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Secondary efficacy endpoints of the pentavalent rotavirus vaccine against gastroenteritis in sub-Saharan Africa

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ABSTRACT

The efficacy of the pentavalent rotavirus vaccine (PRV), RotaTeq®, was evaluated in a double-blind, placebo-controlled, multicenter Phase III clinical trial conducted (April 2007–March 2009) in 3 low-income countries in Africa: Ghana, Kenya, and Mali. In total, 5468 infants were randomized 1:1 to receive 3 doses of PRV/placebo at approximately 6, 10, and 14 weeks of age; concomitant administration with routine EPI vaccines, including OPV, was allowed. HIV-infected infants were not excluded. The primary endpoint, vaccine efficacy (VE) against severe-rotavirus gastroenteritis (RVGE), as measured by Vesikari scoring system (VSS, score ≥11), from ≥14 days following Dose 3 through a follow-up period of nearly 2 years in the combined 3 African countries, and secondary endpoints by total follow-up period have been previously reported. In this study, we report post hoc subgroup analyses on secondary endpoints of public health importance. VE against RVGE of any severity was 49.2% (95%CI: 29.9, 63.5) through the first year of life and 30.5% (95%CI: 16.7, 42.2) through the complete follow-up period. VE against severe-gastroenteritis of any etiology was 21.5% (95%CI: <0, 38.4) through the first year of life and 10.6% (95%CI: <0, 24.9) through the complete follow-up period. Through the complete follow-up period, VE against severe-RVGE caused by (i) vaccine-contained G and P types (G1–G4, P1A[8]), (ii) non-vaccine G types (G8, G9, G10), and (iii) non-vaccine P types (P1B[4], P2A[6]) was 34.0% (95%CI:11.2, 51.2), 81.8% (95%CI:16.5, 98.0) and 40.7% (95%CI:8.4, 62.1), respectively. There was a trend towards higher VE with higher disease severity, although in some cases the numbers were small. In African countries with high under-5 mortality rates, PRV significantly reduced RVGE through nearly 2 years of follow-up; more modest reductions were observed against gastroenteritis of any etiology. PRV provides protection against severe-RVGE caused by diverse rotavirus genotypes, including those not contained in the vaccine.

1. Introduction

Annually, rotavirus gastroenteritis (RVGE) kills more than 453,000 children around the world [1,2]. The highest mortality rates are experienced by children less than 1 year of age in developing countries, particularly in Africa and Asia. Since 2006, children born in the United States and many countries in Latin America and Europe have benefited from life-saving rotavirus vaccines but, without demonstrated efficacy in Africa and Asia, the WHO Strategic Advisory Group of Experts (SAGE) on Immunization recommended that clinical trials be conducted in these areas of the world [3] to demonstrate their immunogenicity and efficacy. Over the last several years, these studies have been performed with both Rotarix® and Rotateq®, the two rotavirus vaccines that are currently on the market [4–6].

Both vaccines were found to be efficacious in African populations [4,5] and in June 2009 SAGE issued its recommendation for the global use of rotavirus vaccines in all infants worldwide [7]. The primary endpoint of the efficacy trials of the pentavalent rotavirus

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vaccine (PRV) in Africa and Asia, protection against severe RVGE as defined by a Vesikari severity score (VSS) of ≥11, regardless of serotype, occurring 14 days or more after the third dose of placebo or vaccine until the end of the study follow-up, as well as secondary outcomes, have previously been reported [5,6]. However, additional understanding of the data could inform public health decisions, including analyses of important outcomes by country and by year of life.

In this manuscript, we describe selected ad hoc supplemental analyses from the Phase III efficacy clinical trial of the PRV (RotaTeq®, Merck, Whitehouse Station, NJ, USA), in sub-Saharan Africa and in each country. The following efficacy endpoints are included (i) efficacy against severe RVGE by individual circulating rotavirus serotypes; (ii) efficacy against RVGE of any severity by country and by year; (iii) efficacy against severe gastroenteritis of any etiology by country and by year; and (iv) efficacy against severe RVGE according to different severity definitions.

