

**Efficacy and immunogenicity of two or three dose rotavirus-vaccine regimen
in South African children over two consecutive rotavirus-seasons: a
randomized, double-blind, placebo-controlled trial.**

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SAM, KMN and ADS were involved in the study conduct; reviewed all relevant literature; were involved in developing study methods; contributed to data analysis and prepared the first draft.

MK, CL, PB, SA played key roles in study conduct; critiqued the study methods and assisted in editing the manuscript; provided several additional critical reviews of the draft manuscript at various stages. AB was involved in study design, development of study organization and methods and part of the study conduct as employee of GSK.

Conflicts of Interest

SAM has received research grants and honoraria from GSK and MERCK.

The primary analysis as per analysis-plan was undertaken by GSK, with additional analysis undertaken by SAM.

Disclaimer

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Disclosure

All authors have approved the final article.

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Abbreviated Title

Rotavirus vaccine in South Africa

Abstract

Background: Human rotavirus vaccine (HRV; i.e., *Rotarix*) reduced the incidence of severe rotavirus gastroenteritis (RVGE) by 77% (95% Confidence interval: 56% to 88%) during the first year of life in South Africa. Persistence of HRV-derived protection against RVGE during subsequent rotavirus seasons, although evident in industrialized settings, remains to be established in African settings. This study reports on the efficacy of HRV against severe RVGE over two consecutive rotavirus seasons in South African children.

Methods: A prospective, double-blind, placebo controlled multi-centered trial in South Africa and Malawi randomly assigned infants in a 1:1:1 ratio to receive either two (10 and 14 weeks; HRV_2D) or three (6, 10 and 14 weeks; HRV_3D) doses of HRV or placebo. The primary analysis involved pooling of HRV_2D and HRV_3D arms. Episodes of gastroenteritis caused by wild-type rotavirus were identified through active follow-up surveillance and graded by the Vesikari scale.

Results: 1,339 infants (447 in the HRV_2D group, 447 in the HRV_3D group and 445 in the placebo group) were enrolled in Year 2 of the study, including 1,035 (77.3%) who were followed up over two consecutive rotavirus seasons (i.e., Cohort 2 subjects). *Rotarix* was associated with ongoing protection against severe RVGE, preventing 2.5 episodes per 100 vaccinated children over two consecutive rotavirus seasons; vaccine efficacy: 59% (95% Confidence interval: 1 to 83). An exploratory analysis indicated better immunogenicity (among Cohort 1 subjects) and a higher point-efficacy estimate over two seasons in the HRV_3D compared to HRV_2D arms of the study in Cohort 2 subjects.

Conclusion: *Rotarix* is associated with significant reductions in severe gastroenteritis episodes through 2 years of life among South African children. Further research is needed to determine the optimal dosing schedule of *Rotarix* in providing long-term protection against rotavirus illness in African children.

Introduction

Rotavirus is a leading cause of under-5 childhood mortality, with an estimated 220,000 (40%) of 527,000 annual deaths attributed to this virus occurring in sub-Saharan Africa.(1) In 2009, the World Health Organization (WHO) recommended that infant immunization with human rotavirus vaccine (HRV) should be introduced in all countries and particularly where greater than 10% of under-5 mortality is attributed to diarrhea.(2) This revised recommendation was supported in part by clinical trials from Africa in which the efficacy of HRV during infancy was established.(3;4)

Although the efficacy of the rotavirus vaccines against severe rotavirus diarrhea in the first year of life, was lower in African studies (61-65%),(3;4) compared to those from more industrialized settings (84-100%),(5-8) the burden of disease prevented in African studies (5.0 per 100 infant-years) exceeded that prevented in studies from Europe,(6) Latin America,(9) and middle-income countries in Asia.(10) Multi-country efficacy studies of *Rotarix*TM (GlaxoSmithKline [GSK] Biologicals) and *RotaTeq*TM (Merck & Co., Inc.), in Africa, however, have also demonstrated between-country differences in vaccine efficacy against severe rotavirus gastroenteritis (S-RVGE).(3;4) While the efficacy of *Rotarix* against S-RVGE was greater in South African

(76.9%) compared to Malawian (49.4%) infants, the attributable reduction of S-RVGE was two-fold greater among Malawian infants.(3) Furthermore, persistence of HRV protection against S-RVGE during the second year of life and/or two consecutive rotavirus seasons has predominantly been established in industrialized settings,(7-10) whereas the sustainability of protection against S-RVGE remains to be established in African settings. Post-introduction effectiveness studies in some Latin American countries have indicated that there is a decrease in protection during the second year of life with *Rotarix* and *RotaTeq*.(11;12) In addition, vaccine efficacy point-estimates against S-RVGE were lower in the second year of life (19.6%) compared to that in the first year of life (64%) with *Rotateq* in Africa.(4)

Based on the differences in rotavirus vaccine-efficacy and epidemiology of infection between South African and Malawian infants during infancy in the Phase 3 *Rotarix* trial,(3) we now report on country-specific data on the extended efficacy evaluation and immunogenicity of HRV. The analyses are specific to secondary study objectives, including: i.) Assessment of the efficacy of HRV in the combined HRV 2-dose (HRV_2D) and 3-dose (HRV_3D) groups (pooled groups) to prevent S-RVGE and all-severity gastroenteritis caused by the circulating wild-type rotavirus strains over two consecutive rotavirus seasons; ii.) To assess the efficacy of HRV in preventing all-cause severe gastroenteritis over two consecutive rotavirus seasons; iii.) To determine the immunogenicity of a HRV_2D and HRV_3D schedule of rotavirus vaccine.

