Evaluation of a Booster Dose of Pentavalent Rotavirus Vaccine Co-Administered with Measles, Yellow Fever and Meningitis A Vaccines in 9-month-old Malian Infants

Fadima C. Haidara[1], Milagritos D. Tapia[2], Samba O. Sow[1,2], Moussa Doumbia[1], Flanon Coulibaly[1], Fatoumata Diallo[1], Awa Traoré[1], Mamoudou Kodio[1], Corey L. Kelly[3], Meagan Fitzpatrick[2], Karen Kotloff[2], John. C. Victor[3], Kathleen Neuzil[2]

[1] Centre pour le Développement des Vaccins – Mali, Bamako, Mali

[2] Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, USA

[3] PATH, Seattle, WA, USA

Corresponding author: Milagritos D. Tapia, MD, Center for Vaccine Development, University of Maryland School of Medicine, 685 West Baltimore St, Rm 480, Baltimore, MD 21201, mtapia@som.umaryland.edu, Telephone: 410-706-5328, Fax: 410-706-6205

Alternate corresponding author: Kathleen Neuzil, Center for Vaccine Development, University of Maryland School of Medicine, 685 West Baltimore St, Rm 480, Baltimore, MD 21201, kneuzil@som.umaryland.edu, Telephone: 410-706-5328, Fax: 410-706-6205

Brief summary: Pentavalent rotavirus vaccine at 9 months of age in Mali increased antibody levels and did not interfere with responses to measles vaccine and meningococcal A conjugate vaccine. Interference with yellow fever vaccine could not be ruled out.

Running title: Booster Rotavirus Vaccine in Infants

Footnote page

Conflict of Interest

MDT, AT and KK receive research funds from Merck and MF receives research funds from the National Institutes of Health. Since the study was completed, Dr. Sow has become the Mali Minister of Health. The remaining authors have no conflicts of interest to declare.

Funding

This work was supported by PATH with a grant from the Bill and Melinda Gates Foundation. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Gates Foundation or PATH.

Prior Research Dissemination

This research was presented in part at the 10th African Rotavirus Symposium on June 2, 2016 in Bamako, Mali and the 65th Annual Meeting of the American Society of Tropical Medicine & Hygiene on November 15, 2016 in Atlanta, GA, USA (Abstract Number 1064).

Request for reprints: Milagritos D. Tapia, MD, Center for Vaccine Development, University of Maryland School of Medicine, 685 West Baltimore St, Rm 480, Baltimore, MD 21201, mtapia@medicine.umaryland.edu, Telephone: 410-706-5328, Fax: 410-706-6205

Abstract

Background: Rotavirus vaccines given to infants are safe and efficacious. A booster dose of rotavirus vaccine could extend protection into the second year of life in low resource countries.

Methods: We conducted an open-label, individual-randomized trial in Bamako, Mali. We assigned 600 9- to 11-month old infants to measles (MV), yellow fever (YFV), and meningococcal A conjugate vaccines with or without pentavalent rotavirus vaccine (PRV). We

assessed non-inferiority of seroconversion and seroresponse rates (≤10% difference) to MV, YFV and MenAV. We compared the seroresponse to PRV.

Results: Seroconversion to measles occurred in 255/261 (97.7%) and 246/252 (97.6%) of the PRV and control group; difference, 0.1% (95% CI, − 4.0 to 4.2). Seroresponse to YFV occurred in 48.1% (141/293) of the PRV group compared to 52.2% (153/293) of the control group; difference − 4.1% (95% CI, −12.2 to 4.0). A 4-fold rise in meningococcal A bactericidal titer was observed in 273/292 (93.5%) PRV recipients and 276/293 (94.2%) controls; difference, −0.7% (95% CI, − 5.2 to 3.8). Rises in anti-rotavirus IgA and IgG geometric mean concentrations were higher among PRV recipients (118 [95% CI, 91 to 154] and 364 [95% CI, 294 to 450]) compared to controls (68 [95% CI, 50 to 92] and 153 [95% CI, 114 to 207].

Conclusions: PRV did not interfere with MV and MenAV; this study could not rule out interference with YFV. PRV increased serum rotavirus antibody levels.

Keywords: Rotavirus vaccine, Mali, infants, booster dose, yellow fever vaccine, measles vaccine

ClinicalTrials.gov Identifier: NCT02286895

Introduction

Rotavirus is the most common cause of severe and fatal diarrhea in young children throughout the world [1, 2]. The importance of rotavirus disease is well-established in the first year of life, and studies in low resource settings demonstrate that severe rotavirus incidence remains high through the second year of life [2, 3]. Oral rotavirus vaccines are currently recommended by the World Health Organization (WHO) Strategic Advisory Group of Experts.

While rotavirus vaccines reduce severe rotavirus disease in the first year of life in low resource settings, declines in efficacy have been reported in the second year of life [3-7].

Improving protection beyond that achieved with the current vaccination schedule could have significant global impact. One strategy to extend protection is the administration of a booster dose of rotavirus vaccine at 9 months of age, concomitant with other routinely recommended Expanded Programme on Immunization (EPI) vaccines. In a study in Bangladesh, human monovalent rotavirus vaccine administered at 9 months of age did not interfere with immune responses to concomitantly-administered vaccines and significantly increased rates of anti-rotavirus IgA and IgG seropositivity [8]. Moreover, a recent model estimates that up to 20,000 additional deaths could be averted with a booster dose at 9 to 12 months of age in medium and high child mortality countries [9].