2. Materials and methods

2.1. Study design

As previously reported [6], this randomized, placebo-controlled trial was conducted from 28 April 2007 to 31 March 2009 in three sites in sub-Saharan Africa. These included a rural site in Kassena Nankana District of Ghana, a rural site in the Karemo Division of Siaya District, Nyanza Province in western Kenya, and urban Bamako, Mali. The study was conducted in accordance with the principles of the Declaration of Helsinki and in compliance with Good Clinical Practice guidelines. After obtaining informed consent, infants were randomized in a 1:1 ratio to receive three oral doses of PRV or placebo, given with other routine pediatric vaccines, including oral poliovirus vaccine (OPV), at approximately 6, 10, and 14 weeks of age. Participants were followed from the moment they were enrolled until the end of the study.

During the surveillance period, participants were visited at least once per month and reminded to seek care at the local health center in the event that gastroenteritis (defined as three or more watery or looser-than-normal stools within a 24-h period and/or forceful vomiting) occurred [6,8]. Upon presentation to a medical facility, stool samples were collected; history of symptoms of the current illness was collected through interview with the parent/guardian; and physical signs were documented by medical staff caring for the subject via direct observation. Diary cards were not used. Each case of gastroenteritis was investigated and different clinical indicators of disease severity were recorded; including temperature, the number and quantity of vomiting and/or diarrhea episodes, hydration status, general activity level, duration of the episode and treatment. These data were used to define severity using the 20-point modified VSS ("mild", "moderate", "severe", very severe", and "extremely severe" were defined as a scores of <7, 7–10, ≥11, ≥15 and ≥19, respectively) [9]; and the 24-point modified Clark Severity Score (CSS) ("mild", "moderate" and "severe" disease as defined as a score of <9, 9–16, and ≥17, respectively) [10].

The surveillance system was observed to need strengthening after the first year of the study in Mali and this was performed by educating and encouraging traditional healers to refer sick children to study health care facilities, and conducting more frequent home visits as described elsewhere in this Supplement [8]. For the evaluation of efficacy, all subjects were followed for severe RVGE from the time they were enrolled until the end of the study.

Enrollment occurred year round and follow-up for the primary timeframe of interest began 14 days after the third dose. Efficacy analyses were also conducted to determine whether PRV confers protection to infants before completion of the 3-dose regimen. These analyses may be of particular interest to health care professionals immunizing infants during, or just prior to, the rotavirus season in countries where there is one. Among infants who ultimately completed the 3-dose vaccination series and were not protocol violators (i.e., the per-protocol population), vaccine efficacy between doses was measured from ≥14 days post dose (PD)1 up to dose 2 and ≥14 days PD2 up to dose 3, consistent with the starting point used to evaluate the per-protocol postdose 3 efficacy of the vaccine.

Efficacy of PRV against severe RVGE by individual rotavirus genotype was evaluated throughout the entire follow-up period, and through the first year and during the second year of follow-up. In addition, efficacy analyses against severe RVGE by vaccine contained G and P types, non-vaccine G types (G8, G9, G10), non-vaccine P types (P1B[4], P2A[6]), and against G8 and G10 genotypes combined were performed for all three follow-up periods described above. Additional analyses performed included: efficacy against severe RVGE by country using different severity scales and/or cut-points, efficacy against RVGE of any severity, efficacy against gastroenteritis of any etiology, and efficacy of PRV against severe RGVE between doses of PRV (before completion of dosing regimen).

A stool sample was collected whenever possible with each diarrhoeal episode. As previously described, stool samples were tested for rotavirus antigen by enzyme immunoassay (EIA) [11], and wild-type rotavirus was confirmed by reverse-transcriptase-polymerase-chain-reaction (RT-PCR) for identification of the VP6 genotype. Identification of rotavirus P and G genotypes was done by RT-PCR [12]. EIA assays were conducted in the laboratory of Dr. Richard Ward at Children's Hospital Medical Center, Cincinnati, OH; RT-PCR assays were conducted at Merck Research Laboratories.

2.2. Statistical analyses

Efficacy was defined as $1 - R_{\text{vaccine}}/R_{\text{placebo}} \times 100\%$, where R represented the incidence for the respective groups. It was assumed that the number of cases (i.e., subjects with the endpoint of interest) in each group followed a Poisson distribution; the statistical analysis then conditioned on the total number of cases from both treatment groups, such that the number of cases in the vaccine group followed a binomial distribution. For analyses of severe endpoints, subjects with multiple episodes, the most severe episode was used for analysis. Exact inference was used, and follow-up time was accounted for in the calculations.

