Safety and Immunogenicity of Sequential Rotavirus Vaccine Schedules

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abstract

BACKGROUND AND OBJECTIVES: Although both licensed rotavirus vaccines are safe and effective, it is often not possible to complete the schedule by using the same vaccine formulation. The goal of this study was to investigate the noninferiority of the immune responses to the 2 licensed rotavirus vaccines when administered as a mixed schedule compared with administering a single vaccine formulation alone.

METHODS: Randomized, multicenter, open-label study. Healthy infants (6–14 weeks of age) were randomized to receive rotavirus vaccines in 1 of 5 different schedules (2 using a single vaccine for all doses, and 3 using mixed schedules). The group receiving only the monovalent rotavirus vaccine received 2 doses of vaccine and the other 4 groups received 3 doses of vaccine. Serum for immunogenicity testing was obtained 1 month after the last vaccine dose and the proportion of seropositive children (rotavirus immunoglobulin A ≥20 U/mL) were compared in all the vaccine groups.

RESULTS: Between March 2011 and September 2013, 1393 children were enrolled and randomized. Immune responses to all the sequential mixed vaccine schedules were shown to be noninferior when compared with the 2 single vaccine reference groups. The proportion of children seropositive to at least 1 vaccine antigen at 1 month after vaccination ranged from 77% to 96%, and was not significantly different among all the study groups. All schedules were well tolerated.

CONCLUSIONS: Mixed schedules are safe and induced comparable immune responses when compared with the licensed rotavirus vaccines given alone.

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Dr Libster coordinated and supervised data collection, data analysis, and writing the manuscript; Ms McNeal directed the laboratory where assays were validated and samples were analyzed, approved all data before reporting, and reviewed and edited the manuscript; Drs Walter, Shane, Winokur, Cress, Berry, Kotloff, Sarpong, Turley, Harrison, Pahud, Marbin, Dunn coordinated and supervised data collection, and reviewed and edited the manuscript; Ms El-Khorazaty conducted statistical analyses for the project, and reviewed and edited the manuscript; Ms Barrett coordinated and supervised the study database development and statistical analysis, and reviewed and edited the manuscript; Dr Edwards designed and supervised the project, analyzed data, and wrote the manuscript; and all authors approved the final version as submitted.

This trial has been registered at www.clinicaltrials.gov (identifier NCT01266850).

WHAT'S KNOWN ON THIS SUBJECT: Rotavirus vaccines (Rotarix and RotaTeq) are safe and effective. In clinical practice it is often not possible for infants to receive the same formulation for all doses. No studies have addressed the interchangeability of the 2 different rotavirus vaccine formulations.

WHAT THIS STUDY ADDS: Mixed rotavirus vaccine schedules are safe and noninferior in immunogenicity when compared with each licensed rotavirus vaccine when administered alone.

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In the United States, before universal rotavirus vaccine use in 2006, rotavirus was the most common cause of severe gastroenteritis among children.[1,2] It was responsible for >410 000 doctor visits, 205 000 emergency department visits, between 55 000 and 70 000 hospitalizations, and 20 to 60 deaths in children younger than 5 years annually.[2] Although mortality attributed to rotavirus is rare in the United States, in developing countries, rotavirus is reported to be associated with >100 000 deaths each year in children ≤5 years of age.[3]

Currently there are 2 rotavirus vaccines approved by the Food and Drug Administration that are licensed for use in the United States: RotaTeq (RV5) and Rotarix (RV1). These vaccines use different principles to achieve broad-range immunity against diverse strains.[4,5] RV5 is a live, oral vaccine that contains a combination of 5 human/bovine reassortant rotaviruses.[6,7] Three doses of the vaccine are recommended. RV1 is a live-attenuated human rotavirus vaccine prepared from a single human strain (G1P[8]),[8,9] and 2 doses are recommended.

Both vaccines have been shown to be highly efficacious and safe in large clinical studies.[2,10–12] In contrast to a previously licensed rotavirus vaccine (Rotashield),[13] only a small increase in cases of intussusception has been reported after both RV1 and RV5 vaccinations.[14]

Although the Advisory Committee on Immunization Practices (ACIP) recommended receipt of the same formulation of rotavirus vaccine for all doses, in clinical practice this is often not possible. The aim of this study was to determine the noninferiority and safety of 3 different mixed schedules of the 2 licensed rotavirus vaccines compared with administration of the same vaccine formulation for each dose.