A booster dose of rotavirus vaccine had never previously been evaluated in African infants, nor were data available for the pentavalent rotavirus vaccine (PRV). In 2014, Mali introduced PRV into their routine immunization program at 6, 10 and 14 weeks of age. To assess the effect of concomitant administration of PRV on measles vaccine (MV) and yellow fever vaccine (YFV), we compared immune responses to these vaccines in Malian infants receiving a supplemental dose of PRV to those observed in the absence of PRV (control group). We also measured anti-rotavirus immune responses within both groups. Finally, since meningococcal A conjugate vaccine (MenAV) was planned to be added to the EPI schedule at the same age, we characterized immune responses to that vaccine in the presence and absence of PRV.

Methods

Study design and Participants

We conducted an open-label, individual-randomized, comparative immunogenicity trial. From October 15, 2014 to December 18, 2014, participants were enrolled at 9 health centers in Bamako, Mali. Eligible infants were aged 9 to 11 months, resided in the study area, were generally healthy, had been fully vaccinated according to the local immunization schedule and verified in the vaccination record and had parents who were willing to follow protocol procedures. Infants were ineligible if they had history of any of the following: prior receipt of MV, YFV, or MenAV; rotavirus vaccine in the past 90 days; known hypersensitivity to any component of the study vaccines and/or following administration of previous vaccines; and any chronic medical condition or medications that might compromise the well-being of the participant, compliance with study procedures or interfere with the outcome of the study. Moderate or severe acute illness at the time of enrollment was a temporary exclusion at the discretion of the investigator.

The protocol was approved by the University of Maryland School of Medicine Institutional Review Board, the Ethical Committee of Faculté de Médecine, Pharmacie et Odontostomatologie of Mali, the Ministry of Health of Mali, Western Institutional Review Board, Puyallup, Washington, and leaders of the involved communities. Parents or guardians of participants provided informed consent prior to initiation of study procedures. The trial was registered with ClinicalTrials.gov (NCT02286895).

Procedures

All participants received MV (Serum Institute of India, Ltd.) by subcutaneous injection, YFV (Federal State Unitary Enterprise on Manufacture of Bacterial and Viral Preparations, Chumakov Institute of Poliomyelitis and Viral Encephalitides, Russian Academy of Medical Sciences) by intramuscular injection, and MenAV, 5 μg (Serum Institute of India, Ltd) by intramuscular injection, at a separate site. Participants were randomly assigned 1:1 by a sequentially assigned numeric code to receipt of oral PRV (Merck & Co., Inc.) or no PRV.

Placebo was not used in this study as PRV is used by the public sector immunization program in Mali and there was no placebo that could be accessed. Laboratory staff performing serologic tests remained masked to individual participant group assignments.

All participants were observed for 30 minutes after vaccination. Local (induration and pain) and systemic reactions (fever, lethargy, irritability, vomiting, diarrhea, loss of appetite, rash, persistent crying, and signs of potential intussusception) were assessed on Days 1 - 5 during home visits by the trained field workers and on Day 7 (+/-1) by a physician in clinic. Unsolicited adverse events (AE) were assessed until Day 28. Serious Adverse Events (SAE) were assessed from Day 0 until the end of study (Day 84).

Blood samples (3 to 5 ml) were collected on Days 0 and 28 for evaluation of antibody responses to all antigens. Anti-measles antibody was measured by commercially-available enzyme-linked IgG immunoassay (EIAs; Wampole Laboratories, Princeton, New Jersey). Yellow fever specific neutralizing antibody titers (NT) were determined using Robert Koch Institute's yellow fever standard operating procedure and relative to international scientific references [10, 11]. A validated serum bactericidal assay using baby rabbit complement (SBA) was used to measure the titer of functional antibody in human sera to *Neisseria meningitidis* group A [12, 13]. Antirotavirus immunoglobulin A (IgA) and immunoglobulin G (IgG) were measured by enzyme-linked immunosorbent assay at the Laboratory of Specialized Clinical Studies at the Cincinnati Children's Hospital Medical Center (Cincinnati, Ohio), as described previously [14-16]. The positive control was pooled sera from subjects that had received a vaccine or a natural rotavirus infection. The negative control was sera shown to have no antibody to rotavirus.

Outcomes

The study had two co-primary outcomes: 1) To evaluate the noninferiority of the anti-measles IgG seroconversion rate 28 days post-vaccination in the PRV group as compared to

the group without PRV. Seroconversion was defined as a positive result at day 28 among participants negative at baseline (defined by measurement ≤ 0.90); and 2) To evaluate the noninferiority of the YF NT response in the two groups. A 4-fold or higher response in the post-vaccination YF NT in reference to pre-vaccination YF NT, regardless of baseline serostatus, was used to define seroresponse to vaccination.

