Immunogenicity, safety and efficacy of tetravalent rhesus–human, reassortant rotavirus vaccine in Belém, Brazil

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A tetravalent rhesus–human reassortant rotavirus (RRV-TV) vaccine (4×10^4 plaque-forming units/dose) was evaluated for safety, immunogenicity and efficacy in a prospective, randomized, double-blind, placebo-controlled trial involving 540 Brazilian infants. Doses of vaccine or placebo were given at ages 1, 3 and 5 months. No significant differences were noted in the occurrence of diarrhoea or vomiting in vaccine and placebo recipients following each dose. Low-grade fever occurred on days 3–5 in 2–3% of vaccinees after the first dose, but not after the second or third doses of vaccine. An IgA antibody response to rhesus rotavirus (RRV) occurred in 58% of vaccinees and 33% of placebo recipients. Neutralizing antibody responses to individual serotypes did not exceed 20% when measured by fluorescent focus reduction, but exceeded 40% when assayed by plaque reduction neutralization.

There were 91 cases of rotavirus diarrhoea among the 3-dose (vaccine or placebo) recipients during two years of follow-up, 36 of them among children given the vaccine. Overall vaccine efficacy was 8% ($P = 0.005$) against any diarrhoea and 35% ($P = 0.03$) against any rotavirus diarrhoea. Protection during the first year of follow-up, when G serotype 1 rotavirus predominated, was 57% ($P = 0.008$), but fell to 12% in the second year. Similar results were obtained when analysis was restricted to episodes in which rotavirus was the only identified pathogen. There was a tendency for enhanced protection by vaccine against illness associated with an average of 6 or more stools per day. These results are sufficiently encouraging to warrant further studies of this vaccine in developing countries using a higher dosage in an attempt to improve its immunogenicity and efficacy.

Introduction

Rotavirus causes more than 125 million cases of infantile diarrhoea and about 1 million deaths worldwide per year, mostly in tropical regions (*1*). The similar incidence of the illness in both industrialized and developing countries suggests that it will not be controlled by improving the water supply, sanitation or hygiene practices, and that an effective vaccine is required (*2*, *3*).

Strategies to develop an oral vaccine range from the "Jennerian approach", using rotavirus strains isolated from animals, to the application of molecular biological techniques (*4*). A promising approach is the construction of human–animal rotavirus reassortants bearing the neutralization specificity of human strains, with the objective of inducing homotypic immunity against each of the four important G serotypes (*5*, *6*).

Trials of single serotype rhesus–human vaccines in Finland and the USA (*7*, *8*) have shown protection following a single dose of 10^4 plaque-forming units (pfu). The protection, which was not necessarily serotype-specific, appeared to last more than 1 year, was greatest in infants with a serum IgA antibody response to the vaccine, and was possibly greater for severe diarrhoeal episodes than for mild illness. In Finland, for example, where rotavirus G serotype 1 was prevalent, rates of protection conferred by rhesus–human vaccines with specificity for G serotype 1 (D × rhesus rotavirus vaccine (RRV)) or 2 (DS1 × RRV) averaged 66% and 38% in the first and second rotavirus epidemic seasons following immunization, respectively (*7*). For infants with a serum IgA antibody response, these figures were 92% and 59%. In contrast, vaccine efficacy in developing countries has been disappointing. In a 2-year trial in Peru (*9*), neither the D × RRV nor the DS1 × RRV vaccine, given in a single dose, evoked significant

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protection against diarrhoea caused by heterotypic or homotypic rotavirus.

Recent efforts have focused on evaluation of the RRV-tetravalent (RRV-TV) vaccine, which contains rhesus–human reassortants specific for G serotypes 1, 2 and 4, and RRV for G serotype 3, in the hope that it would protect against each of the four important G rotavirus serotypes and provide greater overall protection than the individual components.* Safety and immunogenicity of the reassortant RRV vaccines, given either alone or in combination, have been assessed in children and young infants (*11–18*). Only limited data are available, however, on the efficacy of RRV-TV vaccine. In the USA, 57% protection against rotavirus diarrhoea was achieved for 2 years, following three doses (4×10^4 pfu each) of the vaccine (*19*). In contrast, three doses of the vaccine provided only 24% protection for 1 year in Peru (*10*).

The present study was conducted to determine the safety, immunogenicity and efficacy of three doses of RRV-TV vaccine in healthy infants in Belém, Brazil.

Materials and methods

In the field work we have essentially followed the recommendations of Lanata & Black (*20*). The study was approved by the Ethical Review Committee of the Instituto Evandro Chagas, the Regional Council of Medicine, the Secretary of Public Health of Pará State and the Ministry of Health of Brazil, and by the Ethical Review Committee of the World Health Organization.