METHODS

Participants

A randomized, nonblinded (for the subject and study team), multicenter study was conducted at 6 primary Vaccine Testing and Evaluation Unit (VTEU) sites funded by the National Institutes of Health and 4 subcontract sites (Children's Hospital of Oakland, Oakland, CA; Children's Mercy Hospital, Kansas City, MO; Duke University Health System, Durham, NC; Emory University School of Medicine, Emory Children's Center, Atlanta, GA; Group Health Cooperative, Seattle, WA; St Louis University, St Louis, MO; University of Iowa, Iowa City, IA; University of Maryland, Baltimore, MD; University of Texas Medical Branch at Galveston, Galveston, TX; and Vanderbilt University Medical Center, Nashville, TN). The Laboratory for Specialized Clinical Studies, Division of Infectious Diseases at the Cincinnati Children's Hospital Medical Center performed all the immunologic assays.

Eligible subjects were healthy infants (determined by medical history, physical examination, and medical assessment), at least 6 weeks and ≤15 weeks of age at first vaccination. Parents or legal guardians provided signed informed consent.

Subjects were excluded from participation if they had any clinically significant history of gastrointestinal disease or other serious medical conditions, any history of immunodeficiency, known sensitivity to any vaccine components, previous receipt of a rotavirus vaccine, an acute illness at the time of or in the previous 48 hours before vaccine administration (axillary temperature higher than 100.2°F, >3 looser-than-normal stools, or any episodes of vomiting), participation in another study involving an experimental agent, birth at <37 weeks' gestation, receipt of blood and/or blood products (including immunoglobulin) within 4 weeks before vaccine administration, and receipt of any live vaccine within the past 30 days or an inactivated vaccine within the previous 14 days.

Study Objectives

The primary objective of the study was to determine if the proportion of seropositive children, defined as serum anti-rotavirus immunoglobulin A (IgA) ≥20 U/mL at 1 month after the last dose of vaccine,[15,16] in each sequential mixed rotavirus vaccine group, was noninferior to the proportion of seropositive infants in the group receiving a single vaccine formulation matching the first dose for each mixed vaccine group. The secondary objectives were to determine the neutralizing rotavirus antibody responses[17] to the most common rotavirus serotypes (G1–G4 and G9) at 1 month after the last vaccination and to determine if all the schedules were safe with no statistically significant increase in fever, diarrhea, vomiting, or intussusception compared with the recommended schedules of the single vaccine.

Vaccines

RV5 vaccine consists of 5 live reassortant rotaviruses, containing G1, G2, G3, G4, and P[8] genes from human strains on a bovine (WC3 strain) background.[7] RV1 vaccine consists of a live, attenuated human G1P[8] rotavirus.[8]

Study Design

After informed consent, infants were randomized in an unblinded manner to 1 of 5 different rotavirus vaccine study groups. The randomization scheme was prepared by statisticians at Emmes. Randomization was stratified by site and used blocks of either 12 or 13 treatments to balance treatments across the enrollment period. The treatment table was generated using R Foundation for Statistical Computing (R 2.10.0, Vienna, Austria).

All vaccines were administered according to existing ACIP guidelines.[18] All formulations of RV5 and RV1 were tracked by lot numbers and expiration date and were distributed from a central monitoring pharmacy. All rotavirus vaccines were administered concurrently with the other routinely administered childhood vaccines per ACIP guidelines, including diphtheria and tetanus toxoids and acellular pertussis, hepatitis B, *Haemophilus influenzae* type b conjugate, pneumococcal conjugate, and inactivated polio. The concurrent vaccinations were administered outside of the study protocol as part of routine patient care.

Assessment of Immunogenicity

Serum for immunogenicity testing was obtained 1 month after the last dose of vaccine. For the study group 1, RV5 only and the mixed rotavirus vaccine study groups 2, 3, and 5, sera were obtained at ~7 months of age. For the reference RV1 group (group 4), sera were obtained at ~5 months of age.

Determination of Antibody Responses

Enzyme-linked immunoassays (ELISA) were used to detect and quantify rotavirus IgA antibody concentrations.[15,19–21] The assay was validated, shown to be specific for the detection of serum IgA, and had acceptable accuracy, precision, and linearity. Each serum specimen was individually assayed using viral lysate of WC3 rotavirus (backbone for RV5) and 89–12 rotavirus (precursor to RV1) so that the ELISA results would not favor either vaccine. The curve was modeled by using a 4-parameter logistic fit regression function. The lower limit of quantification for the assay was set at 7.5 U/mL during validation of the assay. A subject was considered to have a seropositive result if the IgA antibody concentration determined using either virus was ≥20 U/mL.[16]

Neutralizing antibody was determined against several rotavirus strains representing the common G and P types: Wa(G1P[8]), DS-1(G2P[4]), P(G3P[8]), ST3(G4P[6]), VA70(G4P[8]), and CCHMC-G9P6(G9P[6]) by using a method described previously.[17] A subject was considered to have a seropositive result for neutralizing antibody if the titer determined against any virus was ≥10.