We evaluated additional evidence of PRV interference with the immune response to MV, YFV, and MenAV by comparing the difference between PRV and control groups with regard to the following secondary outcomes: anti-measles IgG seroconversion rates at Day 84; YF geometric mean titers (GMT) at Day 28; YF seroresponse rate (defined as a 2-fold or higher NT increase from baseline) at Day 28; YF NT seroconversion rate (defined as a positive result with titer ≥ 1:8 among those negative at baseline) at Day 28; SBA seroresponse rate (defined as a 4-fold or higher increase from baseline) at Day 28; SBA GMT at Day 28; and anti-rotavirus IgA and IgG seroresponse rates at Day 28, restricted to those at baseline in each group. We also conducted a superiority evaluation of the ratio of anti-rotavirus IgA and IgG GMCs in PRV vs. control groups at Day 28, first inclusive of all participants and then restricted only to subjects with baseline levels <20 units/mL. Secondary outcomes related to safety were descriptive and included the proportion of participants in each group with any of the following: immediate reactions occurring in the first 30 minutes post-vaccination, solicited adverse reactions, adverse events (AEs) or serious adverse events (SAEs).

Statistical Analysis

All immunogenicity analyses and summaries were performed on a per-protocol basis (see below for definition for each outcome). Supportive intention-to-treat analyses were conducted on all enrolled participants who received at least one dose of study vaccines. Safety analyses were conducted on this same intention-to-treat basis.

For the measles outcome, the per-protocol cohort included infants meeting all inclusion and no exclusion criteria, having less than seroprotective levels of measles virus IgG before vaccination, and receiving study vaccines and undergoing blood specimen collection according to schedule. YF virus–associated secondary analyses were conducted on the same per-protocol cohort, except that infants were required to have less than seroprotective levels of anti–YF NT before vaccination instead of measles virus antibody.

For the measles vaccine and YF vaccine immunogenicity primary analyses, proportions of participants reaching seroprotective levels of measles virus IgG or YF NT at the pre-specified time points after vaccination were compared between groups, using the Newcombe-Wilson method without continuity correction. A noninferiority margin of −10% was chosen as the maximal absolute reduction in proportion allowed in the concomitant MV, YFV, MenAV+ PRV group as compared to the MV, YFV, MenAV alone group. Rotavirus and meningococcal A immunogenicity analyses were conducted on the per-protocol cohort without the requirement for less than seroprotective levels of measles virus or yellow fever virus before vaccination. Anti-rotavirus IgA and IgG GMCs and the proportion of infants who were seropositive were compared before vaccination and post vaccination in each group, using the McNemar test for correlated proportions; for GMC calculations, concentrations of <20 U/mL were converted to 10 U/mL.

We assumed 90% seroconversion and seroresponse rates in each arm for each antigen in the two co-primary objectives. For each co-primary outcome, to rule out a noninferiority margin of no more than 10% with 95% power and a one-sided type-one error rate of no more than 2.5%, 237 evaluable subjects were required in each group. (95% power is chosen to give an overall power of at least 90%.) Assuming 79% evaluability (up to 10% baseline seropositivity) and a 12% loss to follow-up rate), a total sample size of 600 vaccinated subjects was required.

Role of the Funding source

The Bill and Melinda Gates Foundation had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

From October 15, 2014 to December 18, 2014, 605 infants were screened and 600 enrolled with 300 receiving PRV. All participants completed all study visits until 5 months after vaccination (Figure 1). Study follow-up was completed on March, 23, 2015. The baseline characteristics among participants in both study groups were similar (Table 1).

Measles Virus

Before vaccination, 85.5% participants were measles seronegative — 87%PRV recipients and 84% controls. A positive result was observed 28 days after vaccination in 255/261 (97.7%) PRV recipients and 246/252 (97.6%) controls for a difference in seroconversion rate of 0.1% (95% CI, −4.0 to 4.2) (Table 2). On Day 84, 210/228 (92.1%) of PRV recipients who seroconverted remained seropositive, compared to 206/218 (94.5%) controls, for a difference of −2.4% (95% CI, −7.5 to 2.7) (Table 2). Similar results were obtained in the ITT population (data not shown). These results met our pre-specified criteria for non-inferiority of the response to MV (Figure 2).

Yellow Fever Virus

At baseline, YFV-specific immune responses were assessed in 586 participants. Four-fold or higher increases in YF PRNT occurred in 141/293 (48.1%) PRV recipients and in 153/293 (52.2%) controls for a difference of −4.1% (95% CI, −12.2 to 4.0). (Table 3) As the lower bound on the confidence interval of the difference exceeds −10, our primary criterion to establish non-inferiority of the response to YFV was not met (Figure 2). When seroresponse

was defined as at least a 2-fold rise, the response among PRV recipients was non-inferior with 202/293 (68.9%) responding, compared to 206/293 (70.3%) controls; difference −1.4% (95% CI, −8.8 to 6.1; Table 3, Figure 2). Furthermore, seroconversion among participants who had tested negative at baseline (titer <1:8) was comparable in both groups; the difference was −2.3% (95%CI, −9.5 to 4.8) (Table 3). Similar results were observed in the ITT population (data not shown). The GMTs were similar in both groups, and the ratio of the Day 28 YF NT GMT in the PRV group relative to the control group was 0.92 (95% CI, 0.77 to 1.09; p = 0.3150).