The study was powered to evaluate the efficacy of the vaccine through the entire efficacy follow-up period of nearly 2 years, which was the primary efficacy follow-up period; it was not powered to evaluate efficacy through the first year or within the second year.

3. Results

3.1. Study profile

The design of the clinical trial with PRV conducted in Africa was recently described [6]. Briefly, 5468 study participants were screened and randomized to receive either vaccine ($n = 2733$ participants) or placebo ($n = 2735$) in a 1:1 ratio. The primary per-protocol efficacy analysis included 86% of participants in the vaccine and placebo groups (2357 and 2348 participants, respectively) [6]. The demographic characteristics of the infants and the proportion of children who received oral poliovirus vaccine (OPV) at birth or concomitantly with the rotavirus vaccine were similar across treatment groups but varied across the country study sites. Nearly all the subjects were followed through at least one year of age with the majority being followed through the second year of life.

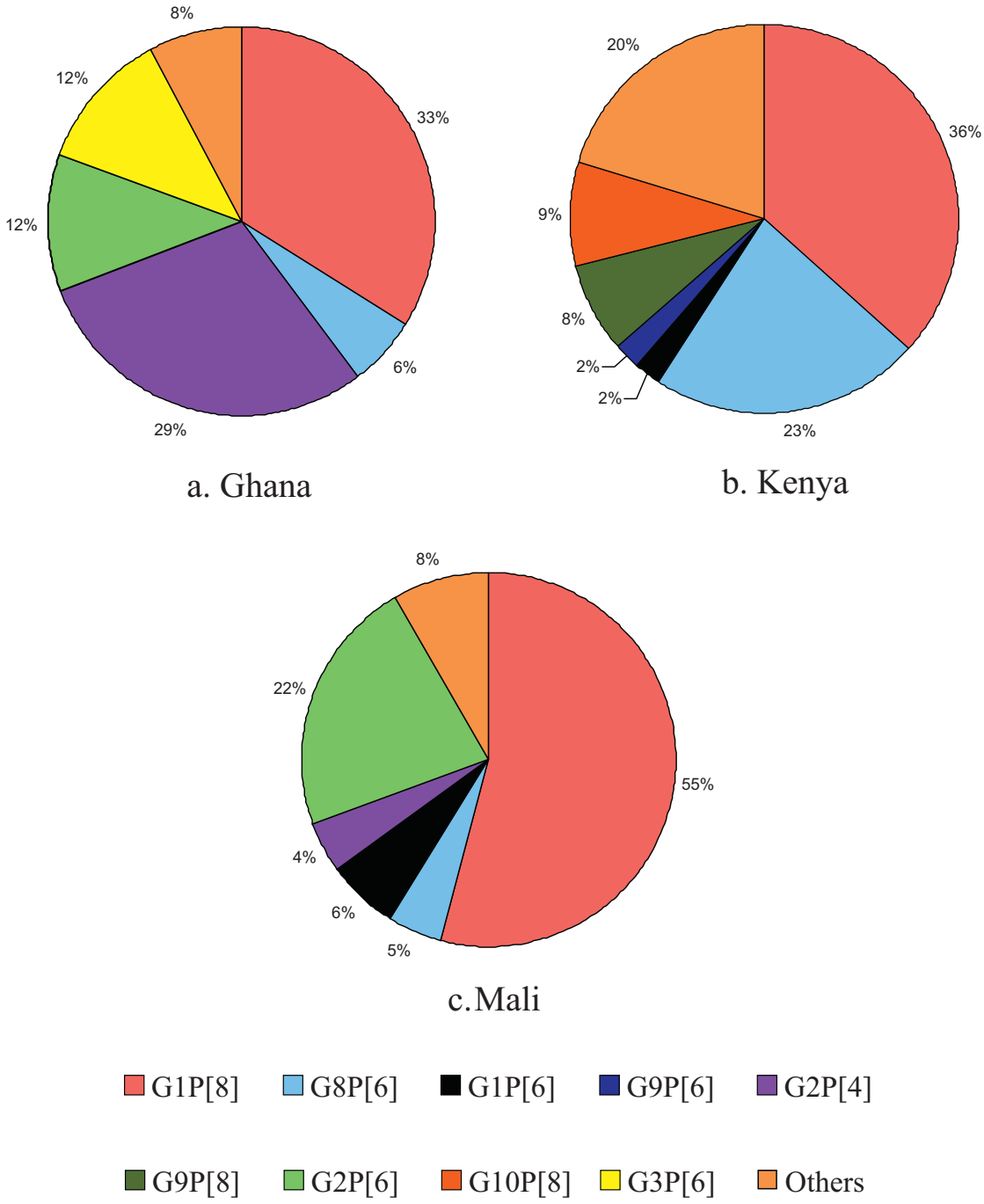

Fig. 1. Percent of rotavirus genotypes identified in stool samples, regardless of gastroenteritis severity, collected during the course of the study in Africa (Ghana, Kenya, and Mali). (a) Ghana, $n = 139$; Ghana: Other Genotype combinations detected: G2P[8], 1.4%; G8P[1], 0.7%; G3P[4], 0.7%; G2P[?], 0.7%; G?P[?] 2.2%; G?P[8], 0.7%; G?P[4], 0.7%; and G?P[6], 0.7%. (b) Kenya, $n = 93$; Kenya: Other Genotype combinations detected: G1P[?], 6.5%; G2P[8], 1.1%; G8P[?], 1.1%; G10P[?], 1.1%; G?P[?], 5.4%; G?P[8], 4.3%; and G?P[6], 1.1%. (c) Mali, $n = 370$. Mali: Other Genotype combinations detected: G1P[4], 0.5%, G1P[?], 1.1%; G2P[8], 0.5%; G2P[5], 0.3%; G2P[?], 0.5%; G9P[8], 1.1%; G?P[?], 2.4%; G?P[8], 0.3%; G?P[4], 0.3%; and G?P[6], 1.4%.

3.2. Rotavirus genotypes