Assessment of Safety

Gastrointestinal and systemic symptoms were recorded for 8 days after each rotavirus vaccination by the parents/legal guardians on a provided memory aid. Diarrhea, vomiting, fever, intussusception, hospitalization, and/or any other event considered severe in the opinion of the investigator were recorded. Approximately 1 week after each vaccination, study staff telephoned the parents/legal guardians, reviewed the completed memory aid, and recorded the findings on the case report form. Adverse events were assessed by a licensed clinician and were graded for severity and relationship to study product. Serious adverse events were reported from enrollment through 1 year of age. A Safety and Monitoring Committee was established to monitor the study progress and address any specific vaccine safety concerns.

Ethical Considerations

The institutional review boards at each participating center approved the protocol and informed consent documents.

Statistical Analysis

The primary analyses focused on comparisons within the subset of children receiving the same vaccine type at the first vaccination. The trial was considered to contain 2 independent, concurrent trials: one where children received RV5 for their first dose and the other where children received RV1 for their first dose. The primary outcome measure was to determine noninferiority, based on a binary outcome of attainment of serum anti-rotavirus IgA levels ≥20 U at 1 month after the last dose of vaccine. Noninferiority was determined by comparing the lower bound of the 2-sided 95% confidence interval for the difference in a mixed minus the corresponding single vaccine schedule to the noninferiority margin of –0.10. Comparisons were based on a per-protocol analysis.

The sample size for this study was calculated based on the projected IgA seropositive response rates (defined as a titer ≥20) with 80% power to establish noninferiority for both substudies: 80% probability of showing groups 2 (RV5-RV1-RV1) and 3 (RV5-RV5-RV1) were noninferior to Group 1 (RV5-RV5-RV5) and also 80% probability of showing Group 5 (RV1-RV5-RV5) was noninferior to Group 4 (RV1-RV1). Previous estimates suggested that seroresponse rates would be at least 90% for RV5 and 80% for RV1, requiring larger group sizes for the RV1 as first dose comparison groups.[2] The total sample size was projected to be 1266 subjects, but due to higher than planned loss to follow-up, the study population was increased to 1385.

RESULTS

Study Population

A total of 1407 infants were screened for participation and 1393 were enrolled and randomized to 1 of the 5 study groups between March 2011 and September 2013. A total of 1063 children were randomized to receive 3 doses and 330 were randomized to receive 2 doses. Of these, 1384 (99%) received the first dose, 1309 (94%) received the second dose, and 958 (90%) of 1063 received the third vaccine dose.

Among all randomized children, 1236 (89%) infants completed the study (Fig 1; Supplemental Table 5). The characteristics of the subjects are shown in Table 1 and no differences were noted among the 5 rotavirus vaccine dosing groups with respect to age, gender, and race.

Immunogenicity

The proportion of seropositive children (IgA ≥20 U/mL) against at least 1 vaccine antigen (WC3 or 89–12) 1 month after vaccination was high (77%–96%), and was similar among all the study groups. All the sequential mixed vaccine schedules were shown to be noninferior when compared with the 2 single vaccine reference groups (Table 2; Supplemental Fig 3). Interestingly, when comparing Groups 4 (RV1 only) and 5 (RV1-RV5-RV5), the proportion of infants with rotavirus IgA ≥20 U/mL against both WC3 and 89–12 was significantly greater in the sequential mixed vaccine schedule group than the 2-dose RV1 schedule ($P < .0001$). Geometric Mean Titers (GMTs) measured by serum anti-rotavirus