Study area and population. Belém is located in North Brazil, near the mouth of the Amazon river, and has a wet tropical climate. Half of its estimated population of 1.5 million live under crowded conditions with poor sanitation. The trial was conducted in an area of 8 km^2 with a population of 350000. Previous studies in this area have shown that rotaviruses account for about 30% of cases of acute diarrhoea among hospitalized infants and young children (*21*), and nearly 10% of acute gastroenteritis episodes in the community (*22*). All four G serotypes circulate in the region, G type 1 being the most prevalent (*23*).

A total of 1064 pregnant women were recruited through home visits from October 1989 to January 1990, of whom 540 were selected to participate in the trial. An effort was made to cluster families geographically in order to facilitate the field work. Following recruitment and until giving birth, the women were visited monthly to sustain their interest and restate the objectives and conditions of the study. Talks on the project were also given regularly in community centres.

Infants were enrolled during the first monthly visit after birth, at which time signed informed consents were obtained from the parents. Infants were excluded from the study if (*a*) consent was not provided, (*b*) they had a chronic illness, or (*c*) any household member was taking an immunosuppressive drug or had an immunodeficiency disorder. From February to September 1990, 540 newborns were recruited, most of them (94%) during March–July.

Study design. The study was a 2-year, prospective, double-blind, placebo-controlled, randomized trial in which infants received three doses of placebo or RRV-TV vaccine. The first, second and third doses were given to 540, 513 and 495 infants, respectively, at approximately 1, 3, and 5 months of age (age ranges: 30–59, 90–119 and 150–179 days, respectively). Venous blood was obtained from 180 children prior to the first dose, and 170, 168 and 165 specimens were collected from the same children 1 month after the first, second and third doses, respectively. Serum samples were frozen at –20 °C until assayed for rotavirus antibody.

Calculation of the sample size was performed as recommended by Lanata & Black (*20*). The study was designed to detect a minimum protective efficacy of 70% (with an alpha level of 5% and a power of 90%) against all episodes of rotavirus diarrhoea during 2 years of surveillance after immunization. The expected incidence in the placebo group was based on previous surveillance in the same area (*22*). At least 200 children per group were required by the end of follow-up. Randomization in blocks of twelve was performed using the children's sequential enrolment numbers; 180 children were randomly selected for immunogenicity studies in the proportion of 3 vaccine recipients to 1 placebo recipient. The vaccine randomization and selection of children for the immunogenicity study were carried out by Wyeth-Ayerst Laboratories, Radnor, PA, USA. The vaccine code was kept at Wyeth-Ayerst Laboratories and WHO, and was not broken until surveillance was completed; data were checked and cleaned, and all outcome data were confirmed as final.

Vaccine and immunization. Each dose of RRV-TV vaccine contained 4×10^4 pfu with equal parts of (*a*) serotype G1 reassortant (D × RRV), (*b*) serotype G2 reassortant (DS1 × RRV), (*c*) serotype G3 RRV, and (*d*) serotype G4 reassortant (ST3 × RRV). Each reassortant strain contained the gene coding for VP7 of human G serotype 1, 2 or 4, the remaining 10 genes being derived from rhesus

rotavirus strain MMU18006 (*4*). Vaccine and placebo (uninfected cell-culture fluid) were manufactured at Wyeth-Ayerst Laboratories, supplied lyophilized and kept at 4 °C until used. Vaccine and placebo vials were numbered serially and were identical in appearance. Vials for children randomly preselected for the immunogenicity study had distinctive pink labels. Reconstitution was with 1.2 ml of distilled water immediately before administration.

From April 1990 to January 1991, vaccine or placebo was given twice weekly at the Instituto Evandro Chagas. Before immunization each child was examined by a paediatrician. Vaccination was rescheduled for children who had fever during the preceding 48 hours or diarrhoea during the preceding 72 hours. Breast- and bottle-feeding were withheld for 1 hour before and after immunization. Immediately before immunization, 30 ml of reconstituted evaporated milk (with no rotavirus neutralizing activity and containing 400 mg of sodium bicarbonate) was given to each child. For infants in the immunogenicity study, 2–5 ml of blood was also obtained by venepuncture. Mothers were encouraged to obtain other routine vaccines from the official public health services. However, as agreed by the local public health authorities, our staff assumed responsibility for giving oral poliomyelitis vaccine to all participating children. This was given at an interval of at least 15 days before or after rotavirus vaccination.