Rotavirus

Anti-rotavirus IgA and IgG seroconversion and seroresponse rates were significantly higher at Day 28 among PRV recipients compared to controls (Table 4). At baseline, 160 (54.8%) and 165 (56.5%) participants in the PRV and control groups had IgA levels <20 U/ml, and 91 (31.1%) and 92 (31.4%) participants in each group had IgG levels <20 U/ml. Among these IgA seronegative participants, 91 (56.9%) in the PRV group and 52 (31.5%) in the No PRV control group had Day 28 levels ≥ 20 U/ml (p < 0.0001). Among all tested participants, regardless of baseline status, 44.9% PRV recipients and 27.4% controls experienced ≥ 3-fold increase in IgA (p < 0.0001), and 74.7% PRV recipients and 58.9% controls had Day 28 levels ≥ 20 U/ml (p < 0.0001). Among all tested participants, the ratio of the Day 28 anti-rotavirus IgA geometric mean concentration in the PRV group relative to the control group was 1.7 (95% CI, 1.2 to 2.4; p = 0.0033). Serum IgG responses were more vigorous than IgA responses, and as with IgA, responses were significantly higher among PRV recipients compared to controls (Table 4).

Serotype A Meningococcus

Serum meningococcal A bactericidal responses were available in 292 PRV recipients and 293 controls. A 4-fold rise in bactericidal titer was observed in 273 (93.5%) PRV recipients and 276 (94.2%) controls, with a difference of −0.7% (95% CI, −5.2 to 3.8; Table 5; Figure 2).

Post-vaccination titers ≥ 8 and ≥ 128 also met non-inferiority criteria (Table 5; Figure 2). The ratio of the Day 28 SBA GMT in the PRV group relative to the control group was 0.9 (95% CI, 0.7 to 1.3; p = 0.6709).

Safety

There were no immediate reactions following vaccination. Systemic reactions occurred in 29/300 (9.7%) PRV recipients and 30/300 (10.0%) controls during the first 7 days of follow-up (p = 1.000). At least one unsolicited AE was observed through day 28 in 103/300 (34.3%) PRV recipients and 125/300 (41.7%) controls (p = 0.0772). Of note, 39/300 (13.0%) PRV recipients and 51/300 (17.0%) controls experienced gastrointestinal illness (p = 0.2083) with complaints of gastroenteritis, vomiting or diarrhea from vaccination until Day 28. A total of 15 participants experienced a single SAE each over the 3-month follow-up period, 7/300 (2.3%) PRV recipients and 8/300 (2.7%) controls (p = 1.000). These events were considered by the investigator to be unrelated to vaccination. There were no fatal SAEs and all resolved without sequelae. There were no cases of intussusception.

Discussion

In this first study to evaluate a booster dose of rotavirus vaccine in African infants, PRV was well-tolerated and elicited robust rotavirus-specific immune responses among 9- to 11-month-old Malian infants. Responses to MV, YFV and MenAV were similar whether or not the infant also received a booster dose of PRV. Our results met our pre-specified non-inferiority outcome for MV and demonstrate that PRV, when co-administered with MV, does not interfere with the immune response to MV for up to at least 3 months after vaccination. We did not meet our pre-specified non-inferiority criterion for YFV, as the CI on the difference between seroresponse rates in PRV and control groups was 12.5% when seroresponse was defined as a ≥ 4-fold increase in titer. This effect was not present when the secondary definition of seroresponse (≥ 2-fold increase in titers) was used or when seroconversion was compared

among those who had a negative titer at baseline. Given the inclusion of MenAV into the Malian EPI schedule beginning in 2017, it is reassuring that concomitant administration of PRV did not interfere with serum bactericidal responses to that antigen.

Infants who received PRV had post-vaccination increases in rotavirus-specific IgA and IgG antibody measurements that were highly statistically significant as compared to infants who did not receive PRV. These increases are particularly important for infants with low levels of antibody at the baseline measurement. Prior to booster vaccination, over half of participants had IgA levels <20 u/ml, consistent with susceptibility to severe disease and indicating that vaccination could be beneficial [17]. Seroconversion rates among these infants with baseline levels < 20 U/ml were superior in the PRV group, including a nearly 2-fold increase in IgA GMC. Of note, based on IgA measurements, 31.5% of controls and 56.9% of vaccinees seroconverted in the month between receipt of vaccine and the follow-up blood draw. Wild type rotavirus circulated during the study period, and likely influenced these results in both groups. Though not statistically significant, there were more adverse events identified as gastrointestinal illness in the control group compared to PRV through the one month post-vaccination time point.

Our results are similar to a prior study of a rotavirus vaccine booster dose using the oral monovalent human rotavirus vaccine and conducted among 9-month-old children in Bangladesh. In that study, rotavirus vaccine was well-tolerated and the proportion of infants with a protective immune response to anti-rotavirus IgA and IgG was significantly higher among infants who received the booster[8]. As with the current study, there was no evidence of interference with MV in the Bangladesh study. While these booster dose studies support that a dose of PRV at 9 months of age could enhance and extend protection in infants, a correlation between individual immune responses to rotavirus vaccines and protection from rotavirus disease has not been established. Data from clinical trials show that, on average, mean levels of anti-rotavirus IgA in a population are related to the population level efficacy against severe

disease [18, 19]. As IgG antibodies may be maternally-derived, they are generally not used in young infants to evaluate immune responses. However, as here, they may be useful measures in older children and adults. Properly designed field studies of the quality required to inform policy are needed to establish the effect of a booster dose on rotavirus disease outcomes.