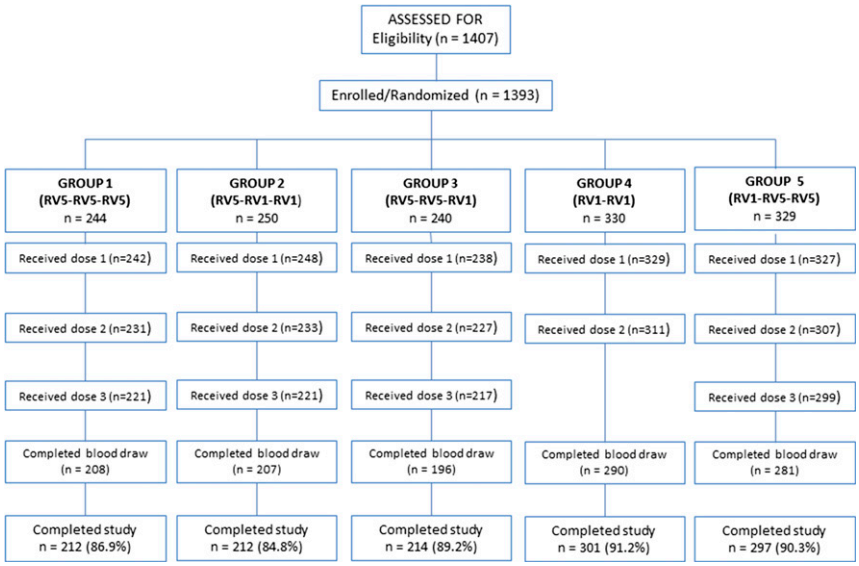

FIGURE 1
Flowchart. Enrollment, randomization, and follow-up of the study participants.

IgA levels against WC3 and 89–12 were higher for sequential mixed vaccine schedule groups 3 and 5 when compared with groups 1 and 4 (Table 2; Fig 2). The Geometric Mean Ratios comparing GMTs between the groups are shown in Supplemental Table 6. The proportion of subjects who were seropositive for serum neutralizing rotavirus antibodies (titer ≥10 post vaccination) against all the evaluated strains was comparable between the groups with sequential mixed schedules shown to be noninferior when comparing with reference single vaccine groups (Table 3). Similar to the IgA results when comparing groups 4 (RV1-RV1) and 5 (RV1-RV5- RV5) where RV1 was given first, a higher proportion

TABLE 1 Demographic Characteristics of the Study Population

	Study Groups					
	Group 1[a]: RV5-RV5-RV5, *n* = 242	Group 2: RV5-RV1-RV1, *n* = 248	Group 3: RV5-RV5-RV1, *n* = 238	Group 4[b]: RV1-RV1, *n* = 329	Group 5: RV1-RV5-RV5, *n* = 327	All Children, *n* = 1384
Gender, *n* (%)						
Boys	118 (48.8)	136 (54.8)	113 (47.5)	153 (46.5)	178 (54.4)	698 (50.4)
Girls	124 (51.2)	112 (45.2)	125 (52.5)	176 (53.5)	149 (45.6)	686 (49.6)
Ethnicity, *n* (%)						
Non-Hispanic	201 (83.1)	199 (80.2)	193 (81.1)	277 (84.2)	260 (79.5)	1130 (81.6)
Hispanic	41 (16.9)	49 (19.8)	45 (18.9)	52 (15.8)	67 (20.5)	254 (18.4)
Race, *n* (%)						
American Indian/ Alaskan	1 (0.4)	0 (0.0)	1 (0.4)	0 (0.0)	1 (0.3)	3 (0.2)
Asian	5 (2.1)	4 (1.6)	4 (1.7)	12 (3.6)	8 (2.4)	33 (2.4)
Black/African American	60 (24.8)	57 (23.0)	52 (21.8)	80 (24.3)	81 (24.8)	330 (23.8)
Multiracial	30 (12.4)	35 (14.1)	37 (15.5)	33 (10.0)	41 (12.5)	176 (12.7)
Hawaiian/Pacific Islander	0 (0.0)	4 (1.6)	1 (0.4)	1 (0.3)	0 (0.0)	6 (0.4)
White	143 (59.1)	143 (57.7)	139 (58.4)	199 (60.5)	195 (59.6)	819 (59.2)
Other/Unknown	3 (1.2)	5 (2.0)	4 (1.7)	4 (1.2)	1 (0.3)	17 (1.2)
Age, wk						
Mean, STD	9.2 (1.4)	9.1 (1.2)	9.2 (1.2)	9.1 (1.2)	9.2 (1.4)	9.2 (1.3)

[a] Reference group for Groups 2 and 3.
[b] Reference group for Group 5.

