Rotavirus vaccines in low-income settings

Lauren Michelle Schwartz

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Reading committee:

M. Elizabeth Halloran, Chair

Ali Rowhani-Rahbar

Jairam Lingappa

Program authorized to Offer Degree:

Epidemiology

University of Washington

Abstract

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Lauren Michelle Schwartz

Chair of the supervisory committee:
M. Elizabeth Halloran
Professor
Biostatistics, Epidemiology

Background: An estimated 200,000 deaths due to rotavirus diarrhea occur annually in children with most of the burden in low-income settings. Rotavirus is a double stranded RNA virus transmitted via the fecal-oral route. Without a vaccine, nearly all children <5 years old become infected with rotavirus and recurrent infection is common. Rotavirus vaccines continue to be the key intervention needed to reduce the global burden of severe rotavirus diarrhea. Two rotavirus vaccines are used worldwide (Rotarix [RV1]) and (RotaTeq [RV5]). Large multi-site randomized controlled trials (RCTs) of both vaccines in sub-Saharan Africa and Asia demonstrated moderate vaccine efficacy (VE) against severe rotavirus diarrhea during the first year of life. As of March 2018, 93 countries, including 43 Gavi-eligible countries, have introduced a rotavirus vaccine into its immunization schedule. This dissertation sought to evaluate the test-negative design as an appropriate epidemiologic study design to measure rotavirus vaccine effectiveness in low-income settings (Aim 1), estimate the population-level impact of rotavirus vaccine introduction in Matlab, Bangladesh (Aim 2), and assess the role of host genetic determinants in rotavirus vaccine failure (Aim 3).

Methods: Each aim of the dissertation uses a separate data source. For Aim 1, test-negative vaccine effectiveness (VE-TND) estimates were derived from three large randomized placebo-controlled trials of RV1 and RV5 in sub-Saharan Africa and Asia. Derived VE-TND estimates were compared to the original RCT vaccine efficacy estimates (VE-RCTs). The core assumption of the TND (i.e., rotavirus vaccine has no effect on rotavirus-negative diarrhea) was also assessed. For Aim 2, interrupted time series were used to estimate the impact of RV1 introduction in Matlab, Bangladesh among children <2 years of age. Analyses were conducted using diarrheal surveillance data collected between 2000 and 2014 within the two service delivery areas (icddr,b service area [ISA] and government service area [GSA]) of the Matlab Health and Demographic Surveillance System. Age-group specific incidence rates were calculated for both rotavirus-positive (RV+) and rotavirus-negative (RV-) diarrhea. For Aim 3, conditional logistic regression was used to assess the relationship between Secretor and Lewis phenotypes and rotavirus vaccine failure. Analyses were conducted among children 3-<24 months of age enrolled within the Vaccine Impact on Diarrhea in Africa (VIDA) study, a large case-control study in The Gambia, Mali, and Kenya assessing diarrheal etiologies.

Results: For Aim 1, TND vaccine effectiveness estimates were nearly equivalent to original RCT estimates. Neither rotavirus vaccine had a substantial effect on rotavirus-negative diarrhea. For Aim 2, we observed a downward trend in RV+ diarrhea incidence among children from ISA villages presenting to Matlab Hospital during approximately 3.5 years of routine RV1 use. Significant impact of RV1 on RV+ diarrhea incidence among children from GSA villages was not observed. Differences in population-level impact between ISA and GSA villages may be due to lower rotavirus vaccine coverage in GSA villages and a lower presentation rate to the hospital. For Aim 3, preliminary results demonstrated null phenotypes reduced the risk of rotavirus

vaccine failure in a population with mostly P[8] infections, but serotype-specific results could not be estimated.

Conclusions: The findings of this dissertation support the TND as an appropriate epidemiologic study design to measure rotavirus vaccine effectiveness in low-income settings, provide additional evidence of a decrease in rotavirus diarrhea burden in Asia after vaccine introduction, and explored a preliminary analysis of the relationship between host genetic determinants and rotavirus vaccine failure. Together these results show the public health success of rotavirus vaccines in reducing averting severe rotavirus diarrhea cases in regions with the greatest burden. The results also highlight the need for additional research to understand the effectiveness and population-level impact of these vaccines in understudied regions and the interplay of risk factors contributing to moderate vaccine effectiveness.

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CHAPTER 1: INTRODUCTION

Diarrheal disease is associated with approximately 446,000 deaths globally in children <5 years of age with most of the burden in low-income settings, including sub-Saharan African and Asia [1]. A variety of pathogens are associated with diarrhea in children, but in most countries rotavirus is the leading cause of severe diarrhea in young children [2,3]. An estimated 200,000 deaths due to rotavirus diarrhea occur annually in children [4].

Rotavirus is a double stranded RNA virus transmitted via the fecal-oral route. Without a vaccine, nearly all children <5 years old become infected with rotavirus and recurrent infection is common [5]. A range of symptoms is associated with infection. While some children are asymptomatic or are symptomatic with mild self-limiting diarrhea, severe cases lead to dehydrating diarrhea with fever and vomiting [5]. Improvements in water and sanitation have not significantly impacted rotavirus transmission, therefore effective rotavirus vaccines continue to be the key intervention needed to reduce the global burden of severe rotavirus diarrhea [6].