Initially, concerns about the association between oral rotavirus vaccines and intussusception led to age restrictions on the administration of rotavirus vaccines. WHO reviewed data on risk and benefits of rotavirus vaccines in 2012, and concluded that the additional lives saved by removing age restrictions would far outnumber excess vaccine-associated intussusception morbidity and mortality [20, 21]. While this recommendation was made in the context of the primary immunization schedule, additional data may allow the same rationale to be applied to a booster dose. This study generated relevant safety data regarding use of PRV at later ages. The vaccine was well-tolerated, and adverse events were observed similarly in both groups. No cases of intussusception were reported in either group, although this study was underpowered to assess that rare outcome.

This study was the first to evaluate the serum bactericidal response when MenAV is administered with PRV. Mali was one of the first countries to introduce MenAV via widespread campaigns in 2010 and 2011 [22]. Since then, meningococcal A disease has dramatically declined in the region [23] and since 2017, a 5 µg dose of MenAV has been used in the routine immunization program at 9 months of age to protect new cohorts of children. Our results demonstrate that seroresponse rates were equally robust in both groups and there was no interference by PRV. These findings are consistent with those observed in a study of a meningococcal C conjugate vaccine [24].

Yellow Fever continues to be a risk in the African region [25] and infant vaccination and maintaining population immunity remain public health priorities. While the clinical significance of failing to meet our predefined non-inferiority criteria is uncertain, the overall lower-than-expected

antibody responses to YFV in both groups are concerning. In a previous study of YFV and MenAV coadministration in Malian infants, over 95% of infants in every group demonstrated an NT titer >1:8 [26], as compared to approximately 75% in our study. Both studies used vaccine from the same manufacturer, however differences in potency of the vaccine lot, improper cold chain handling, or performance of the NT assay could have contributed to the lower responses in the current study. The prior study identified inferior immune responses to YFV when the vaccine is co-administered with a 5 µg dose MenAV, as used here. Mutual interference was also found between YFV and a measles-mumps-rubella (MMR) vaccine in Brazil [27]. The combination of this previous work with our results suggests that further studies to evaluate the kinetics and magnitude of YF antibody responses are warranted.

The burden of severe rotavirus gastroenteritis remains high in Mali and other resource-poor countries in the second year of life [2, 6, 7, 28]. This study of a booster dose of rotavirus vaccine, the first to be conducted in African infants, strongly supports that a PRV booster dose strategy is feasible, well-tolerated, and immunogenic. While these results are compelling, global policymakers will need strong clinical evidence along with cost-effectiveness and expected impact data to recommend immunization schedule changes. In the absence of an immunological correlate of protection, efficacy studies are urgently needed to determine if an additional dose of rotavirus vaccine will be a safe and effective strategy to extend protection from the primary series into the second year of life.

Acknowledgements

The authors thank the participants and their families for the generous contribution of their time and effort.

References

1. Tate JE, Burton AH, Boschi-Pinto C, Parashar UD. Global, Regional, and National Estimates of Rotavirus Mortality in Children <5 Years of Age, 2000-2013. Clin Infect Dis **2016**; 62 Suppl 2: S96-s105.
2. Kotloff KL, Nataro JP, Blackwelder WC, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet **2013**; 382(9888): 209-22.
3. Zaman K, Dang DA, Victor JC, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in Asia: a randomised, double-blind, placebo-controlled trial. Lancet **2010**; 376(9741): 615-23.
4. Isanaka S, Guindo O, Langendorf C, et al. Efficacy of a Low-Cost, Heat-Stable Oral Rotavirus Vaccine in Niger. N Engl J Med **2017**; 376(12): 1121-30.
5. Madhi SA, Kirsten M, Louw C, et al. 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. Vaccine **2012**; 30 Suppl 1: A44-51.
6. Armah GE, Sow SO, Breiman RF, et al. Efficacy of pentavalent human-bovine reassortant rotavirus vaccine against severe rotavirus gastroenteritis in sub-Saharan Africa: a randomized, double-blind, placebo-controlled trial. Lancet **2010**; 376: 606-14.
7. Madhi SA, Cunliffe NA, Steele D, et al. Effect of human rotavirus vaccine on severe diarrhea in African infants. N Engl J Med **2010**; 362(4): 289-98.
8. Zaman K, Fleming JA, Victor JC, et al. Noninterference of Rotavirus Vaccine With Measles-Rubella Vaccine at 9 Months of Age and Improvements in Antirotavirus Immunity: A Randomized Trial. J Infect Dis **2016**; 213(11): 1686-93.
9. Burnett E, Lopman BA, Parashar UD. Potential for a booster dose of rotavirus vaccine to further reduce diarrhea mortality. Vaccine **2017**; 35(51): 7198-203.
10. Clarke E, Saidu Y, Adetifa JU, et al. Safety and immunogenicity of inactivated poliovirus vaccine when given with measles-rubella combined vaccine and yellow fever vaccine and when given via different administration routes: a phase 4, randomised, non-inferiority trial in The Gambia. Lancet Glob Health **2016**; 4(8): e534-47.
11. German Accreditation Body (DAkkS). Assay Accreditation. Vol. D-ML-13113-01-13. Frankfurt, Germany: DAkkS, **2013**.
12. Frasch CE, Borrow R, Donnelly J. Bactericidal antibody is the immunologic surrogate of protection against meningococcal disease. Vaccine **2009**; 27 Suppl 2: B112-6.
13. Borrow R, Balmer P, Miller E. Meningococcal surrogates of protection--serum bactericidal antibody activity. Vaccine **2005**; 23(17-18): 2222-7.
14. Vesikari T, Clark HF, Offit PA, et al. Effects of the potency and composition of the multivalent human-bovine (WC3) reassortant rotavirus vaccine on efficacy, safety and immunogenicity in healthy infants. Vaccine **2006**; 24(22): 4821-9.
15. Ciarlet M, Schodel F. Development of a rotavirus vaccine: clinical safety, immunogenicity, and efficacy of the pentavalent rotavirus vaccine, RotaTeq. Vaccine **2009**; 27 Suppl 6: G72-81.
16. Ward RL, Bernstein DI, Shukla R, et al. Effects of antibody to rotavirus on protection of adults challenged with a human rotavirus. J Infect Dis **1989**; 159(1): 79-88.