TABLE 2 Immunogenicity Response (GMTs) Measured by Serum Anti-Rotavirus IgA Levels and Proportion of Seropositive Children at 3 to 6 Weeks After the Last Dose of Vaccine

	ELISA Titers to WC3			ELISA Titers to 89–12						
	n	GMT (95% CI)	Proportion of Subjects Seroresponding[a] (95% CI)	*n*	GMT (95% CI)	Proportion of Subjects Seroresponding[a] (95% CI)	*n*	Proportion Seropositive to Both WC3 and 89–12[b] (95% CI)	*n*	Proportion Seropositive to Either WC3 or 89–12[c] (95% CI)
Group 1[d]: RV5-RV5-RV5	206	294.03 (231.52 to 373.41)	0.90 (0.86 to 0.95)	206	60.89 (49.36 to 75.12)	0.77 (0.71 to 0.83)	206	0.77 (0.71 to 0.83)	206	0.91 (0.87 to 0.95)
Group 2: RV5-RV1-RV1	206	215.81 (168.36 to 276.65)	0.88 (0.84 to 0.93)	207	115.72 (94.54 to 141.64)	0.89 (0.84 to 0.93)	206	0.86 (0.81 to 0.91)	207	0.91 (0.87 to 0.95)
Group 3: RV5-RV5-RV1	194	305.89 (238.61 to 392.13)	0.90 (0.86 to 0.95)	193	104.04 (83.03 to 130.38)	0.85 (0.80 to 0.91)	193	0.85 (0.80 to 0.90)	194	0.91 (0.86 to 0.95)
Group 4[e]: RV1-RV1	287	38.06 (31.72 to 45.69)	0.67 (0.61 to 0.72)	287	100.21 (80.58 to 124.63)	0.76 (0.71 to 0.81)	287	0.66 (0.60 to 0.71)	287	0.77 (0.72 to 0.82)
Group 5: RV1-RV5-RV5	280	256.90 (214.44 to 307.77)	0.93 (0.90 to 0.96)	280	212.52 (173.82 to 259.83)	0.91 (0.88 to 0.95)	280	0.89 (0.85 to 0.92)	280	0.96 (0.94 to 0.99)

CI, confidence interval; GMT, geometric mean titer.

[a] Seropositivity is defined as serum anti-rotavirus IgA ≥20 U/mL.

[b] Post hoc analysis. Subjects with both measurements were included in this endpoint.

[c] Prespecified primary endpoint. Subjects with at least 1 measurement were included in this endpoint.

[d] Reference group for Groups 2 and 3.

[e] Reference group for Group 5.

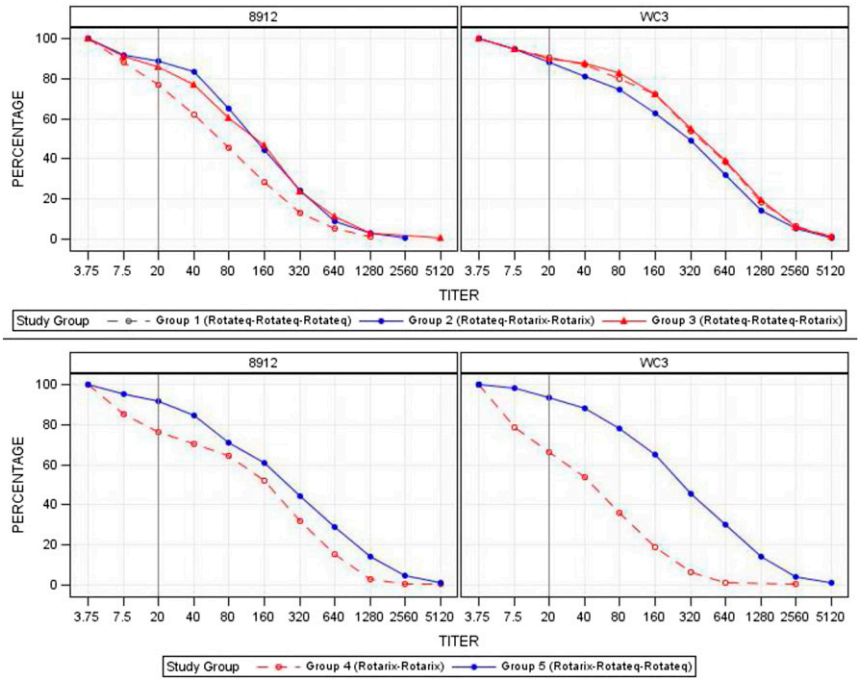

FIGURE 2
Geometric mean titers measured by serum anti-rotavirus IgA levels according the vaccine schedule study groups. Immunogenicity response (GMTs) measured by serum anti-rotavirus IgA levels was comparable for sequential mixed vaccine schedule groups when compared with reference groups against WC3 and 89–12.

of seropositive neutralizing antibody titers was found for the sequential vaccine schedule group, particularly for neutralizing antibodies to DS-1, ST3, VA70, and CCHMC-G9P6 viruses (Table 3)