While the study was being conducted in Africa there was a great diversity of rotavirus genotypes circulating in the population (Fig. 1). In Ghana, the most common rotavirus strains belonged to genotypes G1P[8] (33.8%), G2P[4] (29.5%), G2P[6] (11.5%), G3P[6] (11.5%), and G8P[6] (5.8%). Other strains detected in Ghana belonged to genotypes G2P[8] (1.4%), G8P6[1] (0.7%), G3P[4] (0.7%), and either G or P non-typeable genotypes (5%). In Kenya, the most common rotavirus strains belonged to genotypes G1P[8] (36.6%), G1P[6] (2.2%), G8P[6] (22.6%), G9P[8] (7.5%), G9P[6] (2.2%), and G10P[8] (8.6%). Other strains detected in Kenya belonged to genotypes G1P[?] (6.5%), G2P[8] (1.1%), G8P[?] (1.1%), G10P[?] (1.1%), and either G or P non-typeable genotypes (10.8%). In Mali, the most common rotavirus strains belonged to genotypes G1P[8] (54.3%), G1P[6] (6.2%), G2P[4] (4.3%), G2P[6] (22.2%), and G8P[6] (4.6%). Other strains detected in Mali belonged to genotypes G1P[4] (0.5%), G2P[8] (0.5%), G2P[5] (0.3%), G9P[8] (2.4%), and either G or P non-typeable genotypes (6%).

3.3. Efficacy of PRV against severe RVGE (measured by a VSS score ≥11) regardless of serotype

As previously reported, through the entire efficacy follow-up period of nearly 2 years (primary efficacy follow-up period), the vaccine efficacy against severe RVGE, regardless of serotype, in Africa was 39.3% (95% CI: 19.1%, 54.7%). However, through the first year of life, vaccine efficacy against severe RVGE was 64.2% (95% CI: 40.2%, 79.4%); and during the second year of life, vaccine efficacy against severe RVGE, was 19.6% (95% CI: <0.0%, 44.4%). Overall, the vaccine was efficacious in Africa through the entire follow-up period, as well as through the first year of life [6].

3.4. Efficacy of PRV against severe RVGE by individual rotavirus genotype (as measured by a VSS score ≥11)

Among severe RVGE cases with complete molecular testing results, the majority were found to be caused by rotaviruses with G and/or P genotypes covered by PRV (95.1% [78/82] in Ghana, 88.9% [16/18] in Kenya, and 97.1% [99/102] in Mali) [6]. By individual rotavirus genotype, the estimates of efficacy against severe RVGE through the complete follow up period, the first year of life and during the second year of life are shown in Table 1.