17. Cheuvart B, Neuzil KM, Steele AD, et al. Association of serum anti-rotavirus immunoglobulin A antibody seropositivity and protection against severe rotavirus gastroenteritis: analysis of clinical trials of human rotavirus vaccine. Hum Vaccin Immunother **2014**; 10(2): 505-11.
18. Armah GE, Breiman RF, Tapia MD, et al. Immunogenicity of the pentavalent rotavirus vaccine in African infants. Vaccine **2012**; 30 Suppl 1: A86-A93.
19. Patel M, Glass RI, Jiang B, Santosham M, Lopman B, Parashar U. A systematic review of anti-rotavirus serum IgA antibody titer as a potential correlate of rotavirus vaccine efficacy. J Infect Dis **2013**; 208(2): 284-94.
20. World Health Organization. Rotavirus vaccines WHO position paper, **2013** Feb 1.
21. Patel MM, Clark AD, Sanderson CF, Tate J, Parashar UD. Removing the age restrictions for rotavirus vaccination: a benefit-risk modeling analysis. PLoS Med **2012**; 9(10): e1001330.
22. Djingarey MH, Diomande FV, Barry R, et al. Introduction and Rollout of a New Group A Meningococcal Conjugate Vaccine (PsA-TT) in African Meningitis Belt Countries, 2010-2014. Clin Infect Dis **2015**; 61 Suppl 5: S434-41.
23. Lingani C, Bergeron-Caron C, Stuart JM, et al. Meningococcal Meningitis Surveillance in the African Meningitis Belt, 2004-2013. Clin Infect Dis **2015**; 61 Suppl 5: S410-5.
24. Vesikari T, Karvonen A, Borrow R, et al. Results from a randomized clinical trial of coadministration of RotaTeq, a pentavalent rotavirus vaccine, and NeisVac-C, a meningococcal serogroup C conjugate vaccine. Clin Vaccine Immunol **2011**; 18(5): 878-84.
25. Kraemer MU, Faria NR, Reiner RC, Jr., et al. Spread of yellow fever virus outbreak in Angola and the Democratic Republic of the Congo 2015-16: a modelling study. Lancet Infect Dis **2017**; 17(3): 330-8.
26. Roy Chowdhury P, Meier C, Laraway H, et al. Immunogenicity of Yellow Fever Vaccine Coadministered With MenAfriVac in Healthy Infants in Ghana and Mali. Clin Infect Dis **2015**; 61 Suppl 5: S586-93.
27. Nascimento Silva JR, Camacho LA, Siqueira MM, et al. Mutual interference on the immune response to yellow fever vaccine and a combined vaccine against measles, mumps and rubella. Vaccine **2011**; 29(37): 6327-34.
28. Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. Lancet Infect Dis **2012**; 12(2): 136-41.

Figure legends

Figure 1. Study Profile.

MV: Measles vaccine; YFV: yellow fever vaccine; PRV: pentavalent rotavirus vaccine; MenAV: meningococcal A conjugate vaccine

Figure 2. Non-inferiority of immune responses to concomitantly administered vaccines, PRV vs control groups, per-protocol population.

Blue line indicates no difference, red line indicates the non-inferiority margin of 10%. MV: measles vaccine; YFV: yellow fever vaccine; MenAV: meningococcal A conjugate vaccine; SBT: serum bactericidal titer.

Table 1. Baseline characteristics of vaccinated participants, per-protocol population

Characteristic	MV + YFV + MenAV + PRV	MV + YFV + MenAV
	n = 300	n = 300
Age, months	9.7 (0.7)	9.7 (0.7)
Male sex	149 (49.7)	167 (55.7)
Length (cm)	69.5 (2.5)	69.6 (2.6)
Weight (kg)	8.1 (1.0)	8.2 (1.0)

Data are no. (%) of children or mean value (SD).

