serotypes G1–4 were detected in 24%–36% and 16%–36% of one- and three-dose vaccine recipients, respectively, with 48% of the former and 64% of the latter group showing a rise to at least 1 serotype. Only 12% of 25 placebo recipients had neutralizing antibody responses to at least 1 serotype (one- and three-dose vaccine recipients vs. placebo recipients, $P < .001$; table 3). Vaccinees without detectable neutralizing antibody (titer <1:40) to any given serotype in their prevaccination sera were more likely to have a serologic response to that serotype than were vaccinees who had titers ≥1:40 (26/51 vs. 33/149, $P = .001$).

Shedding of vaccine virus was also examined in the first 25 three-dose RRV-TV vaccine recipients for whom a specimen was available 4–6 days after each dose. This subset of 25 vaccinees had an overall IgA serologic response of 76%, similar to the 75% response of the larger sample of three-dose vaccine recipients (table 2). Shedding was detected in 36%, 24%, and 12% after the first, second, and third dose, respectively, with a cumulative shedding rate of 60%.

VE. The rate of diarrhea (episodes/child-year) was 8.3 in the placebo group and 8.6 in both the one- and three-dose vaccine groups. There were 9718 diarrheal episodes during the surveillance period, and samples from 6443 of the episodes (66%) were tested by ELISA for rotavirus: Rotavirus was identified in 222 (3.4% of those tested). One and three doses of RRV-TV vaccine failed to induce significant VE against any rotavirus diarrhea (table 4). Three doses of the vaccine were significantly protective against more severe rotavirus diarrhea, as indicated by the presence of fever (VE, 35%; 95% CI, 2%–57%), vomiting (VE, 40%; 95% CI, 10%–60%), and ≥6 liquid or semi-liquid stools in 24 h at any time during the episode (VE, 40%; 95% CI, 7%–62%). Using a severity score of ≥9, there was no significant efficacy with either one or three doses of RRV-TV vaccine.

Ninety (41%) of the 222 rotavirus-positive stool samples from the diarrheal episodes had at least 1 additional enteropathogen: 9 had *Campylobacter* species, 35 had EAEC, 25 had ETEC, and 21 had ≥2 of these different agents. *Shigella* and *Cryptosporidium* species were not found in any of the 222 samples. The vaccine had no efficacy against these mixed infections (data not shown). In the analysis utilizing diarrheal episodes with rotavirus as the only pathogen isolated, VE against more severe episodes was seen only with one vaccine dose. These more severe diarrheal episodes were associated with vomiting (VE, 56%; 95% CI, 11%–78%) and a high number of liquid or semi-liquid stools (VE, 59%; 95% CI, 12%–81%); three doses offered a slightly lower protection, which did not reach statistical significance (table 4). Using a severity score of ≥9, there was no significant VE.

Serotype-specific protection. Of the 222 fecal samples from rotavirus-positive diarrheal episodes, 175 (79%) could be G serotyped by ELISA, and 20 (9%) that were not serotyped by ELISA were serotyped by solid-phase immune electron mi-

Table 3. Rotavirus serotype–specific neutralizing antibody rises, as determined by plaque reduction assay, in study subjects after receipt of placebo or RRV-TV vaccine at 2, 3, and 4 months of age.

	No. (%) of subjects with ≥4-fold antibody rise against indicated strains (serotypes)				
Study group	Wa (1)	DS-1 (2)	P (3)	ST3 (4)	Any*
Placebo	2 (8)†	1 (4)†	0†	0†	3 (12)†
RRV-TV, 1 dose	9 (36)	6 (24)	9 (36)	7 (28)	12 (48)
RRV-TV, 3 dose	9 (36)	4 (16)	8 (32)	7 (28)	16 (64)

NOTE. First 25 placebo recipients with sufficient amounts of sample sera before and after dose 1 and after dose 3 were tested; first 25 1- and 3-dose RRV-TV recipients who had serologic responses by IgA ELISA and sufficient sample amounts of all 3 sera were tested.

* Includes all subjects with antibody rise to ≥1 serotype.