Surveillance for side-effects and diarrhoeal episodes. Following immunization, each child was visited daily for seven consecutive days. At each visit the following were noted and recorded: (*a*) number and consistency of stools in the past 24 hours; (*b*) frequency of vomiting; (*c*) rectal temperature; and (*d*) occurrence of respiratory symptoms. Beginning 7 days after the first dose, and continuing for 2 years, each child was visited twice weekly at home to detect diarrhoeal episodes, defined as three or more liquid or semi-liquid motions in a 24-hour period. When diarrhoea was detected, daily visits were made until the episode ended, i.e. when the child, during 48 hours or more, passed less than three liquid or semi-liquid stools in each 24-hour period. During diarrhoeal episodes, the following clinical parameters were regularly recorded: (*a*) number of bowel movements in each 24-hour period (including watery stools); (*b*) duration of diarrhoea; (*c*) presence of blood or mucus in stools; (*d*) frequency of vomiting; (*e*) fever measured rectally; and (*f*) signs of dehydration using WHO criteria (*39*). Whenever signs of dehydration were present, children were visited by a physician from our staff and, if required, referred to either a medical unit or a public hospital.

Laboratory procedures. Stool specimens or rectal swabs were obtained as soon as possible after an episode of diarrhoea was detected. Specimens were obtained while diarrhoea continued and up to 48 hours after it had stopped. During 1990–91 only faecal specimens were collected; in 1992, rectal swabs were taken whenever a faecal sample could not be obtained. Faeces or rectal swabs were placed in sterile saline for rotavirus examination and in two screw-capped vials of Cary-Blair medium, one containing Skirrow's antimicrobial supplement (*24*). A sample was also placed in a vial containing merthiolate, iodine and formalin (MIF) solution for parasitological examination. Samples were transported to the laboratory within 4 hours in insulated boxes with cold packs and processed the same day. All samples were routinely assayed for rotavirus antigen using DAKOPATTS ELISA (enzyme-linked immunosorbent assay) kits (Copenhagen, Denmark) (*25*). G serotyping of rotavirus strains was routinely performed using monoclonal antibodies, essentially as described by Taniguchi et al. (*26*). Results were confirmed at the Department of Hygiene and Epidemiology, Sapporo Medical College, Sapporo, Japan. Rotavirus strains not typable by this method were serotyped at the Istituto di Malatie Infetive, University of Pavia, Italy, by solid phase immunoelectron microscopy (*27*).

Only rotavirus-positive faecal samples were subsequently examined for the presence of other diarrhoeal pathogens, using standard techniques.[a] Enterotoxin production by *Escherichia coli* was tested by commercial kits: the heat-labile toxin (LT) by the reversed passive latex agglutination test (VET-RPLA, Unipath Ltd, Basingstoke, Hampshire, England), and the heat-stable toxin (ST) by a competitive ELISA (*E. coli* ST EIA kit, Unipath Ltd, Basingstoke, Hampshire, England). In addition, all enteropathogenic *E. coli* identified in this study (by standard serological procedures) were further assayed for the presence of enteroadherence factor (EAF) by gene probe, as previously described (*28*). Rotavirus-positive faecal samples were routinely examined by conventional parasitological techniques, as well as for the presence of *Cryptosporidium* sp. using a modified Ziehl-Nielsen and auramine staining method (*29*).

Pre- and post-immunization sera were tested for rotavirus antibody at United Medical Laboratories

[a] **World Health Organization.** *Manual for laboratory investigations of acute enteric infections.* Unpublished WHO document CDD/83.3 (Rev. 1), 1987 (available upon request from Division of Child Health and Development, World Health Organization, 1211 Geneva 27, Switzerland).

(McLean, VA, USA). Anti-rotavirus IgA was measured by an enzyme immunoassay using RRV as the capture antigen, as previously described (*30*, *31*). All sera obtained from a subject were tested simultaneously, starting at a dilution of 1:50. Rotavirus serotype-specific neutralizing antibodies were determined by a modified fluorescent focus reduction (FFR) assay (*32*). All sera were tested against serotypes G1 (strain Wa), G2 (strain DS1), G3 (strain P), G4 (strain ST3) and RRV. Neutralizing antibodies to strains WA, DS1, SC2 (a WC3-based G2 reassortant), RRV, WI78-8 (G3 type), and BrB9 (a WC3-based G4 reassortant) were also measured in sera from 20 RRV-TV recipients by plaque-reduction neutralization (PRN), essentially as described elsewhere (*33*).

Data analysis. Of 540 infants who received the first dose of vaccine or placebo, 495 (92%) were fully immunized and, of these, 466 (94%) were followed up for the full 2-year study period. Loss to follow-up was caused by death (8 children), withdrawal of consent (1 child), and migration from the study area (65 children). When determining vaccine efficacy, only diarrhoeal episodes were considered that began at least 15 days after the third dose of vaccine or placebo. Children who were not fully immunized were excluded from the analysis. Vaccine efficacy was calculated for all rotavirus-positive diarrhoeal episodes and for episodes of "pure" rotavirus infection, i.e. those in which no other enteropathogen (*Salmonella*, *Shigella*, *Aeromonas* sp., *Campylobacter* sp., *Vibrio* sp., enterotoxigenic *E. coli*, EAF-positive *E. coli*, *Cryptosporidium* sp., *Giardia intestinalis* or *Entamoeba histolytica*) was identified. Efficacy was also assessed against indicators of clinical severity, using a modified, 20-point scoring system (CS), as proposed by Flores et al. (*34*).