Materials and Methods

Study design and participants

A Phase 3, double-blind, randomized placebo-controlled multicenter study was undertaken in South Africa and Malawi as reported.⁽³⁾ Briefly, the study included two cohorts in South Africa who were consecutively enrolled from October 2005 through January 2006 (Cohort 1: 1,828 subjects) and November 2006 to February 2007 (Cohort 2: 1,339 subjects). The interruption of enrollment between Cohort 1 and Cohort 2 subjects in South Africa was based on targeting completion of study-vaccine vaccination before the anticipated start of the rotavirus season in South Africa, which generally occurs between March and June.⁽¹³⁾ Children were randomly assigned individually in a 1:1:1 ratio to receive at 6, 10, and 14 weeks either a dose of placebo followed by two doses of HRV (HRV_2D); three doses of HRV (HRV_3D); or three doses of placebo.

Vaccine used in the study was the same as the commercial formulation of *Rotarix* and the placebo the same as vaccine-formulation without the viral antigen.⁽¹⁴⁾ The study was conducted in a double-blind manner with respect to vaccine or placebo and HRV dosing schedule. The parents/guardians of the subjects, the study personnel, and the investigator were unaware of the administered treatment. Blinding was maintained for the whole study period.

Study exclusion criteria included use of any investigational or non-registered product (drug or vaccine) other than the study vaccine(s) during the study period, chronic systemic administration (defined as more than 14 days) of immunosuppressant during the study period, administration of a vaccine not foreseen by the study protocol during the period starting from 14 days before each

dose of study vaccine(s) and ending 14 days after, or administration of immunoglobulins and/or any blood products during the study period.

A detailed description of testing of infants for HIV, active weekly surveillance for gastroenteritis episodes, analysis of stool samples, and safety assessment has been described.(3) Briefly, the parents or legal guardians of children were trained to complete diary cards documenting any episode of gastroenteritis and the clinical course, which were retrieved by trained surveillance officers during weekly home-visits. Stool samples were also collected from the date of first study-vaccine dose, with each episode of gastroenteritis defined as the passage of 3 or more stools that were looser than normal within a 24-hour period. Stool samples were to be collected as soon as possible after the symptoms began and preferably not later than 7 days after the start of diarrhea. Stool samples were tested for rotavirus by enzyme-linked immunoglobulin assay (ELISA; Rotaclone, Meridian Bioscience).

Rotavirus-positive samples were tested at DDL Diagnostic Laboratory (Voorburg, the Netherlands) by reverse transcriptase polymerase chain reaction (RT-PCR), followed by reverse hybridization assay and/or sequencing in order to determine the rotavirus G and P genotypes and to differentiate presence of wild-type G1 rotavirus from the vaccine-strain virus.(15)

**Assessment of vaccine efficacy and type-specific protection over two rotavirus seasons
(Cohort 2 subjects only)**

Vaccine efficacy in the first year of life has been reported for both cohorts in the initial analysis,(3) however, Cohort 1 subjects were not included in the second-year efficacy follow-up period as they had terminated study participation before the protocol was amended to evaluate

the efficacy of HRV over 2 consecutive rotavirus seasons. This report consequently focuses on vaccine efficacy over two consecutive rotavirus seasons in Cohort 2 of the study, which involved follow-up until the end of the 2007 rotavirus season. The severity of all gastroenteritis episodes was evaluated with the use of the 20-point Vesikari scale, on which a score of 11 or more indicates severe gastroenteritis.(16) Vaccine efficacy was also measured for rotavirus-confirmed gastroenteritis of any severity, all-cause gastroenteritis, and all-cause severe gastroenteritis.

Assessment of immunogenicity (Cohort 1 subjects only)

Blood samples were collected from approximately 10% of infants in Cohort 1 prior to the first dose of study drug and one month after the last dose of study drug had been administered, to determine serum concentrations of anti-rotavirus immunoglobulin A (IgA) antibody. We have previously reported on the IgA seropositivity rates for the pooled analysis of either 2 or 3 doses of HRV,(3) however, we now extend this analysis to report on the immunogenicity of the HRV_2D and HRV_3D arms of the study. Serum from blood samples were stored at -70 degrees Celsius until being analyzed by ELISA at GlaxoSmithKline Biologicals, with the assay cutoff point set at 20U per milliliter.(17;18)

Statistical considerations

A randomization list was generated at GSK Biologicals, Rixensart, using a standard SAS® program (SAS Institute, Cary, North Carolina, USA). A randomization blocking scheme (1:1:1 ratio) was used to ensure that balance between treatments was maintained throughout the study. The vaccine doses were distributed to each study center while respecting the randomization block size.

The targeted sample size of 4,950 participants between the South African and Malawi sites was based on evaluating the primary objective of determining if HRV (pooled HRV_2D and HRV_3D groups) given concomitantly with routine childhood vaccines could prevent S-RVGE (≥ 11 on the 20-point Vesikari scoring system)(16) caused by the circulating wild-type RV strains during the period from 2 weeks after the last dose of HRV vaccine or placebo until 1 year of age (after the first rotavirus season). Estimates used in the sample size calculation included an assumption of an underlying incidence of S-RVGE of 2%, which required 3,960 evaluable infants to provide 84% power to detect a statistically significant difference in rates of S-RVGE between the *Rotarix*-pooled group and the placebo group if vaccine efficacy was 60% and upward. The sample size included adjustment to allow for 20% of infants not being evaluable for the primary analysis. The higher than anticipated attack rates of S-RVGE during infancy alone, 3.3% in South Africa and 7.9% in Malawi, favored post-hoc country-specific estimates of vaccine efficacy, despite not being planned *a priori* in the sample size calculations.