Safety

Vaccines were well tolerated among all study groups; the proportion of subjects with solicited symptoms is shown in Table 4. No statistically significant differences were found when comparing solicited symptoms (fever, diarrhea, and vomiting) between sequential schedule groups 2 (RV5-RV1-RV1) and 3 (RV5-RV5-RV1) versus the single

TABLE 3 Proportion of Subjects Who Were Seropositive for Serum Neutralizing Rotavirus Antibodies (Titer ≥10 at 1 Month After Vaccination)

Strains of Virus in Neutralization Assays	Wa G1P[8]		DS-1 G2P[4]		P G3P[8]		ST3 G4P[6]		VA70 G4P[8]		CCHMC-G9P6 G9P[6]	
	n/N	Proportion of Seropositive Subjects (95% CI)	*n/N*	Proportion of Seropositive Subjects (95% CI)	*n/N*	Proportion of Seropositive Subjects (95% CI)	*n/N*	Proportion of Seropositive Subjects (95% CI)	*n/N*	Proportion of Seropositive Subjects (95% CI)	*n/N*	Proportion of Seropositive Subjects (95% CI)
Group 1[a]: RV5-RV5-RV5	187/206	0.91 (0.87 to 0.95)	127/206	0.62 (0.55 to 0.69)	108/205	0.53 (0.46 to 0.60)	146/206	0.71 (0.64 to 0.77)	161/206	0.78 (0.72 to 0.84)	110/206	0.53 (0.46 to 0.60)
Group 2: RV5-RV1-RV1	192/207	0.93 (0.89 to 0.97)	112/207	0.54 (0.47 to 0.61)	134/207	0.65 (0.58 to 0.71)	136/207	0.66 (0.59 to 0.72)	152/206	0.74 (0.68 to 0.80)	109/207	0.53 (0.46 to 060)
Group 3: RV5-RV5-RV1	176/194	0.91 (0.86 to 0.95)	116/194	0.60 (0.53 to 0.67)	111/194	0.57 (0.50 to 0.64)	145/194	0.75 (0.68 to 0.81)	150/190	0.79 (0.73 to 0.85)	112/194	0.58 (0.51 to 0.65)
Group 4[b]: RV1-RV1	260/287	0.91 (0.87 to 0.94)	61/287	0.21 (0.16 to 0.26)	179/287	0.62 (0.57 to 0.68)	68/287	0.24 (0.19 to 0.29)	114/287	0.40 (0.34 to 0.46)	71/287	0.25 (0.20 to 0.30)
Group 5: RV1-RV5-RV5	260/280	0.93 (0.90 to 0.96)	165/280	0.59 (0.53 to 0.65)	189/280	0.68 (0.62 to 0.73)	195/280	0.70 (0.64 to 0.75)	216/279	0.77 (0.72 to 0.83)	154/280	0.55 (0.49 to 0.61)

CI, confidence interval.
[a] Reference group for Groups 2 and 3.
[b] Reference group for Group 5.

vaccine reference in group 1 (RV5-RV5-RV5). Interestingly, the overall proportion of subjects with fever, vomiting, and any solicited symptom was significantly higher in group 5 (RV1-RV5-RV5) when compared with group 4 (RV1-RV1) reference group. However, when the associations between group and presence of solicited symptoms were stratified by vaccine dose, there were no statistically significant differences between the 2 groups for the first or second doses of rotavirus vaccine. Irritability was the most frequently reported adverse event among all groups.

During the study period, 70 infants were hospitalized, but only 1 of these hospitalizations was classified to be associated with the study product. That subject was a 2-month-old girl in Group 4 (RV1-RV1) who was hospitalized for 48 hours at 5 days after the first vaccine dose with a diagnosis of gastroenteritis that resolved without any sequelae. The infant was also confirmed to have an *Escherichia coli* urinary tract infection.

In addition, hematochezia was reported in 33 patients and, among those, 14 were attributed to the vaccines: 2 in group 1, 1 in group 2, 2 in group 3, 2 in group 4, and 7 in group 5. All episodes except 1 were mild and resolved without sequelae. One episode of intussusception was reported 91 days after the last vaccination in an infant who belonged to group 3, but it was determined to be unrelated to the vaccine.