Table 2 shows the efficacy of PRV against severe RVGE by genotypes (P and G) contained in the vaccine, G genotypes not contained in the vaccine, P genotypes not contained in the vaccine, and by genotypes G8 and G10 combined. The vaccine provided significant protection against severe RVGE caused by rotavirus genotypes contained in the vaccine as well as rotavirus genotypes not contained in the vaccine (i.e., G8, G10, P[4], and P[6]) through the first year of life and the entire efficacy follow-up period of nearly 2 years. The efficacy of the vaccine in the second year of life was not statistically significant. The efficacy against the rotavirus genotype G8 appeared even higher than the efficacy against individual rotavirus genotypes contained in the vaccine, but the study was not designed to differentiate relative efficacy against individual genotypes.

3.5. Efficacy of PRV against severe gastroenteritis of any etiology (as measured by a VSS score ≥11)

Although not statistically significant, the vaccine also showed efficacy against severe gastroenteritis of any etiology (10.6% [95% CI: <0, 24.9] and 21.5% [95% CI: <0, 38.4] through the entire follow-up period and the first year of life, respectively) (Table 3). Although a drop in efficacy was expected in the second year of life, the study was not powered to evaluate the efficacy of the vaccine in the second year alone.

3.6. Efficacy of PRV against severe RVGE between doses (as measured by a VSS score ≥11)

There were few RVGE cases that occurred before the 3-dose regimen was fully administered, and the evaluation of efficacy between doses did not yield statistically significant results. There were 4 cases of severe RVGE in the vaccine group and 0 in the placebo group between doses 1 and 2, and there were 2 cases of severe RVGE in the vaccine group and 1 in the placebo group between doses 2 and 3.

3.7. Efficacy of PRV against RVGE of any severity and different severities (as measured by a VSS and CSS)

Table 4 shows the efficacy of PRV against RVGE of any severity. Overall, an efficacy of 49.2% (95%CI: 29.9, 63.5) and 30.5% (95%CI: 16.7, 42.2) was observed in the first year of life and throughout the entire follow-up period, respectively. Table 5 shows the efficacy of PRV against RVGE of different severities through the first year of life, during the second year of life, and through the entire follow-up period in Africa. There was a slight trend towards higher efficacy between severe and very severe RVGE. Among those with extremely severe RVGE (>19 by the VSS), there were only 6 versus 3 cases creating a point estimate with very wide confidence intervals.

A full comparison of the two clinical scoring systems – Vesikari and Clark – are described in detail in another manuscript in this supplement [13].

4. Discussion

Rotavirus vaccines are efficacious in Africa and, with the recent announcement of financial support for the GAVI Alliance for new vaccines, several countries in the region are planning ahead to introduce these vaccines into their routine immunization programs in the near future. Although higher efficacy was observed against severe RVGE cases and especially those that occur in the first year of life, efficacy against any severity of RVGE into the second year of life was also observed. The decrease of vaccine efficacy in the second year of life did not result in a decrease of public health benefit, as the number of severe gastroenteritis cases prevented through the first year of life and during the second year of life are additive, resulting in additional benefit over the entire follow-up period (data not shown).

This observation is important from a public health perspective, as study subjects experienced severe RVGE in the second year of life and prevention of these cases in an African setting would be greatly beneficial. Even though morbidity from RVGE decreased during the second year of life compared to the first year, childhood illness at any age places a tremendous toll on the economic resources of a family, and places an undue burden on the family. In many instances, a parent or family member would give up their usual employment to care for a sick child or use their very limited resources to seek care and provide medications for the ill child [14,15]. The modest reductions of severe gastroenteritis of any etiology observed during this trial are also important; these were higher in the first year of life and may have an impact on the long-term nutritional status of these children. Repeated episodes of gastroenteritis put children at risk for malnutrition which has long-term implications [16]. This vaccine has the potential to curb some of those cases and spare some of the long term effects, as well as the economic burden alluded to earlier.

The lower efficacy of the vaccine in the second year of life is likely due to a number of factors, including the lower incidence of severe rotavirus gastroenteritis noted in the initial studies [5,6]. However, there appears to be a waning of immunity in developing country populations as reported from rotavirus vaccine demonstration projects in El Salvador and Nicaragua [17,18], in comparison to the long-term protection seen in the United States [19]. Additional studies are underway to elucidate how to improve the performance of live oral attenuated vaccines with respect to this, including studies evaluating additional doses, micronutrient supplementation and a booster dose of rotavirus vaccine.