Vaccine efficacy analysis was performed on the according-to-protocol (ATP) efficacy cohort, which included the first episode of any specified event occurring at least 2 weeks after the third dose of assigned study vaccine. For a specific event, vaccine efficacy for each HRV group and for pooled HRV groups was primary computed as $VE = \text{vaccine efficacy} = (1 - RR) * 100 = (1 - (ARV/ARU)) * 100$ Where: ARU = number of subjects reporting at least one event / total number of subjects in the placebo group; ARV = number of subjects reporting at least one event / total number of subjects in the HRV vaccine group; Relative risk (RR) = ARV/ARU. The same transformation was used to derive the exact confidence interval (CI) boundaries from those

obtained for the relative risk. The CI for the relative risk was based on the method described by Tang et al.(19) This primary analysis was complemented by: (i) Two-sided Fisher's exact test, (ii) Vaccine efficacy derived from a Cox regression model on the time to first event with censoring at end of study for subjects without event (the model included the group as fixed effect, (iii) Incidence rate in a group (P) was computed as the number of subjects reporting at least 1 event (n)/total follow-up time to a first event or censored at end of study visit (T). The associated 95% CI was obtained considering that n followed a Poisson distribution with $P \cdot T$ parameter. The number of events prevented by 100 vaccinated infant-years was obtained from 100 times the difference in incidence rate. This associated CI was derived using the method by G. Y. Zou and A. Donner.(20)

For the immunogenicity analysis seropositivity/seroconversion rates and their exact 95% CI were tabulated and the geometric mean concentrations (GMCs) and their 95% CI were calculated. The 95% CI for the mean of log-transformed concentration was first obtained assuming that log-transformed concentrations were normally distributed with unknown variance. The 95% CI for the GMC was then obtained by exponential transformation of the 95% CI for the mean of log-transformed concentration. The analysis included the *a priori* comparison of the pooled HRV groups versus placebo group. In addition, an exploratory analysis was performed for following groups: each HRV group versus placebo group and the HRV_2D group versus HRV_3D group.

Ethical considerations:

The study protocol, including all amendments, informed consent forms, and other information that required pre-approval were reviewed and approved by the South African Medicine Control Council, three investigational centers' independent ethics committees or institutional review boards in South Africa, and the World Health Organization (WHO) ethics committee. This study was conducted in accordance with Good Clinical Practice guidelines and all applicable regulatory requirements, including, where applicable, the Declaration of Helsinki. Written informed consent was obtained from each parent /guardian prior to the performance of any study-specific procedures.

Results

A total of 1,340 children were enrolled in Cohort 2 (447 subjects in the HRV_2D group, 447 subjects in the HRV_3D group and 445 subjects in the placebo group; Figure 1). One child did not receive any study vaccine dose post-randomization and was excluded from all subsequent analyses. Eighty-eight (6.6%) children from Cohort 2 were excluded from the ATP analysis for measuring vaccine efficacy for reasons indicated in Figure 1; and a further 227 (17.0%) children did not enter into the second-season surveillance period. The mean age of vaccination for the three study-vaccine doses were at 6.2, 11.0, and 15.9 weeks in Cohort 2 subjects, and the mean age at end of follow-up was 13.8 months, which did not differ by group.

Concomitant oral polio vaccine was administered in greater than 99% of subjects at each of the study-vaccine doses (Table 1). No differences were observed in the characteristics described in Table 1 between the HRV_2D and HRV_3D groups (data not shown). Overall, HIV-PCR testing

was undertaken with parental consent in 725 (54.1%) Cohort 2 children, of whom 45 (6.2%) were determined to be HIV-infected (Table 1).

Vaccine efficacy over two consecutive rotavirus seasons in Cohort 2.

The attack rate of S-RVGE was 3.2% (95% CI: 1.7 to 5.4) over 2 consecutive rotavirus seasons in placebo recipients, with a 59% ($p = 0.047$) reduction observed among the pooled-HRV group. HRV efficacy in prevention of S-RVGE was 32% ($p = 0.487$) in the HRV_2D as compared to placebo and 85% ($p = 0.006$) in the HRV_3D group as compared to placebo. The relative efficacy of HRV_3D vs. HRV_2D was 78% (95% CI: 0 to 95; $p = 0.031$). Similarly, although significant reduction in any-severity RVGE was observed in the HRV_2D group (49%; $p = 0.007$), the observed reduction was lower than that in HRV_3D group (68%; $p < 0.001$); the relative efficacy of HRV_3D vs. HRV_2D was 43% (95% CI: 10 to 63; $p = 0.013$).

In addition, a 44% (95% CI: 9 to 66) reduction in all-cause severe gastroenteritis was observed in the HRV_3D group ($p = 0.018$), whereas there was no significant reduction in the HRV_2D group ($p = 0.986$). No reduction in all-cause gastroenteritis of any severity between the HRV and placebo groups was observed (Table 2). The specific incidence of S-RVGE among placebo recipients during the second rotavirus season was 1.2%; Table 3. The study was not powered to evaluate the efficacy of HRV specifically during the second year of life, and none of the efficacy estimates during the second rotavirus season were statistically significant (Table 3).

The number of serotypes causing RVGE of any severity during Year 2 in the HRV_2D, HRV_3D and placebo groups were 3, 1, and 5, respectively for G1P[8]; 2, 2, and 4 respectively for G2/P[4] or P[6]; and 1 case of G12P[6] in a HRV_2D recipient.

Immunogenicity of rotavirus vaccine in Cohort 1 subjects

The ATP analysis for seroconversion consisted of 205 subjects from Cohort 1 (70 subjects in the HRV_2D group, 66 subjects in the HRV_3D group and 69 subjects in the placebo group) from whom blood had been obtained prior to the first dose and 1 month following the third dose of study vaccine. The seroconversion rate in the HRV_3D group was moderately higher (66.7%; 95% CI: 54.0-77.8%), although not significantly, than in the HRV_2D group (57.1%; 95% CI: 44.7-68.9%). Similarly, a trend toward higher GMCs was observed in the HRV_3D group (94.3 U/ML; 95% CI: 56.5-157.4 U/ML) than the HRV_2D group (59.4 U/ML; 95% CI: 37.5-93.9 U/ML).