DISCUSSION

Since RV5 and RV1 were licensed, millions of doses have been delivered worldwide.[4,22] During routine rotavirus immunization of young children it is likely that mixed schedules of the 2 vaccines are administered to infants. In fact, a recently published study

TABLE 4 Proportion of Subjects With Solicited Symptoms

	N	Fever[a]			Diarrhea[b]			Vomiting[c]			Any Symptom	
	n	Proportion (95% CI)	Difference[d] (95% CI)	*n*	Proportion (95% CI)	Difference[d] (95% CI)	*n*	Proportion (95% CI)	Difference[d] (95% CI)	*n*	Proportion (95% CI)	Difference[d] (95% CI)
Group 1[e]: RV5-RV5-RV5	241	37	0.15 (0.11 to 0.20)	—	22	0.09 (0.05 to 0.13)	—	23	0.10 (0.06 to 0.13)	—	64	0.27 (0.21 to 0.32)	—
Group 2: RV5-RV1-RV1	245	36	0.15 (0.10 to 0.19)	0.01 (−0.06 to 0.07)	27	0.11 (0.07 to 0.15)	−0.02 (−0.08 to 0.04)	22	0.09 (0.05 to 0.13)	0.01 (−0.05 to 0.06)	70	0.29 (0.23 to 0.34)	−0.02 (−0.10 to 0.06)
Group 3: RV5-RV5-RV1	236	31	0.13 (0.09 to 0.18)	0.02 (−0.04 to 0.09)	17	0.07 (0.04 to 0.11)	0.02 (−0.03 to 0.07)	18	0.08 (0.04 to 0.11)	0.02 (−0.04 to 0.07)	56	0.24 (0.18 to 0.29)	0.03 (−0.05 to 0.11)
Group 4[f]: RV1-RV1	326	29	0.09 (0.06 to 0.12)	—	28	0.09 (0.05 to 0.12)	—	17	0.05 (0.03 to 0.08)	—	64	0.20 (0.15 to 0.24)	—
Group 5: RV1-RV5-RV5	325	49	0.15 (0.11 to 0.19)	−0.06[g] (−0.11 to −0.01)	32	0.10 (0.06 to 0.13)	−0.01 (−0.06 to 0.03)	33	0.10 (0.07 to 0.14)	−0.05[g] (−0.09 to −0.01)	94	0.29 (0.24 to 0.34)	−0.09[g] (−0.16 to −0.02)

CI, confidence interval.

[a] Fever: Axillary temperature ≥100.4°F.

[b] Diarrhea: ≥3 looser than normal stools a day.

[c] Vomiting: ≥2 events of vomiting a day.

[d] Estimates of difference in proportions are not provided for Groups 1 and 4 as they are the reference groups.

[e] Reference group for Groups 2 and 3.

[f] Reference group for Group 5.

[g] Statistically different.

evaluating rotavirus vaccine uptake in private US practices showed that 3% received a combination of the 2 vaccines.[23] However, no data exist, to our knowledge, on the impact of sequential schedules on safety or immunogenicity when compared with administration of a single vaccine type in the recommended schedule.

In this study we showed that mixed sequential schedules were both safe and noninferior with respect to immunogenicity measured by serum IgA and neutralizing antibody titers when compared with their corresponding single vaccine standard schedules. These encouraging data are supported by an earlier study involving precursors of both vaccines and natural rotavirus infections. The backbone of the RV5 vaccine, bovine rotavirus strain WC3, was administered as a vaccine to young children during the 1988–1989 rotavirus season and 25 (12.1%) of 206 children had already experienced a natural rotavirus infection.[24] When 8 of these 25 children were administered the WC3 vaccine, 7 seroconverted to the G1P[8] Wa strain (a prototype rotavirus that belongs to the same G and P types as the circulating rotaviruses strains found that season) with 9.7-fold rises in average G1P[8] neutralizing antibody.[25] However, these 7 subjects also had average rises in neutralizing antibodies of between 11.5-fold and 13.7-fold against prototype rotaviruses of other serotypes, including the G2P[4] DS-1 strain, which is completely heterotypic (not cross reactive) relative to both the G1P[8] circulating strains and the G6P[5] WC3 vaccine strain. These results suggested that if RV1 (based on a human G1P[8] strain and therefore similar to natural infection with a G1 rotavirus) and RV5 (a WC3-like vaccine) were administered as RV1 first and RV5 later, the immune responses could contain high-titer,

broadly reactive neutralizing antibodies to both of the common rotavirus serotypes.

Supporting these previous observations, we showed that a higher proportion of seropositive infants was found for the sequential vaccine schedule group that received a first dose of RV1 (group 5), particularly for neutralizing antibodies to DS-1, ST3, VA70, and CCHMC-G9P6 viruses.