Abbreviations: MV, measles vaccine; YFV, yellow fever vaccine; MenAV, meningococcal A conjugate vaccine; PRV, pentavalent rotavirus vaccine.

Table 2. Anti-measles IgG seroconversion rates in infants who received yellow fever, measles and meningitis A conjugate vaccines with or without pentavalent rotavirus vaccine, per-protocol population

	MV + YFV + MenAV + PRV		MV + YFV + MenAV		Difference	95% CI
	n/N	% (95% CI)	n/N	95%CI		
Seroconversion[a], Day 28	255/261	97.7 (95.9 to 99.5)	246/252	97.6 (95.7 to 99.5)	0.1	−4.0 to 4.2
Seroconversion[a], Day 84	210/228	92.1 (88.6 to 95.6)	206/218	94.5 (91.5 to 97.5)	−2.4	−7.5 to 2.7

Abbreviations: IgG, immunoglobulin class G; MV, measles vaccine; YFV, yellow fever vaccine; MenAV, meningococcal A conjugate vaccine; PRV, pentavalent rotavirus vaccine.

[a] Seroconversion was defined as a positive response among those who had negative result at baseline.

Table 3. Yellow fever plaque reduction neutralizing responses in infants who received yellow fever, measles and meningitis A conjugate vaccines with or without pentavalent rotavirus vaccine, per-protocol population

	MV + YFV + MenAV + PRV		MV + YFV + MenAV		Difference	95% CI
	n/N	% (95% CI)	n/N	% (95% CI)		
Seroresponse[a], ≥ 4-fold	141/293	48.1 (42.4 to 53.8)	153/293	52.2 (46.5 to 57.9)	−4.1	−12.2 to 4.0
Seroresponse[a], ≥ 2-fold	202/293	68.9 (63.6 to 74.2)	206/293	70.3 (65.1 to 75.5)	−1.4	−8.8 to 6.1
Seroconversion[b], ≥ 1:8	210/287	73.2 (68.0 to 78.3)	219/290	75.5 (70.6 to 80.5)	−2.3	−9.5 to 4.8
	GMT (95% CI)		GMT (95% CI)		Ratio of PRV/ no PRV group (95% CI)	p-value
Baseline	2.49 (2.37 to 2.62)		2.40 (2.31 to 2.49)		--	--
Day 28	15.03 (13.31 to 16.97)		16.82 (14.80 to 19.11)		0.92 (0.77 to 1.09)	0.3150

Abbreviations: MV, measles vaccine; YFV, yellow fever vaccine; MenAV, meningococcal A conjugate vaccine; PRV, pentavalent rotavirus vaccine; GMT, geometric mean titer.

[a] Seroresponse was defined as the indicated fold increase in titer from baseline to Day 28.

[b] Seroconversion was defined as a positive result (titer ≥1:8) at Day 28 among participants with negative (titer <1:8) at baseline.

Table 4: Antirotavirus IgA and IgG responses in infants who received yellow fever, measles and meningitis A conjugate vaccines with or without pentavalent rotavirus vaccine, per-protocol population

	MV + YFV + MenAV + PRV		MV + YFV + MenAV			
	n/N	% (95% CI)	n/N	% (95% CI)	Difference	p-value
Per-protocol population						
IgA						
≥ 3-fold increase in level from baseline to Day 28	131/292	44.9 (39.2 to 50.6)	80/292	27.4 (22.3 to 32.5)	17.5	< 0.0001
≥ 20 U/ml	218/292	74.7 (69.7 to 79.6)	172/292	58.9 (53.3 to 64.5)	15.8	< 0.0001
≥ 20 U/ml among those with <20 U/ml at baseline	91/160	56.9 (49.2 to 64.5)	52/165	31.5 (24.4 to 38.6)	25.4	< 0.0001
	GMC (95% CI)		GMC (95% CI)		Ratio of PRV/ no PRV group	p-value
Baseline	25.3 (19.7 to 32.6)		23.7 (18.6 to 30.3)		--	--
Day 28	118.4 (90.9 to 154.3)		67.9 (49.9 to 92.3)		1.7	0.0033

IgG						
≥ 3-fold increase in level from baseline to Day 28	168/293	57.3 (51.7 to 63.0)	77/293	26.3 (21.2 to 31.3)	31.1	< 0.0001
≥ 20 U/ml	275/293	93.9 (91.1 to 96.6)	223/293	76.1 (71.2 to 81.0)	17.7	< 0.0001
≥ 20 U/ml among those with <20 U/ml at baseline	76/91	83.5 (75.9 to 91.1)	29/92	31.5 (22.0 to 41.0)	52.0	< 0.0001
		GMC (95% CI)		GMC (95% CI)	Ratio of PRV/ no PRV group	p-value
Baseline		62.5 (49.6 to 78.8)		58.9 (47.1 to 73.7)	-	-
Day 28		363.6 (293.6 to 450.4)		153.3 (113.8 to 206.5)	2.3	<0.0001

Abbreviations: MV, measles vaccine; YFV, yellow fever vaccine; MenAV, meningococcal A conjugate vaccine; PRV, pentavalent rotavirus vaccine; GMC, geometric mean concentration.