Discussion

This analysis confirmed protection against severe RVGE by *Rotarix* over 2 consecutive rotavirus seasons in South African children for the combined endpoint of infants who had received either a 2-dose or 3-dose HRV schedule during infancy. The 59% reduction of severe RVGE over 2 consecutive rotavirus seasons in the pooled cohort of HRV recipients was lower than the point-estimate observed during the first rotavirus season (77%; 95% CI: 56 to 88), which also included a combined analysis of Cohort 1 and Cohort 2 subjects enrolled in the study in South Africa. Interestingly, these results are similar to that observed in another vaccine study in 3 African countries with the pentavalent rotavirus vaccine.(4) In that study, efficacy against severe rotavirus diarrhea during the first two years of age in 3 African countries, was 39.3%; although vaccine efficacy against severe rotavirus diarrhea in the first year of life was 64.2%. This is

distinct from the situation reported in Latin America, the US, Europe, or middle-income countries in Asia, where the level of clinical protection was maintained at very similar levels over 2 years.(7-10)

One of the possible explanations for this difference, besides the higher immunogenicity and higher point-estimate of efficacy in the European and pan-American studies, is the age at which children are infected with rotavirus. In Africa, rotavirus infections occur commonly in young infants between 3–12 months of age, where > 75% of children with severe rotavirus gastroenteritis from hospital-based studies are observed(13;21-23) and only approximately 10% of rotavirus disease requiring a visit to hospital or the outpatient clinic was in the 12- to 18-month-old group in several African countries.(24) On the other hand, studies from Europe indicate that while rotavirus infection peaks in children 6-24 months of age,(25) 40% of infection occurs in the group 12-23 months of age.(26) In Latin American studies, this pattern of higher incidence of rotavirus infection in the second year was also noted, where the incidence of severe rotavirus infection in unvaccinated children during the second year of life compared to the first was almost double (0.8% vs. 1.5%, respectively).(9) Finally, high infection rates of rotavirus evaluated by serological screening (40%) have been documented in Malawian infants less than 6 months of age.(3)

Although our study was not powered to examine schedule-specific HRV efficacy, an exploratory analysis indicated that vaccine efficacy over 2 consecutive rotavirus seasons was observed to be higher in the HRV_3D than in the HRV_2D groups. Consistently, the point-efficacy estimate of HRV_3D was higher than that of HRV_2D for outcomes of severe RVGE, any severity-RVGE

(albeit not significant), and all-cause severe gastroenteritis. In the previously published efficacy data during the first year of life, there was likewise a trend for greater severe RVGE efficacy with 3 doses of vaccine in the South African cohort (81.5% [95% CI: 55.1-93.7] efficacy HRV_3D vs 72.2% [95%CI: 40.1-88.4] efficacy HRV_2D).(3) An implication of the higher vaccine efficacy observed in the HRV_3D compared to HRV_2D group over 2 consecutive rotavirus seasons in this study indicates the need for protection beyond the first year of life against severe RVGE. The attack rate of severe RVGE during the second rotavirus season (1.2%) was a one-third of the overall attack rate of 3.2% seen over the 2 consecutive rotavirus seasons among the placebo group.

Our study was also not designed to explore for differences in vaccine efficacy between the first and second years of life, however, it is worth noting that lower point-estimates of vaccine efficacy over two consecutive rotavirus seasons compared to that seen in the first season was observed in the HRV_2D arm, which is the licensed schedule for *Rotarix* use. Several possibilities exist to explain the lower efficacy observed in the HRV_2D group over two consecutive rotavirus seasons.

First, children in the placebo group may have developed protection against severe RVGE through natural exposure to wild-type rotavirus during the first year of life in South Africa. However, exposure of placebo recipients to wild-type rotavirus would also have been expected to occur in other settings such as in clinical trials in Europe and Latin America, where efficacy against S-RVGE persisted in the second year of life, but as noted, the incidence rates in the first year of life in Europe and Latin America were lower.(7;9) In addition, vaccine efficacy was 85%

over the 2 consecutive rotavirus seasons in the HRV_3D arm in our study. This suggests that protection of the placebo recipients through wild-type infection in the first year of life was unlikely to be the main reason for the lack of efficacy in the HRV_2D arm over the full follow-up period.

A second possibility for lower efficacy over 2 consecutive seasons in the HRV_2D compared to HRV_3D group may relate to a trend toward inferior immunogenicity, as indicated by the trend of lower IgA GMCs in the HRV_2D compared to HRV_3D group. This may have resulted in accelerated waning of immunity in the HRV_2D group, and consequently lack of efficacy over a 2-year period. The immunogenicity of a HRV_2D compared to HRV_3D in settings such as ours, however, needs further validation as our study was not powered to address this.

A third further possible reason for decreased efficacy of *Rotarix* in our setting over 2 consecutive seasons may relate to the possibility of severity of gastroenteritis episodes in which rotavirus is identified during the second year being due to co-infection with other enteric pathogens. In this study, co-infections were not evaluated. Co-infection with rotavirus and other bacterial and viral enteropathogens has been observed in infants and toddlers in similar settings, and occurs in about 20% of cases.(27;28) As it is not possible to rule out the possibility of co-infection contributing to severe gastroenteritis symptoms rather than rotavirus *per se* being responsible for the illness severity in our study, this also needs to be evaluated further. The possibility of co-infection contributing to more severe illness in subsequent years is corroborated by the observation that rotavirus infections after the first natural rotavirus infection are significantly less severe than first rotavirus infection.(21) As HRV mimics natural rotavirus infection, theoretically subsequent rotavirus infection in vaccinees should be less severe. However, the persistence of protection

observed in the HRV_3D group make it unlikely that this is a major reason for the diminished vaccine efficacy over 2 consecutive rotavirus seasons in the HRV_2D group.