Our data demonstrate that PRV is efficacious against severe RVGE caused by a broad range of rotavirus P and G genotypes through the first year of life and the entire follow-up period of nearly 2 years. The efficacy of PRV was demonstrated against individual rotavirus genotypes contained in the vaccine and in non-vaccine type strains, although in some cases the efficacy was not statistically significant (the study was not designed to differentiate relative efficacy against individual genotypes). The P and G genotypes of the majority of the rotavirus strains identified in the stool samples from study participants were contained in PRV, and the

Table 1
Type-specific efficacy of PRV against severe RVGE (as measured by a score ≥11 according to the Vesikari Clinical Scoring System).

Rotavirus genotype	First year of life				Second year of life				Total follow-up period[b]			
	Total cases		Efficacy		Total cases		Efficacy		Total cases		Efficacy	
	Vaccine	Placebo	%	95% CI	Vaccine	Placebo	%	CI	Vaccine	Placebo	%	CI
G1[a]	10	26	61.8	18.1, 83.6	31	36	14.8	<0.0, 49.0	42	62	32.3	<0.0, 55.4
G2[a]	9	19	52.9	<0.0, 81.2	23	24	5.2	<0.0, 48.9	32	44	27.1	<0.0, 55.2
G3[a]	0	3	100.0	<0.0, 100.0	3	5	40.5	<0.0, 90.8	3	8	62.3	<0.0, 93.6
G8	1	7	85.8	<0.0, 99.7	0	1	100.0	<0.0, 100.0	1	8	87.5	6.5, 99.7
G9	1	1	0.3	<0.0, 98.7	0	1	100.0	<0.0, 100.0	1	2	49.7	<0.0, 99.1
P[8][a]	11	28	60.9	19.1, 82.5	29	36	20.3	<0.0, 52.9	41	64	36.1	4.0, 57.9
P[4]	7	13	46.3	<0.0, 81.9	6	3	<0.0	<0.0, 57.6	13	16	18.2	<0.0, 63.8
P[6]	4	16	75.2	23.0, 94.0	18	26	31.5	<0.0, 64.6	22	42	47.7	10.4, 70.3

Note 1: Individual genotype analyses are not mutually exclusive because RVGE cases could be of more than one genotype.
Note 2: There was also one G10 severe RVGE case (in the placebo group), for which serotype-specific efficacy was not calculated. The G10 case occurred during the first year of life.

[a] Rotavirus genotypes included in the pentavalent rotavirus vaccine, PRV. No rotavirus strains belonging to genotype G4 (a type contained in the vaccine) were detected in the study.

[b] The data for the total follow-up period type-specific efficacy were previously published [6].

Table 2
Efficacy of PRV against severe RVGE (as measured by a score ≥11 according to the Vesikari Clinical Scoring System) according to vaccine types, non-vaccine G types, non-vaccine P types and types G8 and G10.

	First year of life				Second year of life				Total follow-up period			
	Total cases		Efficacy		Total cases		Efficacy		Total cases		Efficacy	
	Vaccine	Placebo	%	95% CI	Vaccine	Placebo	%	CI	Vaccine	Placebo	%	CI
Vaccine types[a]	19	48	60.8	32.0, 78.2	57	67	16.1	<0.0, 42.1	77	116	34.0	11.2, 51.2
Non-Vaccine G types[b]	2	9	77.9	<0.0, 97.7	0	2	100.0	<0.0, 100.0	2	11	81.8	16.5, 98.0
Non-Vaccine P types[c]	11	30	63.6	25.3, 83.5	24	29	18.1	<0.0, 54.4	35	59	40.7	8.4, 62.1
G8 and G10[d]	1	8	87.5	7.3, 99.7	0	1	100.0	<0.0, 100.0	1	9	88.9	19.6, 99.7

Note: Individual genotype analyses are not mutually exclusive because RVGE cases can be of more than one genotype. No rotavirus strains belonging to genotype G4 (a type contained in the vaccine) were detected in the study.

[a] G1, G2, G3, P[8].

[b] G8, G9, G10.

[c] P[4] and P[6].