† *P*, for placebo vs. 1 and 3 doses of RRV-TV: Wa, $P = .01$; DS-1, $P < .10$; P, $P = .001$; ST3, $P < .01$; and any serotype, $P < .001$. There was no significant difference between 1- and 3-dose RRV-TV groups.

croscopy. Only 27 samples (12%) from rotavirus-positive diarrheal episodes could not be serotyped. Of the 195 samples that were serotyped, 124 were of G1 (60%), 66 of G2 (32%), 3 of G3, 8 of G4, and 2 of G9 rotavirus serotypes. Eight fecal samples from diarrheal episodes had >1 rotavirus serotype identified and were counted more than once in the analysis of serotype-specific vaccine protection. Neither one nor three vaccine doses gave significant protection against diarrhea caused by any G1 or G2 rotavirus (table 5). There were too few cases with G3 (3, all in placebo group) or G4 (4 each in placebo and one-dose vaccine groups) to assess protection.

Table 4. Rotavirus (RV) diarrheal episodes and protective efficacy of one or three doses of RRV-TV vaccine by type of episode.

		RRV-TV	
Characteristic of diarrheal episode	Placebo	1 dose	3 doses
Any RV diarrhea	87	71 (18)	64 (24)*
Any RV diarrhea with			
Fever	52	40 (22)	33 (35)†
Vomiting	55	41 (25)	32 (40)†
≥6 liquid stools/24 h	48	31 (35)*	28 (40)†
Dehydration	21	19 (9)	23 (0)
Health service use	38	28 (26)	30 (20)
Severity score ≥9‡	38	24 (36)*	26 (30)
RV-only diarrhea§	39	25 (35)*	28 (26)
RV-only diarrhea§ with			
Fever	21	15 (28)	16 (22)
Vomiting	25	11 (56)†	13 (47)*
≥6 liquid stools/24 h	22	9 (59)†	12 (44)
Dehyration	7	6 (13)	10 (0)
Health service use	13	8 (38)	12 (5)
Severity score ≥9‡	15	7 (53)	12 (18)

NOTE. No. of child-years in placebo group = 386 and in 1- and 3-dose RRV-TV groups = 382 and 376, respectively. Data are no. of subjects (% protective efficacy compared with placebo group).

$P <$ *.10 or †.05, vaccine vs. placebo groups.

‡ 20 points total.

§ Negative for other enteropathogens.

For rotavirus-only diarrhea, one dose of the vaccine was 53% protective (95% CI, 9%–76%) against G1 rotavirus diarrhea and 66% protective (95% CI, 8%–88%) against G1 rotavirus diarrhea with a high number of liquid stools (table 5). In addition, three doses of RRV-TV had a significant efficacy (VE, 47%; 95% CI, 5%–71%) against any G1 rotavirus diarrhea with vomiting or more frequent liquid stools. There was a tendency for a higher VE against more severe serotype-specific diarrheal episodes and against G1 rather than G2 rotavirus diarrhea.

Discussion

This study, which to our knowledge is the first to evaluate the RRV-TV vaccine in a developing country, attempted to determine the safety, immunogenicity, and protective efficacy with the combination of the RRV (G3) and the G1, G2, and G4 reassortant rotavirus vaccines. The vaccine was well tolerated but failed to induce significant protection against all rotavirus diarrhea. There was a moderate level of vaccine protection against more severe rotavirus diarrhea and against G1 rotavirus diarrhea, especially when other enteropathogens were not isolated.

These findings are similar to those with one dose containing 10^4 pfu of the RRV vaccine in the same community [33]. This similarity suggests that the addition of the reassortant rotavirus vaccines to the RRV vaccine did not add to vaccine protection afforded by RRV alone. Furthermore, three doses did not offer any advantage over one dose of the tetravalent vaccine.

The three-dose regimen resulted in a higher cumulative serologic response and GMT, as measured by IgA ELISA, than did the one-dose regimen; however, the percentage of neutralizing antibody responses (16%–36%) to each of the 4 serotypes represented in the vaccine was comparably low in both vaccine groups. Indeed, this study may have overestimated the number of serotype-specific responses within the one- and three-dose vaccine arms in that the subgroups studied for neutralizing

Table 5. Rotavirus (RV) diarrheal episodes of G serotype 1 or 2 and efficacy of one or three doses of RRV-TV vaccine by type of episode.