These data, together with the exploratory analysis which indicated higher point estimate of sero-conversion rates in the HRV_3D group (66.7%) than HRV_2D group (57.1%), indicate that a 3-dose schedule of *Rotarix* may have an advantage in providing longer-term protection against severe RVGE and severe all-cause gastroenteritis than a 2-dose schedule. The sero-conversion rates are similar to those observed in an earlier immunogenicity study in South Africa, which also reviewed the 2-dose schedule at 10 and 14 weeks of age and the 3-dose schedule at 6, 10 and 14 weeks of age.(18)

Although South Africa has introduced a 2-dose schedule of *Rotarix*, based on its licensure conditions, the dosing schedule being used includes a dose at 6 and 14 weeks of age, rather than the 10 and 14 weeks of age schedule evaluated in our study. The rationale for this dosing schedule included the aim of conferring protection as early as possible with the first dose of vaccine being at 6 rather than 10 weeks of age and minimizing missing the opportunity of vaccination at the earliest well-baby visit. The continued use of oral polio vaccine at 6 weeks of age in South Africa, coupled with presence of anti-rotavirus maternal derived antibody, may however negatively affect the immunogenicity and efficacy of this particular dosing schedule in South African children. The immunogenicity and effectiveness of this 2-dose schedule is currently being evaluated in South Africa.

The public-health importance of targeting the prevention of severe RVGE during the second year of life may vary between settings based on prevailing epidemiology, as well as possibly whether herd-protection is induced when a high proportion of the targeted infant groups have been vaccinated with HRV.(19;29) Although there are limited longitudinal studies on the burden of rotavirus in Africa across age-groups, symptomatic rotavirus infection has been shown to be greatest in African infants between 6-12 months of age.(22;23;30) In a longitudinal study of rotavirus infection in Guinea Bissau, 60% of infection in infants between 9 and 12 months of age were symptomatic, while after 18 months all infections were asymptomatic. Primary rotavirus infection was shown to offer 52% protection against symptomatic re-infection.(30)

In addition to the prevention of severe RVGE, our study also indicated that the overall reduction in severe all-cause gastroenteritis was greater than that of severe RVGE in the pooled analysis (4.5 vs. 2.5 per 100 infant years, respectively) as well as among the HRV_3D group (7.9 vs. 4.0 per 100 infant years). These differences illustrate the potential limitations in the sensitivity of our diagnostic methods, including modest sensitivity of the assay used for children reporting late in the course of illness(31) for detecting the actual burden of severe gastroenteritis prevented by *Rotarix*, which would also have implications in calculation of the cost-effectiveness of HRV in settings such as ours.

In conclusion, this study indicates the potential benefits of rotavirus vaccination in an African setting where good efficacy was demonstrated against severe rotavirus gastroenteritis in the first year of life, when most symptomatic rotavirus infection occurs in African infants. In addition, there was also modest protection in the second year of life and an overall reduction of all-cause

gastroenteritis was also observed. Interestingly, this clinical protection was observed in populations where the immune seroconversion would be considered modest (57-67%) when compared to that observed in other parts of the world. In settings where there is high burden of disease occurring at a young age, such as in Africa, the advantages of a 3-dose schedule of *Rotarix* should be further investigated to confirm the findings of our exploratory analysis.

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Rotarix is the trademark of GlaxoSmithKline group of companies; *RotaTeq* is the trademark of Merck & Co., Inc; *Rotaclone* is a trademark of Meridian Bioscience.

Reference List

- (1) Parashar UD, Burton A, Lanata C, Boschi-Pinto C, Shibuya K, Steele D et al. Global mortality associated with rotavirus disease among children in 2004. *J Infect Dis* 2009; 200 Suppl 1:S9-S15.
- (2) Meeting of the immunization Strategic Advisory Group of Experts, April 2009-- conclusions and recommendations. *Wkly Epidemiol Rec* 2009; 84(23):220-236.
- (3) Madhi SA, Cunliffe NA, Steele D, Witte D, Kirsten M, Louw C et al. Effect of human rotavirus vaccine on severe diarrhea in African infants. *N Engl J Med* 2010; 362(4):289-298.
- (4) Armah GE, Sow SO, Breiman RF, Dallas MJ, Tapia MD, Feikin DR et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in sub-Saharan Africa: a randomised, double-blind, placebo-controlled trial. *Lancet* 2010; 376(9741):606-614.
- (5) Ruiz-Palacios GM, Perez-Schael I, Velazquez FR, Abate H, Breuer T, Clemens SC et al. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. *N Engl J Med* 2006; 354(1):11-22.

- (6) Vesikari T, Matson DO, Dennehy P, Van DP, Santosham M, Rodriguez Z et al. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. *N Engl J Med* 2006; 354(1):23-33.
- (7) Vesikari T, Karvonen A, Prymula R, Schuster V, Tejedor JC, Cohen R et al. Efficacy of human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in European infants: randomised, double-blind controlled study. *Lancet* 2007; 370(9601):1757-1763.
- (8) Tate JE, Cortese MM, Payne DC, Curns AT, Yen C, Esposito DH et al. Uptake, impact, and effectiveness of rotavirus vaccination in the United States: review of the first 3 years of postlicensure data. *Pediatr Infect Dis J* 2011; 30(1 Suppl):S56-S60.
- (9) Linhares AC, Velazquez FR, Perez-Schael I, Saez-Llorens X, Abate H, Espinoza F et al. Efficacy and safety of an oral live attenuated human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in Latin American infants: a randomised, double-blind, placebo-controlled phase III study. *Lancet* 2008; 371(9619):1181-1189.
- (10) Phua KB, Lim FS, Lau YL, Nelson EA, Huang LM, Quak SH et al. Safety and efficacy of human rotavirus vaccine during the first 2 years of life in Asian infants: Randomised, double-blind, controlled study. *Vaccine* 2009; 27(43):5936-5941.
- (11) Patel M, Pedreira C, De Oliveira LH, Tate J, Orozco M, Mercado J et al. Association between pentavalent rotavirus vaccine and severe rotavirus diarrhea among children in Nicaragua. *JAMA* 2009; 301(21):2243-2251.