[d] Includes only one (1) G10 case (in placebo group), which occurred in the first year of life.

vaccine was demonstrated to be efficacious against severe RVGE caused by the composite human rotavirus G and P genotypes contained in the vaccine (G1-G4, P[8]). In addition, PRV was efficacious through the entire efficacy follow-up against severe RVGE caused by heterologous rotavirus G and P types not contained in the vaccine.

This is an important finding because there is a broad range of G and P rotavirus genotypes encountered in Africa, including strains belonging to genotypes G8, G9 and G10 [20–23], and this aspect of rotavirus epidemiology has been considered a challenge for vaccine performance [24]. In our study, there were few cases caused by G10 rotavirus strains, but there were sufficient cases to demonstrate efficacy against severe RVGE caused by G8 rotavirus strains throughout the entire follow up period. In fact, efficacy against severe RVGE caused by G8 rotavirus strains was numerically higher (87.5%) than the efficacy against severe RVGE caused by rotavirus strains whose genotypes are covered by PRV. The reason for this finding requires further study but these data demonstrate heterotypic protection against RVGE caused by G8 rotavirus strains, which were associated with genotype P[6], also not contained in the vaccine.

Although complete molecular characterization of some of the rotavirus strains recovered in this clinical trial is underway, it is possible that the G8P[6] strains circulating in humans in Africa

Table 3
Efficacy of PRV against severe gastroenteritis (as measured by a score ≥11 according to the Vesikari Clinical Scoring System) of any etiology in all countries.

	First year of life				Second year of life				Total follow-up period			
	Total cases		Efficacy		Total cases		Efficacy		Total cases		Efficacy	
	Vaccine	Placebo	%	95% CI	Vaccine	Placebo	%	CI	Vaccine	Placebo	%	CI
Africa[a]	125	156	21.5	<0.0, 38.4	73	75	3.6	<0.0, 31.1	252	278	10.6	<0.0, 24.9
Ghana[b]	49	78	40.0	13.1, 58.9	22	18	−14.3	<0.0, 41.5	80	101	25.3	<0.0, 45.0
Kenya[c]	21	22	3.4	<0.0, 49.5	3	4	21.4	<0.0, 88.5	25	29	10.6	<0.0, 49.8
Mali[d]	55	56	3.4	<0.0, 34.6	48	53	7.4	<0.0, 38.7	147	148	0.9	<0.0, 21.6

[a] *N* in the vaccine group and placebo group were 2057 and 2028 evaluable subjects in the first year of life, 1261and 1245 in the second year of life and 2016 and 1992 for the entire follow-up period.

[b] *N* in the vaccine group and placebo group were 753 and 737 evaluable subjects in the first year of life, 486 and 453 in the second year of life and 747 and 725 for the entire follow-up period.

[c] *N* in the vaccine group and placebo group were 481 and 477 evaluable subjects in the first year of life, 331 and 341in the second year of life and 472 and 472 for the entire follow-up period.

[d] *N* in the vaccine group and placebo group were 823 and 814 evaluable subjects in the first year of life, 444 and 451 in the second year of life and 797 and 795 for the entire follow-up period.

Table 4
Efficacy of PRV against RVGE of any severity regardless of serotype in all countries.

	First year of life				Second year of life				Total follow-up period			
	Total cases		Efficacy		Total cases		Efficacy		Total cases		Efficacy	
	Vaccine	Placebo	%	95% CI	Vaccine	Placebo	%	CI	Vaccine	Placebo	%	CI
Africa[a]	59	115	49.2	29.9, 63.5	147	179	19.0	<0.0, 35.4	206	294	30.5	16.7, 42.2
Ghana[b]	31	70	56.4	32.5, 72.4	15	18	19.6	<0.0, 62.3	46	88	48.8	26.0, 65.0
Kenya[c]	6	21	71.5	26.9, 90.6	3	3	<0.0	<0.0, 86.2	9	24	62.0	15.3, 84.4
Mali[d]	22	24	9.3	<0.0, 51.5	129	158	19.2	<0.0, 36.4	151	182	17.6	<0.0, 34.1

[a] *N* in the vaccine group and placebo group were 2401 and 2405 evaluable subjects in the first year of life, 2080 and 2052 in the second year of life and 2383 and 2392 for the entire follow-up period.
[b] *N* in the vaccine group and placebo group were 981 and 989 evaluable subjects in the first year of life, 828 and 803 in the second year of life and 982 and 989 for the entire follow-up period.
[c] *N* in the vaccine group and placebo group were 575 and 573 evaluable subjects in the first year of life, 414 and 413 in the second year of life and 569 and 568 for the entire follow-up period.
[d] *N* in the vaccine group and placebo group were 845 and 843 evaluable subjects in the first year of life, 838 and 836 in the second year of life and 832 and 835 for the entire follow-up period.