Vaccine efficacy and 95% confidence intervals were determined as previously described (*20*). Differences between both placebo and vaccine groups were analysed using the Mantel-Haenszel χ^2 test of association or Fisher's exact test, as appropriate ($P < 0.05$ was regarded as significant).

Results

Reactogenicity. RRV-TV vaccine was well tolerated. The incidences of diarrhoea and vomiting during 7 days after the first, second and third doses, and of fever following the second and third doses, did not differ significantly for vaccine and placebo recipients. Low-grade fever (rectal temperature ≥38 °C) occurred in 1.6–2.9% of infants on days 3–5 after the first dose of RRV-TV vaccine, but in only 0.4–1.2% of placebo recipients ($P < 0.05$ by χ^2 test).

Serology. Pre-vaccination and post-third dose serum samples were available from 161 infants (40 given placebo and 121 given vaccine). Results of ELISA IgA and neutralizing antibody seroconversion, after three doses, are summarized in Fig. 1. ELISA IgA seroconversion was significantly more frequent ($P = 0.005$) among vaccinees (58%) than infants given placebo (33%). In contrast, the serotype-specific seroconversion rates among vaccine recipients for neutralizing antibodies measured by FFR assay were less than 20%, except for the response to RRV (62% and 7.5% seroconversion for vaccine and placebo groups, respectively, $P < 0.001$). Significant differences between vaccine and placebo groups were observed only for serotype 2 (19% vs. 5%, $P = 0.03$) and for all four human serotypes combined (18% vs. 2.5%, $P = 0.01$). Geometric mean titres (GMTs) of neutralizing antibody against G serotypes 1, 2, 3 and 4 were also low, reaching a maximum value of 62 against combined human serotypes in the vaccine group. In contrast, GMTs of RRV-antibody were 410 and 24 for the vaccine and placebo groups, respectively ($P < 0.005$).

PRN antibody responses to rotavirus G1–4 serotypes after each dose of RRV-TV vaccine are summarized in Table 1. Pre-immunization rates of seropositivity to all G types/strains, except the G2/SC2-9 strain, were greater than 60%. In addition, seroresponse rates after any dose ranged from 45% to 75% for the G1/WA and G3/RRV strains, respectively.

Fig. 1. **Seroconversion rates in fully immunized infants.** Seroconversion is defined as ≥4-fold rise in ELISA IgA or FFR neutralizing antibody comparing pre-immunization sera with sera obtained one month after the third dose of vaccine or placebo. ST indicates the serotype for the neutralizing antibody response. Statistical comparisons are summarized in the text.

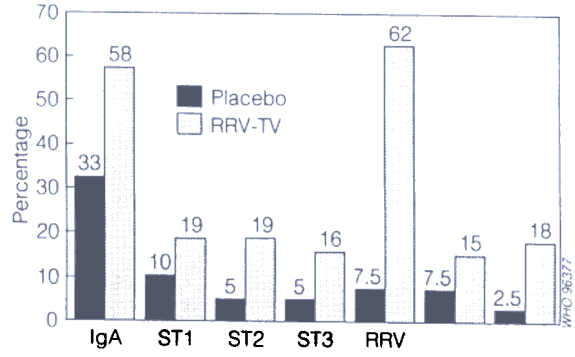

Table 1: **Serotype-specific serological responses[a] of RRV-TV recipients measured by plaque reduction neutralization assay**

G types/strains	No. of positive sera / No. tested: Prior to 1st dose	No. of seroresponses / No. of tested sera: After 1st dose	After 2nd dose	After 3rd dose	After any dose
G1/WA	15/20 (75)[b]	3/20 (15)	4/20 (20)	3/20 (20)	
G2/DS1	15/20 (75)	4/19 (21)	4/20 (20)	3/20 (15)	
G2/SC2-9	6/20 (30)	5/20 (25)	6/20 (30)	5/20 (25)	
G3/RRV	16/20 (80)	8/20 (40)	7/20 (35)	4/20 (20)	
G3/WI78-8	14/19 (74)	3/20 (15)	7/20 (35)	3/20 (35)	
G4/BrB9	11/18 (61)	2/20 (10)	6/20 (30)	5/20 (25)	

[a] An increase in titre (determined as the dilution giving 50% plaque reduction) between bleedings of ≥3-fold, a cumulative increase of 3-fold over two or more bleedings, or an increase in titre from <50 to ≥125.
[b] Figures in parentheses are percentages.