- (12) de Palma O, Cruz L, Ramos H, de BA, Villatoro N, Pastor D et al. Effectiveness of rotavirus vaccination against childhood diarrhoea in El Salvador: case-control study. *BMJ* 2010; 340:c2825.
- (13) Steele AD, Peenze I, de Beer MC, Pager CT, Yeats J, Potgieter N et al. Anticipating rotavirus vaccines: epidemiology and surveillance of rotavirus in South Africa. *Vaccine* 2003; 21(5-6):354-360.
- (14) O'Ryan M, Linhares AC. Update on Rotarix: an oral human rotavirus vaccine. *Expert Rev Vaccines* 2009; 8(12):1627-1641.
- (15) van Doorn LJ, Kleter B, Hoefnagel E, Stainier I, Poliszczak A, Colau B et al. Detection and genotyping of human rotavirus VP4 and VP7 genes by reverse transcriptase PCR and reverse hybridization. *J Clin Microbiol* 2009; 47(9):2704-2712.
- (16) Ruuska T, Vesikari T. Rotavirus disease in Finnish children: use of numerical scores for clinical severity of diarrhoeal episodes. *Scand J Infect Dis* 1990; 22(3):259-267.
- (17) Ward RL, Bernstein DI, Shukla R, Young EC, Sherwood JR, McNeal MM et al. Effects of antibody to rotavirus on protection of adults challenged with a human rotavirus. *J Infect Dis* 1989; 159(1):79-88.
- (18) Steele AD, Reynders J, Scholtz F, Bos P, de Beer MC, Tumbo J et al. Comparison of 2 different regimens for reactogenicity, safety, and immunogenicity of the live attenuated oral rotavirus vaccine RIX4414 coadministered with oral polio vaccine in South African infants. *J Infect Dis* 2010; 202 Suppl:S93-100.

- (19) Tang ML, Ng HK. Comment on: confidence limits for the ratio of two rates based on likelihood scores: non-iterative method. *Stat Med* 2004; 23(4):685-690.
- (20) Zou GY, Donner A. Construction of confidence limits about effect measures: a general approach. *Stat Med* 2008; 27(10):1693-1702.
- (21) Fischer TK, Valentiner-Branth P, Steinsland H, Perch M, Santos G, Aaby P et al. Protective immunity after natural rotavirus infection: a community cohort study of newborn children in Guinea-Bissau, west Africa. *J Infect Dis* 2002; 186(5):593-597.
- (22) Cunliffe NA, Kilgore PE, Bresee JS, Steele AD, Luo N, Hart CA et al. Epidemiology of rotavirus diarrhoea in Africa: a review to assess the need for rotavirus immunization. *Bull World Health Organ* 1998; 76(5):525-537.
- (23) Seheri ML, Dewar JB, van der ML, Geyer A, Tumbo J, Zwegarth M et al. Prospective hospital-based surveillance to estimate rotavirus disease burden in the Gauteng and North West Province of South Africa during 2003-2005. *J Infect Dis* 2010; 202 Suppl:S131-S138.
- (24) Mwenda JM, Ntoto KM, Abebe A, Enweronu-Laryea C, Amina I, Mchomvu J et al. Burden and epidemiology of rotavirus diarrhea in selected African countries: preliminary results from the African Rotavirus Surveillance Network. *J Infect Dis* 2010; 202 Suppl:S5-S11.
- (25) Pediatric ROTavirus European CommitTee (PROTECT). The paediatric burden of rotavirus disease in Europe. *Epidemiol Infect* 2006; 134(5):908-916.

- (26) Giaquinto C, Van DP. Age distribution of paediatric rotavirus gastroenteritis cases in Europe: the REVEAL study. *Scand J Infect Dis* 2010; 42(2):142-147.
- (27) Moyo SJ, Gro N, Matee MI, Kitundu J, Myrmel H, Mylvaganam H et al. Age specific aetiological agents of diarrhoea in hospitalized children aged less than five years in Dar es Salaam, Tanzania. *BMC Pediatr* 2011; 11:19.
- (28) Steele AD, Geyer A, Alexander JJ, Crewe-Brown HH, Fripp PJ. Enteropathogens isolated from children with gastro-enteritis at Ga-Rankuwa Hospital, South Africa. *Ann Trop Paediatr* 1988; 8(4):262-267.
- (29) Cortese MM, Tate JE, Simonsen L, Edelman L, Parashar UD. Reduction in gastroenteritis in United States children and correlation with early rotavirus vaccine uptake from national medical claims databases. *Pediatr Infect Dis J* 2010; 29(6):489-494.
- (30) Fischer TK, Aaby P, Molbak K, Rodrigues A. Rotavirus disease in Guinea-Bissau, West Africa: a review of longitudinal community and hospital studies. *J Infect Dis* 2010; 202 Suppl:S239-S242.
- (31) Ajjampur SS, Rajendran P, Ramani S, Banerjee I, Monica B, Sankaran P et al. Closing the diarrhoea diagnostic gap in Indian children by the application of molecular techniques. *J Med Microbiol* 2008; 57(Pt 11):1364-1368.