The proportion of seropositive children (rotavirus IgA ≥20 U/mL) against at least 1 homologous vaccine antigen (WC3 [RV5] or 89–12 [RV1]) at 1 month after vaccination was high and similar among all study groups. In addition, mixed vaccine schedules were shown to be noninferior when compared with the 2 standard vaccination schedules with RV5 or RV1 alone. Moreover, we observed that the percentage of seropositive infants was significantly higher for the sequential rotavirus schedule (RV1-RV5-RV5) against both WC3 and 89–12 when compared with the scheme that used RV1 vaccine as a first dose (RV1-RV1).

As this study was not aimed to evaluate vaccine efficacy and there is a gap of knowledge regarding a precise correlate of protection for rotavirus vaccine, the clinical relevance of the differences in immunogenicity is unclear. However, our study has clearly shown that there is not an inhibition in immunogenicity with the sequential schedule and supports that vaccines may be given interchangeably.

Interestingly, the overall proportion of subjects with fever, vomiting, and any solicited symptom was significantly higher in group 5 (RV1-RV5-RV5) when compared with the RV1-RV1 reference group. However, when the analysis was stratified by the specific number of doses, there were no statistical differences for these solicited symptoms for the first and second dose, showing that this pattern could be explained by the presence of a third vaccine dose in the group 5 that was absent in the reference group 4.

Our study has several limitations. The study was conducted in an unblinded manner, but it is unlikely that this influenced reporting assessments. The study lost 11% of subjects to follow-up, requiring a decision to expand enrollment during study conduct to meet the projected sample size. Although this study was not aimed to address what would be a protective antibody level and a GMC (Geometric Mean Concentration) >90 has been reported previously as a possible cutoff point for protection,[26] we selected an IgA value of ≥20 U/mL as a measure of seropositivity because this was the value used for earlier RV1 trials.[16] Last, this study was not designed to conduct surveillance for the occurrence of rotavirus gastroenteritis during the study period, and it is possible that some naturally occurring rotavirus infections could have influenced immunogenicity results.

The study also has significant strengths. State-of-the art laboratory techniques were used to evaluate immunogenicity, and the study followed every participant until the age of 1 year for safety outcomes.[15,19–21] The sequential mixed vaccine schedules were shown to be comparably safe because the proportion of solicited, unsolicited, and serious adverse events reported were similar across all 5 of the study groups. No increased risk of intussusception was noted among the sequential rotavirus vaccine groups, but the sample size of the study was inadequate to comprehensively evaluate this adverse event.

We have shown that, should the recommended rotavirus vaccine schedules administration not be possible, sequential mixed rotavirus formulation represents a safe and an immunogenic choice.

CONCLUSIONS

Mixed rotavirus vaccine schedules are safe and noninferior in immune responses when compared with those in which a single formulation of licensed rotavirus vaccines is administered alone.

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ABBREVIATIONS

ACIP: Advisory Committee on Immunization Practices
ELISA: enzyme-linked immunosorbent assay
IgA: immunoglobulin A
RV1: Rotarix
RV5: RotaTeq
VTEU: Vaccine Testing and Evaluation Unit

POTENTIAL CONFLICT OF INTEREST: Dr Libster has served as a consultant to Merck on an unrelated topic. Dr Walter has served as a consultant to Merck, as a DSMB (Data and Safety Monitoring Board) member for Novartis, and has served as an investigator for clinical studies funded by bioCSL, GlaxoSmithKline, Merck, Pfizer, Novartis, and Novavax. Ms McNeal has laboratory service agreements with Merck and GSK. Dr Edwards has served as a DSMB member for Novartis and has research funding from Novartis for unrelated vaccine studies. Dr Berry has served as an investigator for clinical studies funded by GlaxoSmithKline, Novartis, Pfizer, and Sanofi Pasteur. Dr Sarpong has served as a clinical investigator to clinical trials for unrelated trials with GlaxoSmithKline, Merck, and Sanofi Pasteur. Dr Harrison has served as investigator for clinical studies funded by GlaxoSmithKline, Pfizer Inc, and Gilead. Dr Pahud has served on advisory boards for Pfizer and Sanofi and has served as investigator for clinical studies funded by GlaxoSmithKline, Pfizer Inc, and Gilead. Dr Kotloff is an investigator on a study funded by Merck. Dr Turley has served as investigator for unrelated trials with GlaxoSmithKline, Merck, Pfizer, and Sanofi Pasteur, and holds publicly traded Abbott and Pfizer stock. The other authors have indicated they have no potential conflicts of interest to disclose.

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