Protective efficacy. During the follow-up period (starting 15 days after completion of three doses), from July 1990 to June 1992, there were 264 363 child–days of surveillance, corresponding to 724 child–years. Specifically, for placebo and vaccine groups, there were 363 and 361 child–years of observation, respectively. A total of 4197 episodes of diarrhoea was recorded, yielding rates of 6.0 and 5.6 episodes per child–year in placebo and vaccine recipients, respectively. Thus, the 2-year vaccine efficacy against any diarrhoeal illness was 8% (95% CI, 3–13%; $P = 0.005$).

Faecal samples or rectal swabs from 1990 episodes of diarrhoea (48% of all episodes) were tested by ELISA for the presence of rotavirus antigen. Altogether, 131 episodes of rotavirus diarrhoea were detected during the study period, of which 91 began at least 15 days after the third dose (Fig. 2). Thus, rotavirus was detected in 4.6% of diarrhoeal episodes (91 of 1990 cases), either as the only pathogen or in association with other enteropathogens. The rates of rotavirus diarrhoea during the period of July–September, for the three years combined, were significantly higher ($P = 0.0007$) than during the rest of the year (Fig. 3). Mixed infections accounted for about 30% of cases, if *G. intestinalis* and enteropathogenic *E. coli* (EPEC), defined only by O-specific antisera, were included. If *Giardia* was excluded, however, and only enteroadherent factor-positive EPEC strains were considered, the proportion of mixed infections was 18%. The latter definition of mixed infection has been used for efficacy analysis. Of the 91 rotavirus strains, 86 (94%) were successfully serotyped. G serotypes 1, 2, 3, 4 and 9 accounted for 65% (58/86), 30% (24/86), 1.2% (1/86), 4.6% (4/86), and 1.2% (1/86) of serotyped strains, respectively.

Table 2 summarizes the protective efficacy of

Fig. 2. **Monthly distribution of 91 rotavirus diarrhoea cases among the study participants from July 1990 to June 1992.** Cases are those with onset at least 15 days after the third dose of vaccine or placebo.

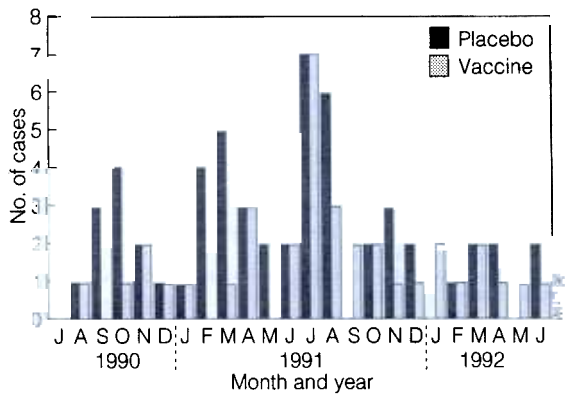

Fig. 3. **Monthly rates of rotavirus diarrhoea in study participants from July 1990 to July 1992.** Cases are those with onset at least 15 days after the third dose of vaccine or placebo.

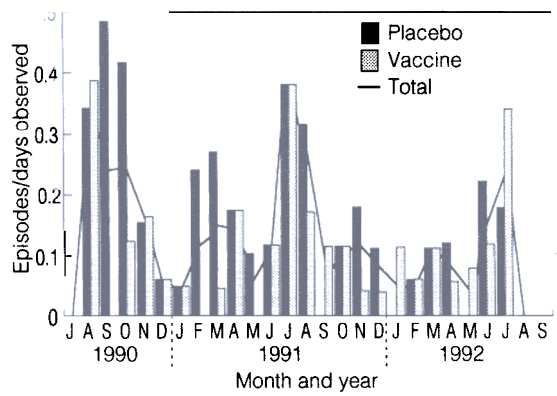

Table 2: **Protective efficacy of RRV-tetravalent vaccine against all diarrhoea cases, and pure and serotype-specific rotavirus (RV) diarrhoea cases**

RV diarrhoea episodes, by year of follow-up	No. on placebo	No. on vaccine	Percentage protection	P^a
Two years (7/90–6/92):	(363)[b]	(361)		
All cases	55	36	35	0.03
Pure RV cases	46	29	37	0.04
All ST1 RV cases[c]	32	24	25	0.27
Pure ST1 RV cases	27	19	29	0.23
All ST2 RV cases[c]	15	9	40	0.22
Pure ST2 RV cases	13	5	61	0.06
First year (7/90–6/91):	(160)	(157)		
All cases	28	12	57	0.008
Pure RV cases	23	11	51	0.03
All ST1 RV cases	20	9	55	0.03
Pure ST1 RV cases	17	8	52	0.07
All ST2 cases	3	2	32	0.51
Pure ST2 RV cases	3	2	0	0.98
Second year (7/91–6/92):	(203)	(204)		
All cases	28	26	12	0.64
Pure RV cases	23	18	22	0.40
All ST1 RV cases	12	15	0	—
Pure ST1 RV cases	10	11	0	—
All ST2 cases	12	7	42	0.23
Pure ST2 RV cases	11	7	37	0.33

[a] By analysis of variance.
[b] Figures in parentheses are the child–years of surveillance after three doses.
[c] ST1 = G serotype 1; ST2 = G serotype 2.