Figure 1: Children enrolled into efficacy trial of *Rotarix* in South Africa.

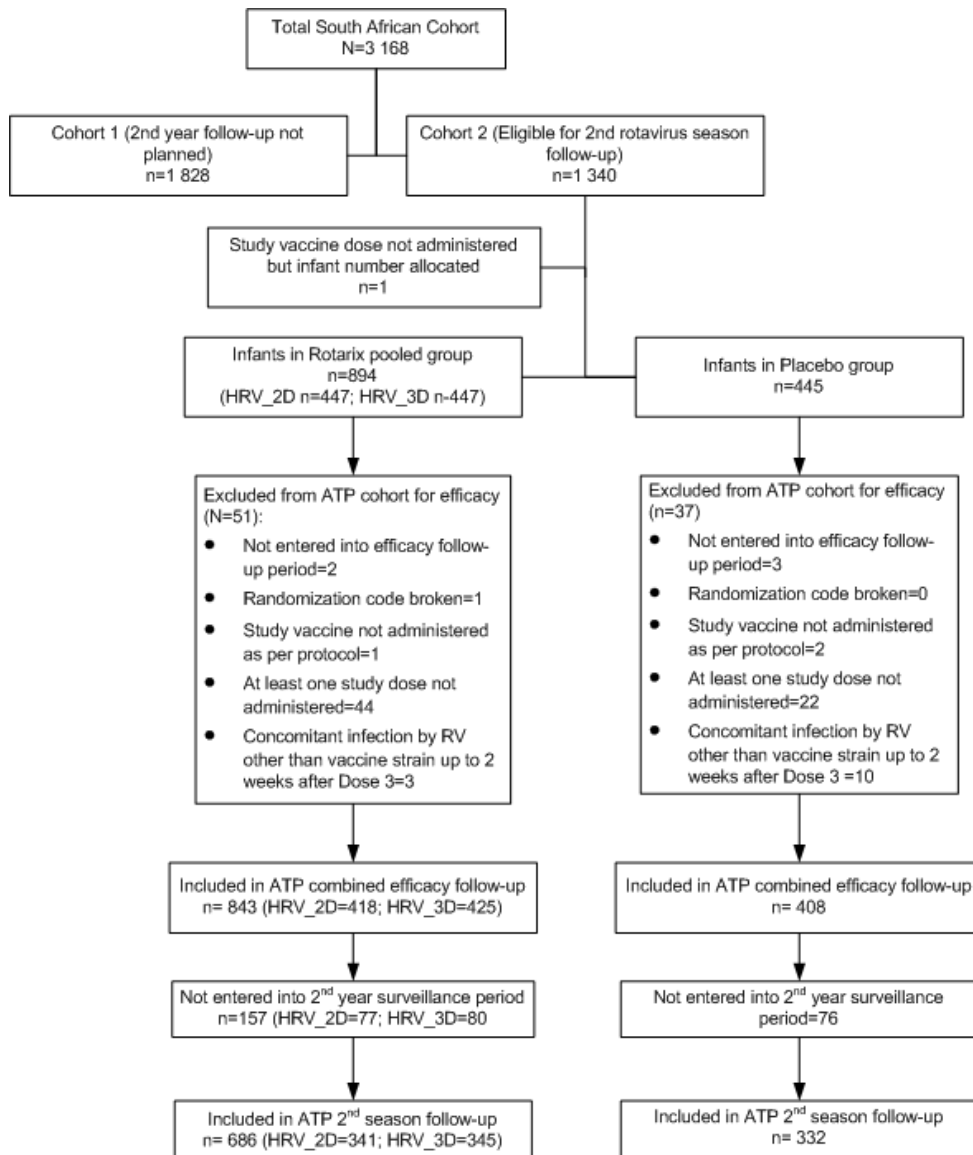
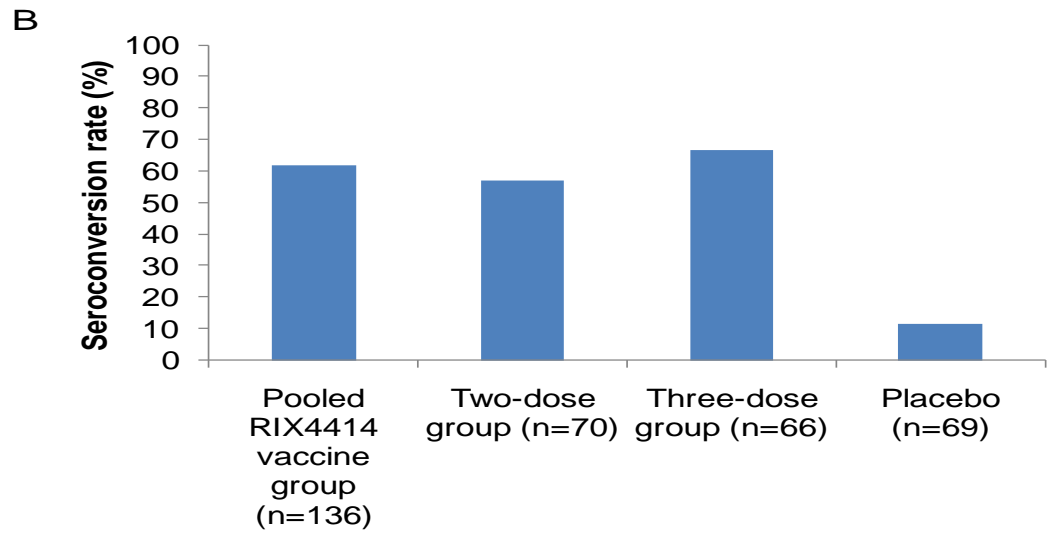


Figure 2: Anti-rotavirus IgA seroconversion rates (95% confidence intervals) in the ATP cohort for Cohort-1 infants.