Table 5. Meningococcal A serum bactericidal responses in infants who received yellow fever, measles and meningitis A conjugate vaccines with or without pentavalent rotavirus vaccine, per-protocol population

	MV + YFV + MenAV + PRV		MV + YFV + MenAV		Difference	95% CI
	n/N	% (95% CI)	n/N	% (95%CI)		
Seroresponse[a]	273/292	93.5 (90.7 to 96.3)	276/293	94.2 (91.5 to 96.9)	−0.7	−5.2 to 3.8
Serum bactericidal titer, ≥ 8	282/292	96.6 (94.5 to 98.7)	282/293	96.2 (94.1 to 98.4)	0.3	−3.8 to 4.4
Serum bactericidal titer, ≥ 128	276/292	94.5 (91.9 to 97.1)	276/292	94.2 (91.5 to 96.9)	0.3	−4.1 to 4.8
	GMT (95% CI)		GMT (95% CI)		Ratio of PRV/ no PRV group (95% CI)	p-value
Baseline	3.22 (2.68 to 3.88)		2.81 (2.40 to 3.28)		--	--
Day 28	2014.25 (1626.21 to 2494.89)		2097.03 (1693.18 to 2597.20)		0.94 (0.69 to 1.26)	0.6709

Abbreviations: MV, measles vaccine; YFV, yellow fever vaccine; MenAV, meningococcal A conjugate vaccine; PRV, pentavalent rotavirus vaccine; GMT, geometric mean titer.

[a] Seroresponse was defined as a ≥4-fold increase in titer from baseline to Day 28.

Figure 1.

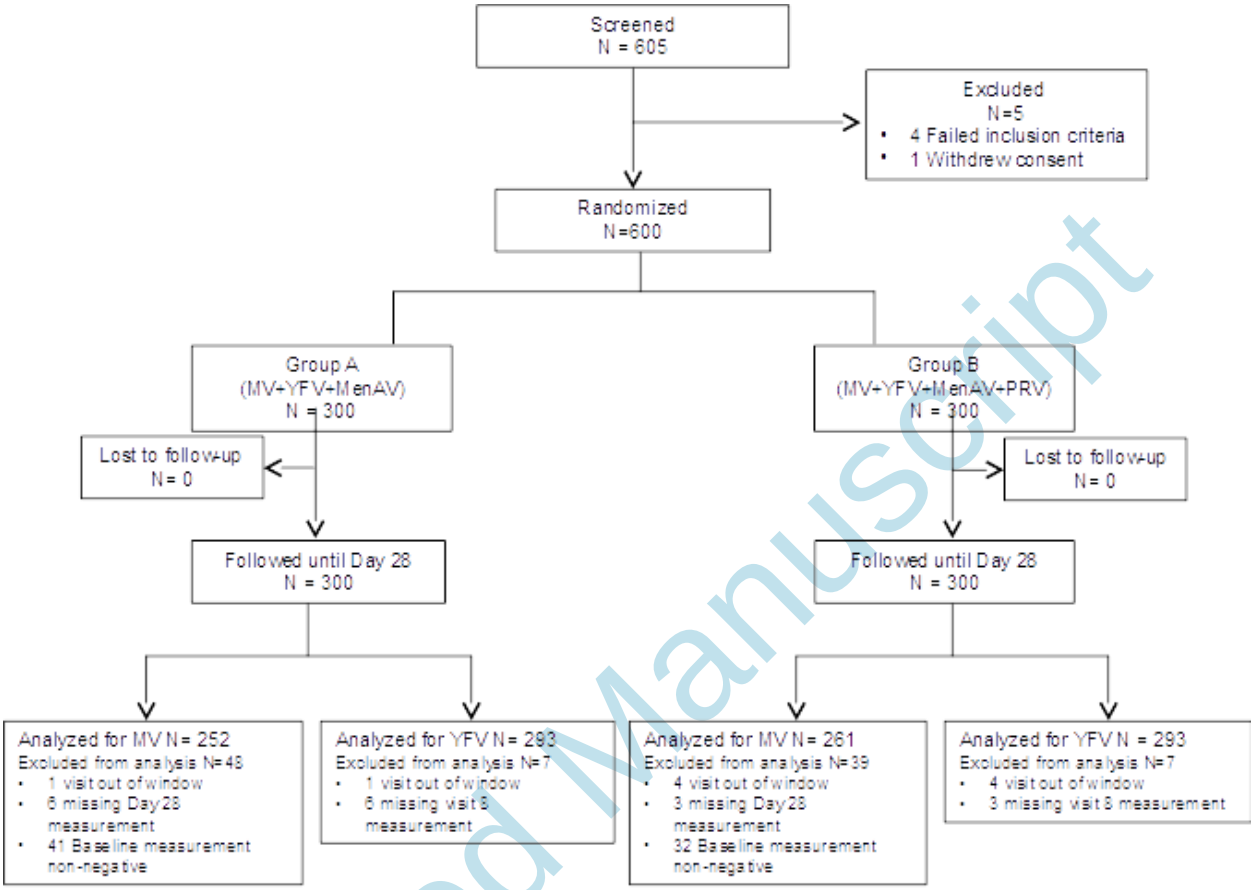

Figure 2.