RRV-TV vaccine against all episodes and "pure" episodes of rotavirus diarrhoea, and those caused by serotypes 1 or 2. There were 55 and 36 rotavirus episodes in the placebo and vaccine groups, respectively, yielding an overall vaccine efficacy for 2 years against all rotavirus diarrhoea of 35% (P = 0.03, χ^2 test; 95% CI, 2–56%). Protective efficacy for two years against pure rotavirus diarrhoeal episodes was 37% (P = 0.04; 95% CI, 1–59%).

During the first year of follow-up (July 1990 to June 1991), vaccine efficacy was greater than 50% against all episodes of rotavirus diarrhoea and against episodes associated with serotype 1. Protection against serotype 2 episodes could not be assessed owing to the small number of cases. During the second year of follow-up there was no protection against serotype 1 rotavirus diarrhoea. There was moderate protection against serotype 2 illness, but this was not statistically significant.

Vaccine-induced protection against specific indicators of clinical severity in children with rotavirus diarrhoea is summarized in Tables 3 and 4. Table 3 shows that, during 2 years of follow-up, RRV-TV vaccine induced 47% (P = 0.02) and 58% (P = 0.03) protection against a *maximum number* of liquid stools (MLS) ≥6 in any 24-hour period and a *mean number* of liquid stools (mLS) ≥6 per 24 hours, respectively, for all rotavirus diarrhoeal episodes; for the latter outcome, days of observation ranged from 1 to 10 (mean = 4.3) and 1 to 8 (mean = 3.9) for placebo and vaccine recipients, respectively. It also induced 46% protection (P = 0.01) against diarrhoeal episodes with a CS (clinical severity score) ≥9 and a trend for protection against vomiting. In the "pure" rotavirus diarrhoeal episodes, significant protection was detected against vomiting (41%, P = 0.03), MLS ≥6 per 24 hours (59%, P = 0.003), mLS ≥6 per 24 hours (65%, P = 0.02), and episodes with a CS ≥9 (56%, $P < 0.005$).

With respect to year one of follow-up (Table 4), the vaccine was significantly protective against vomiting (52%, P = 0.04), MLS ≥6 per 24 hours (59%, P = 0.02) and mLS ≥6 per 24 hours (84%, P = 0.004) for any rotavirus diarrhoea. For "pure" rotavirus diarrhoea, significant protection was noted against MLS ≥6 per 24 hours (60%, P = 0.02) and mLS ≥6 per 24 hours (82%, P = 0.01).

Table 3: **Protective efficacy of RRV-tetravalent vaccine against individual symptoms in all cases of rotavirus (RV) diarrhoea during the two-year follow-up (July 1990 to June 1992)**

RV diarrhoea episodes	No. on placebo	No. on vaccine	Percentage protection	P^a
Two years:	(363)[b]	(361)		
All RV episodes with:[c]				
RT ≥ 38.0°C	7	8	0	—
Vomiting	44	29	34	0.06
MLS ≥ 6	34	18	47	0.02
mLS ≥ 6	19	8	58	0.03
Dehydration	6	4	33	0.53
HCV/hosp.	28	22	21	0.40
CS ≥ 9	39	21	46	0.01
Pure RV episodes with:[c]				
RT ≥ 38.0°C	7	5	28	0.40
Vomiting	39	23	41	0.03
MLS ≥ 6	32	13	59	0.003
mLS ≥ 6	17	6	65	0.02
Dehydration	6	2	67	0.16
HCV/hosp.	26	18	31	0.22
CS ≥ 9	34	15	56	0.005

[a] By analysis of variance.
[b] Figures in parentheses are the child–years of surveillance after three doses.
[c] RT = rectal temperature; MLS = maximum number of liquid stools ≥6/24 h; mLS = mean number of liquid stools ≥6/24 h; dehydration = moderate or severe dehydration; CS = clinical severity score; HCV/hosp. = health centre visit/hospitalization.