Characteristic of diarrheal episode	Serotype 1 RV diarrhea			Serotype 2 RV diarrhea		
	Placebo	1 dose	3 doses	Placebo	1 dose	3 doses
Any RV diarrhea	50	39 (21)	35 (28)	23	21 (8)	22 (2)
Any RV diarrhea with						
Fever	28	21 (24)	19 (30)	19	12 (36)	13 (30)
Vomiting	31	27 (12)	16 (47)*	18	9 (49)	14 (20)
≥6 liquid stools/24 h	31	19 (38)	16 (47)*	13	10 (22)	11 (13)
Severity score ≥9[†]	22	13 (40)	14 (35)	11	7 (36)	11 (0)
RV-only diarrhea[‡]	26	12 (53)*	15 (41)	8	8 (0)	10 (0)
RV-only diarrhea[‡] with						
Fever	13	7 (46)	9 (29)	6	5 (16)	6 (0)
Vomiting	15	9 (39)	7 (52)	7	2 (71)	5 (27)
≥6 liquid stools/24 h	15	5 (66)*	7 (52)	6	4 (33)	5 (14)
Severity score ≥9[†]	10	5 (49)	6 (38)	3	2 (33)	5 (0)

NOTE. No. of child-years in placebo group = 386 and in 1- and 3-dose RRV-TV groups = 382 and 376, respectively. Data are no. of subjects (% protective efficacy compared with placebo group).

* $P < .05$, vaccine vs. placebo group.

[†] 20 points total.

[‡] Negative for other enteropathogens.

antibody responses were selected on the basis of an IgA response. This selection bias may have contributed to the lack of difference in the number of serotype-specific responses between the one- and three-dose vaccine groups, because the predominant effect of additional doses of vaccine was to induce IgA serologic responses in first-dose nonresponders rather than to boost responses in first-dose responders.

One dose (4×10^4 pfu) of tetravalent vaccine appeared to be less immunogenic in subjects in the present study than in subjects of similar age in Venezuela [29, 30] and Turkey [50]: In Peru, Venezuela, and Turkey, 50%, 74%, and 63% of the subjects, respectively, had rotavirus-specific IgA ELISA responses. Vaccine virus shedding after the first dose of RRV-TV in the three-dose group in Peru was detected less frequently than after one dose in Venezuela, although these studies are not strictly comparable because stool specimens were obtained only once, 4–6 days after immunization, in Peru and 3 and 6 days after immunization in the two studies in Venezuela [29, 30].

Breast-feeding is unlikely to have contributed to the lower prevalence of serologic responses in Peru, as it is the predominant method of feeding in the three geographic locations noted above. However, a difference in preexisting antibody levels in the infants may have played a role. In one of the Venezuelan studies [30], only 18% of infants had a detectable IgA response (titer ≥1:50) before vaccination, compared with 40% in Turkey [50] and 35% in Peru. It is also possible that Peruvian subjects had other enteric infections at the time of vaccination and that such infections interfered with the rotavirus vaccine immunogenicity.

The contribution of serotype-specific neutralizing antibodies in eliciting a response against rotavirus vaccine is unclear. Previous trials have shown an association between lack of serotype-specific responses and vaccine failures [17], and a study of natural rotavirus infection suggested that serotype-specific neutralizing antibody may be an important determinant of protection against rotavirus diarrhea [20]. Furthermore, a recent study in Peru that evaluated the efficacy of one dose of serotype 1 or 2 human-RRV reassortants or one dose of RRV vaccine showed only minimal protection in the RRV vaccine group, despite the predominance of serotype 1 and 2 strains [33]. A possible explanation for the lack of efficacy was the low prevalence of serotype-specific responses [33]. In contrast, recent studies in Finland and Rochester, New York, have shown a protective efficacy of 66%–77% against homotypic and heterotypic rotavirus strains after one dose of these monovalent vaccines [16, 51, 52].

These data show the difficulty of assessing the importance of serotype-specific immune responses, which in the face of declining levels of maternally acquired antibody, may also be hard to detect after vaccination in young infants. Significant protection with three doses (given at 2, 4, and 6 months of age) of the identical 4×10^4 RRV-TV vaccine has been demonstrated in the United States [22], where a similar level of protection was found with 4×10^5 RRV-TV [23]. This study shows that it is much more difficult to provide high-level protection against rotavirus diarrhea in a developing-country setting. Enhancements, such as higher titers of the vaccine strains, should be evaluated. The tendency in this study toward greater protection against more severe rotavirus diarrhea is consistent with results of other studies [11, 22, 23] and suggests that this vaccine might have more substantial efficacy in a trial focussed on severe diarrhea.

Acknowledgments

We thank Giuseppe Gerna for serotyping by immune electron microscopy, Hector Verastegui for data analysis, and field workers, supervisors, and doctors for their great care and efficiency in conducting this study.

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