Table 5
Efficacy of PRV against severe RVGE using different severity score cut-points, as measured with the VSS and CSS scoring systems in Africa.

	First year of life				Second year of life				Total follow-up period			
	Total cases		Efficacy		Total cases		Efficacy		Total cases		Efficacy	
	Vaccine	Placebo	%	95% CI	Vaccine	Placebo	%	CI	Vaccine	Placebo	%	CI
Severe (VSS, ≥11)	21	58	64.2	40.2, 79.4	57	70	19.6	<0.0, 44.4	79	129	39.3	19.1, 54.7
Very severe (VSS, ≥15)	7	26	73.4	37.0, 90.2	ND	ND	ND	ND	28	47	40.7	3.3, 64.2
Severe (CSS, ≥17)	4	10	60.1	<0.0, 90.9	ND	ND	ND	ND	12	18	33.0	<0.0, 70.6

ND: Not done.

may represent recent zoonotic events and these human G8 viruses may have originated from ruminants, as recently described [25,26]. Therefore, these "heterotypic" strains may share a genomic constellation similar to the bovine backbone of PRV [27], which may explain why the protection against these strains was high. However, experience has shown that heterotypic protection may not always be consistent [28]; therefore, it is important to monitor the effectiveness of PRV, once implemented, because these strains have not been common but with the pressure of vaccine introduction, their relative frequency could change and impact the overall performance of the vaccines.

Although the data collected during this trial did not permit us to precisely assess the efficacy of 1 and 2 doses of pentavalent rotavirus vaccine, this information is likely to be of great importance in the African setting. For instance, although routine immunization programs call for the immunization of infants at 6, 10 and 14 weeks of age, many infants in the African region complete these vaccine schedule when older [29]. This is further complicated by the fact that, due to concerns of intussusception, infants older than 32 weeks of age should not receive further doses of rotavirus vaccines as advised by WHO [3]. Therefore, infants will likely experience longer periods of time between doses or will only be eligible to receive 1 or 2 doses of vaccine and will be at risk for rotavirus for longer periods of time than was encountered by participants in this trial. This aspect is likely to challenge the performance of PRV and is best explored in observational studies after vaccine introduction which are likely to provide critical information regarding the potential public health impact of this vaccine. Effectiveness trials in other countries have demonstrated decreased performance than that observed in well controlled efficacy trials and this "real world" application of rotavirus vaccines is likely to be a critical piece of information as decision makers in Africa move forward [30,31]. Our data demonstrate that rotavirus continues to be a public health problem in the second year of life and the performance of 1 or 2 doses of vaccine in that setting is also likely to yield important results.

The major limitation of this post hoc analysis is that the study was not powered for these supplemental analyses, including by country or by year of life. Nevertheless, the potential benefits of introducing rotavirus vaccines in Africa are substantial and far-reaching. In the continent where the highest rates of rotavirus mortality per capita are found, the introduction of these vaccines into the routine childhood immunization schedule would have a profound public health impact. African countries have responded to their need for these vaccines and almost 20 countries in the region have applied for GAVI support to subsidize vaccine procurement. Now, we should look towards studying the effectiveness of this vaccine when it is introduced into routine EPI immunization schedules, and assess how to improve its performance in the field.

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Conflict of Interest Statement: SOS received Merck funding as a member of the Advisory Board for Pediatric Vaccines and Vaccine New Products; MC was an employee of Merck when the clinical trial was conducted and owned equity in the company. MML is a paid advisory board member for NIH Vaccine Center, Center for Clinical Vaccinology and Tropical Medicine at Oxford University, AlphaVax, International Vaccine Institute, Centre de Recerca en Salut Internacional de Barcelona, AfriChol, and the Pasteur Institute STOPENTERICS program, and has received consultancies from Novartis and Merck. No other conflicts of interest are declared.

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