Tables

Table 1: Characteristics of total vaccinated cohort of study population eligible for follow-up over two consecutive rotavirus seasons (Cohort-2 only).

Characteristics	Rotarix pooled group	Placebo group	Total
Infants (N)	894	445	1339
Mean Age (weeks)			
At Dose 1	6.2	6.2	6.2
At Dose 2	11.0	11.0	11.0
At Dose 3	15.9	16.0	15.9
Mean duration of efficacy follow-up (months)	1.26	1.25	
Gender (%)			
Male	449 (50.2)	230 (51.7)	679 (50.7)
Female	445 (49.8)	215 (48.3)	660 (49.3)
Mean age at end of follow-up (S.D.)- months.	13.8 (5.5)	13.8 (5.4)	13.8 (5.5)
Combined efficacy total follow-up time of ATP cohort-years.	1064.1 (HRV_2D:532) (HRV_3D: 532)	511	Not applicable
Race (%)			
African	805 (90.0)	399 (89.7)	1204 (89.9)
White - Caucasian	1 (0.1)	0 (0.0)	1 (0.1)
Other - colored / mixed	88 (9.8)	46 (10.3)	134 (10.0)
OPV co-administered (%)			
At Dose 1	891 (99.7)	445 (100)	1336 (99.8)
At Dose 2	854 (99.1)	425 (99.1)	1279 (99.1)
At Dose 3	845 (99.6)	422 (99.8)	1267 (99.7)

Growth parameters			
Mean Height at Dose 1 (cm)	54.6	54.7	54.6
Mean Weight at Dose 1 (kg)	4.8	4.9	4.8
Mean BMI at Dose 1 (kg/m ²)	16.2	16.3	16.2
HIV testing			
*Percentage of infants with consent given for HIV testing (N=)	54.1 (484)	54.2 (241)	54.1 (725)
Percentage HIV-Positive (N=)	6.4 (31)	5.8 (14)	6.2 (45)

*HIV tests were performed only for those infants whose parents gave consent

N = Number of infants in each group; ± = mean ± standard deviation

Table 2: Vaccine efficacy of *Rotarix* over two consecutive rotavirus seasons from 2 weeks after the last dose up until after the end of the second rotavirus season (Cohort-2 only).

Type of gastroenteritis	<i>Rotarix</i> ; N=843 pooled		Placebo		Efficacy (95%CI)	P value	*Rate Difference per 100 infants per year (95% CI)
	HRV_2D N=418		N=408				
	HRV_3D N=425						
	N	% Attack rate (95% CI)	N	Attack rate (95% CI)			
Severe RVGE: Pooled	11	1.3 (0.7 to 2.3)	13	3.2 (1.7 to 5.4)	59% (1 to 83)	0.047	2.5 (0.3 to 6.3)
HRV_2D	9	2.2 (1.0 to 4.0)			32% (-71 to 75)	0.487	1.6 (0.2 to 5.3)
HRV_3D	2	0.5 (0.1 to 1.7)			85% (35 to 98)	0.006	4.0 (1.2 to 7.4)
Any RVGE: Pooled	41	4.9 (3.5 to 6.5)	48	11.8 (8.8 to 15.3)	59% (36 to 73)	<0.001	11.1 (5.9 to 17.4)
HRV_2D	25	6.0 (3.9 to 8.7)			49% (16 to 70)	0.007	9.6 (3.4 to 16.2)
HRV_3D	16	3.8 (2.2 to 6.0)			68% (43 to 83)	<0.001	12.7 (7.0 to 19.1)
All-cause severe GE: Pooled	76	9.0 (7.2 to 11.2)	48	11.8 (8.8 to 15.3)	23% (-12 to 47)	0.179	4.5 (-0.9 to 10.8)
HRV_2D	48	11.5 (8.6 to 14.9)			2% (-49 to 36)	0.986	1.0 (-5.9 to 8.0)
HRV_3D	28	6.6 (4.4 to 9.4)			44% (9 to 66)	0.018	7.9 (1.8 to 14.4)

Table 3: Vaccine efficacy of *Rotarix* over two consecutive rotavirus seasons in fully vaccinated children (Cohort 2) during the second rotavirus season only.

Type of gastroenteritis	<i>Rotarix</i> group		Placebo		Efficacy (95%CI)	P value ¹
	N	% Attack rate (95% CI)	N	Attack rate (95% CI)		
Severe RVGE: Pooled (N=686)	5	0.2 (0.2 to 1.7)	4	1.2 (0.3 to 3.1)	40 (-204 to 87)	0.664
HRV_2D (N=341)	4	1.2 (0.3 to 3.0)			3 (-43 to 82)	1.0
HRV_3D (N=345)	1	0.3 (0.0 to 1.6)			76 (-143 to 100)	0.351
Any RVGE: Pooled	9	1.3 (0.6 to 2.5)	9	2.7 (1.2 to 5.1)	52 (-38 to 83)	0.191
HRV_2D	6	1.8 (0.6 to 3.8)			35 (-104 to 81)	0.571
HRV_3D	3	0.9 (0.2 to 2.5)			68 (-29 to 94)	0.128
All-cause severe GE: Pooled	42	6.1 (4.4 to 8.2)	27	8.1 (5.4 to 11.6)	25 (-27 to 55)	0.305
HRV_2D	26	7.6 (5.0 to 11.0)			6 (-66 to 48)	0.992
HRV_3D	16	4.6 (2.7 to 7.4)			43 (-10 to 71)	0.305

¹ Exact P-value conditional to number of cases