Table 4: **Protective efficacy of RRV-tetravalent vaccine against individual symptoms in cases of rotavirus (RV) diarrhoea during the first year (7/90–6/91) and second year (7/91–6/92) of follow-up**

RV diarrhoea episodes	No. on placebo	No. on vaccine	Percentage protection	P^a
First year:	(160)[b]	(157)		
All RV episodes with:[c]				
RT ≥ 38.0 °C	3	4	0	—
Vomiting	21	10	52	0.04
MLS ≥ 6	20	8	59	0.02
mLS ≥ 6	13	2	84	0.004
Dehydration	2	1	49	0.57
HCV/hosp.	15	8	46	0.14
CS ≥ 9	17	9	46	0.11
Pure RV episodes with:[c]				
RT ≥ 38.0 °C	3	3	0	—
Vomiting	17	9	46	0.11
MLS ≥ 6	18	7	60	.0.02
mLS ≥ 6	11	2	82	0.01
Dehydration	2	1	49	0.51
HCV/hosp.	14	7	49	0.12
CS ≥ 9	14	8	42	0.20
Second year:	(203)[b]	(204)		
All RV episodes with:[c]				
RT ≥ 38.0 °C	4	4	0	
Vomiting	23	19	18	0.50
MLS ≥ 6	14	10	29	0.39
mLS ≥ 6	6	6	0	—
Dehydration	4	3	25	0.70
HCV/hosp.	13	14	0	—
CS ≥ 9	21	12	43	0.09
Pure RV episodes with:[c]				
RT ≥ 38.0 °C	4	2	50	0.34
Vomiting	21	14	34	0.21
MLS ≥ 6	14	6	57	0.06
mLS ≥ 6	6	4	34	0.51
Dehydration	4	1	75	0.18
HCV/hosp.	12	11	9	0.82
CS ≥ 9	19	7	63[d]	0.01

[a] By analysis of variance.
[b] Figures in parentheses are the child–years of surveillance after three doses.
[c] See footnote *c*, Table 3.
[d] 91% protection ($P = 0.003$) against rotavirus serotype 2.

No significant protection was observed for specific symptoms in rotavirus diarrhoea episodes (either all or "pure" episodes) during the second year of surveillance (Table 4). However, the vaccine had 63% protective efficacy ($P = 0.01$) against "pure" rotavirus diarrhoea with a CS ≥9 (Table 4). For rotavirus serotype 2 diarrhoeal episodes with a CS ≥9, vaccine efficacy was 91% ($P = 0.003$).

Discussion

RRV-TV vaccine, in a dose of 4×10^4 pfu, was well tolerated by 1-month-old infants, and no increase was noted in vomiting or diarrhoea following any dose of the vaccine. A modest febrile response was observed on days 3–5, but only after the first dose. These findings accord with reports from Israel, Peru, USA, and Venezuela, in which the same dose of RRV-TV vaccine caused no adverse effects or only transient and mild febrile reactions about four days after immunization (*10, 11, 15, 18*). Subsequent studies in the USA and Venezuela with vaccine doses of 4×10^5 or 4×10^6 pfu have given similar results, confirming that the vaccine is safe (*19, 35*).

The immunogenicity of RRV-TV vaccine in our study resembled that observed in other studies of the vaccine, or its monovalent components, in developing countries: responses detected by IgA ELISA or FFR neutralization of RRV occurred in about 60% of infants, whereas FFR antibody responses to the VP7 antigen of G serotypes 1–4 occurred in only 15–19% of infants (*9, 10, 34*). Neutralizing antibody responses measured by FFR assay appear to occur more frequently in infants in developed countries, reaching 50% for homotypic responses to monovalent vaccine of G serotype 1 (*8, 17*). Possible explanations for reduced immunogenicity of the vaccine in developing countries include a suppressive effect of maternal antibodies transferred in breast milk or transplacentally, or competition from other enteric viruses. Although there is growing evidence that neutralizing antibodies specific for VP7 are not the sole mediator of immunity, vaccine-induced protection does correlate with seroconversion for IgA antibody to RRV detected by ELISA (*7*). This has led to efforts to improve vaccine immunogenicity and efficacy by increasing the dose of RRV-TV vaccine to 4×10^5 pfu (*35*).

In contrast with the above, serotype-specific PRN antibody seroconversion rates were greater than 40% after any dose, thus approaching the rates observed in Venezuela (*15*). The PRN results also show that seroresponses to rotavirus G serotypes 1–4 continued to occur following the second and third doses of vaccine, thus supporting the view that a three-dose immunization schedule improves the immunogenicity of RRV-TV.

Various studies have shown that the efficacy of rhesus–human reassortant rotavirus vaccines is often greater in developed than developing countries. For example, a single dose of D × RRV vaccine (serotype 1, 1×10^4 pfu) evoked 67–77% protection against all episodes of rotavirus diarrhoea (caused mostly by serotype 1) for 1 year in Finland and the USA, but no protection in Peru (*7–9*). Similarly, three doses of RRV-TV vaccine (4×10^4 pfu/dose) evoked 57% protection for 1 year against all episodes of rotavirus diarrhoea in the USA, but only 24% protection in Peru (*10, 19*). Similar geographic

differences in efficacy were reported for live oral vaccines based on attenuated bovine rotavirus strains (*36–38*).

The efficacy of RRV-TV vaccine observed in this study should be considered promising, especially as the study population reflected the low socioeconomic conditions and high diarrhoeal disease incidence typical of many developing countries. Protection against all episodes of rotavirus diarrhoea was 57% during the first year of follow-up and exceeded 80% for disease in which the mean number of liquid stools per day was six or more. Although enhanced protection was not seen for other features of severe disease, this observation suggests that vaccine efficacy may be somewhat greater for severe illness than for all episodes, an observation also made in other trials with monovalent or tetravalent rhesus–human vaccine (*7, 19, 30*). This feature of vaccine efficacy is especially important, as the principal public health benefit of a rotavirus vaccine would be its ability to prevent severe, potentially life-threatening disease. Current trials that focus on cases detected at health facilities rather than by frequent household visits, as in the present study, should be better able to define the extent of vaccine-induced protection against such episodes.

Protection during the second year of follow-up was substantially less than during the first year: 12% vs. 57% for all episodes of rotavirus diarrhoea. A similar time-related decline in vaccine-induced protection has been seen in some reported studies with rhesus–human reassortant vaccines (*7*), but not in others (*8, 19*). Since more than half of rotavirus episodes detected during this study occurred in the second year, this suggests that efforts are needed to improve the duration of vaccine-induced protection, possibly by increasing the titre of virus in each dose, or providing a booster dose early in the second year of life, or both.

As in other trials, most episodes of rotavirus diarrhoea in this study were caused by G serotype 1 and it was only possible to show significant protection against this serotype during the first year of follow-up. G serotype 2 caused about one-third of rotavirus diarrhoeal episodes, mostly during the second year of follow-up, thus also raising the question as to whether the decline in vaccine efficacy might reflect lower protection against this G serotype. Although vaccinees showed a 42% reduction in diarrhoea associated with serotype 2 during the second year, this was not statistically significant.

The results of this trial should encourage further evaluation of RRV-TV vaccine, especially in developing countries where rotavirus is the most important cause of life-threatening watery diarrhoea in infants and young children. Most important would be trials in which the vaccine dose is increased to at least 4×10^5 pfu, in an effort to achieve increased and prolonged protection, and the trial design emphasizes assessment of protection against severe, potentially life-threatening illness. Such a trial is at present under way in Venezuela.

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Résumé

Immunogénicité, innocuité et efficacité d'un vaccin antirotavirus tétravalent obtenu par réassortiment génétique rhésus-homme: essai à Belém, Brésil

L'innocuité, l'immunogénicité et l'efficacité d'un vaccin antirotavirus tétravalent obtenu par réassortiment génétique rhésus-homme (RRV-TV) (4×10^4 unités formant plage/dose) ont été évaluées lors d'un essai prospectif randomisé en double aveugle contre placebo chez 540 nourrissons brésiliens. Des doses de vaccin ou de placebo ont été administrées à l'âge de 1, 3 et 5 mois. Aucune différence significative entre le groupe vacciné et le groupe placebo n'a été observée en ce qui concerne les diarrhées ou les vomissements suivant l'administration des doses. Une fébricule a été observée les jours 3–5 chez 2 à 3% des vaccinés à la suite de la première dose mais non des deux autres. Une réponse en IgA au rotavirus de rhésus (RRV) a été observée chez 58% des vaccinés et 33% des sujets ayant reçu le placebo. Les réponses en anticorps neutralisants contre les sérotypes individuels ne dépassaient pas 20% lorsqu'elles étaient mesurées par réduction des foyers de fluorescence, mais étaient supérieures à 40% par neutralisation de la réduction des plages.

Pendant les 2 ans de suivi, 91 cas de diarrhée à rotavirus ont été observés chez les enfants ayant reçu les trois doses (de vaccin ou de placebo), dont

36 chez des enfants vaccinés. L'efficacité globale du vaccin contre les diarrhées en général était de 8% (p = 0,005) et contre les diarrhées à rotavirus de divers sérotypes, de 35% (p = 0,03). Pendant la première année de suivi, alors que le rotavirus G de sérotype 1 était prédominant, la protection était de 57% (p = 0,008), pour tomber à 12% la deuxième année. Des résultats similaires ont été obtenus lorsque l'analyse était limitée aux épisodes dans lesquels un rotavirus était le seul agent pathogène identifié. Le vaccin paraissait offrir une meilleure protection contre les affections comportant en moyenne 6 selles ou plus par jour. Ces résultats sont suffisamment encourageants pour justifier la poursuite de l'étude de ce vaccin dans les pays en développement, en utilisant une plus forte dose pour tenter d'améliorer son immunogénicité et son efficacité.

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