Starting in 2006, two rotavirus vaccines were introduced worldwide: GlaxoSmithKline's live-attenuated human monovalent vaccine (Rotarix [RV1]) and Merck's live-attenuated pentavalent human-bovine reassortant vaccine (RotaTeq [RV5]). Large multi-site randomized controlled trials (RCTs) of both vaccines demonstrated moderate vaccine efficacy (VE) against severe rotavirus diarrhea during the first year of life in Africa (RV5 [VE: 64.2, 95% confidence interval (CI): 40.2-79.4)], RV1 [VE: 61.2, 95%CI: 44.0-73.2]) and Asia (RV5 [VE: 51.0, 95%CI: 12.8-73.3]) [7–9]. These results were lower than RCTs assessing the same vaccines in high-resource settings (VE: 98.0, 95%: CI: 88.3-11) [10]. However, due to the substantial burden of rotavirus diarrhea incidence and mortality in low-income settings, the WHO recommended worldwide use of rotavirus vaccines in 2009 [11]. As of March 2018, 93 countries including 43 Gavi-eligible

countries have introduced a rotavirus vaccine into its national immunization schedule [12]. Most countries have introduced RV1 or RV5 while India, China, and Vietnam have also licensed locally developed vaccines [13]. Many high-burden countries have not introduced the vaccine and approximately 70% of the world's infants still do not have access to a rotavirus vaccine [14].

Accurate post-introduction monitoring of effectiveness measures after vaccine introduction is important as results can influence the adoption of rotavirus vaccines in new areas and sustain support in countries where vaccines have been introduced. Case-control studies are an efficient means to monitor effectiveness and provide confidence in vaccine performance. In low-income settings, identifying community controls, either using a demographic surveillance system or sampling the community in-person, can be impractical and expensive. Hospital controls can be used to minimize bias due to healthcare seeking behavior. However, for rotavirus vaccine studies, careful consideration must be made to use hospital controls without diarrhea or any illness associated with vaccine-preventable diseases. The test-negative design (TND) can theoretically overcome the limitations of both traditionally-used control groups by using diarrhea patients that test negative for rotavirus to estimate underlying vaccine coverage in the population [15].

De Serres et al. validated the TND for influenza vaccine utilizing RCT databases to verify the accuracy and precision of TND estimates and to test the assumption that the vaccines had no effect on non-influenza respiratory illness [16]. The TND is being increasingly used to estimate rotavirus vaccine effectiveness in middle- and low-income settings due to its low cost and feasibility [17–38], but little has been done to assess this design in the context of rotavirus vaccine effectiveness in low-income settings.

Most rotavirus vaccine studies have been conducted in sub-Saharan Africa and show significant rotavirus vaccine effectiveness and population-level impact against all-cause and rotavirus diarrhea in children within 2-3 years of initiation of routine use [23,28–32,35–46]. Despite the WHO recommendation for rotavirus vaccine use worldwide, only 2 of 11 countries in the WHO South East Asian region have introduced a rotavirus vaccine [12]. Thailand introduced RV1 sub-nationally in September 2012 [47]. In 2016, India began a phased introduction of Rotavac [48]. Limited data on vaccine effectiveness and population impact may have slowed the introduction of rotavirus vaccines in Asia [49]. The only multi-site RCT of RV5 in Asia demonstrated moderate vaccine efficacy against severe rotavirus gastroenteritis in the first two years of life (Bangladesh VE: 42.7%, 95% CI: 10.4-63.9, Vietnam VE: 63.9%, 95% CI: 7.6-90.9, Combined VE: 48.3, 95% CI: 22.3-66.1) [9] and similar efficacy results were observed in India when assessing Rotavac and Rotasiil [48,50].

A two-year cluster-randomized trial (CRT) of RV1 was conducted in Matlab, Bangladesh, between 2008 and 2010 [51]. Overall effectiveness, which assesses the overall reduction in incidence of acute rotavirus diarrhea regardless of vaccination status, was 29.0% (95%CI: 11.3-43.1) in children <2 years of age. This study provided initial evidence of the potential population impact of rotavirus vaccine use in Bangladesh. To our knowledge, no observational studies of routine rotavirus vaccine use have been conducted in Asia. An improved understanding of the population-level impact of routine rotavirus vaccine use in Asia is essential.

Regardless of world region, studies in low-income settings have consistently observed moderate rotavirus vaccine effectiveness in children <2 years of age. It is a highly problematic issue that the direct effect of these vaccines is lowest in the countries with the greatest diarrheal burden. A

better understanding of risk factors for rotavirus vaccine failure, that is developing rotavirus diarrhea after a full course of immunizations, is needed to guide interventions that could improve vaccine performance in these settings. Previously-studied risk factors for rotavirus vaccine failure include high maternal rotavirus antibodies during pregnancy and breastfeeding [52–59], concomitant oral polio vaccine administration [60–65], malnutrition [66,67], a high burden of co-enteric pathogens [68–70], and the microbiome [71,72]. However, these factors alone do not fully explain the reduced rotavirus efficacy in low-income settings. One novel risk factor is the potential role of genetically determined susceptibility to rotavirus infection in rotavirus vaccine failure.

Specifically, individuals with genetic mutations associated with susceptibility to rotavirus infection may be at increased risk for rotavirus vaccine failure. Previous studies identified *FUT2* (Secretor) and *FUT3* (Lewis) genes and the associated development of histo-blood group antigens (HBGAs) to be important for rotavirus infection [73–77]. Rotavirus serotype diversity [78–80] coupled with an increased frequency of these genetic mutations in sub-Saharan Africa [81,82] may lead to a greater risk of rotavirus vaccine failure in this region. Limited studies have examined the association between genetically determined resistance to rotavirus, as measured by Secretor and Lewis phenotypes, and rotavirus vaccine failure in low-income settings. Understanding these genetic mutations as potential risk factor for rotavirus vaccine failure helps answer important remaining scientific questions related to low vaccine efficacy in sub-Saharan Africa and may inform future rotavirus vaccine development.

This dissertation addresses several key questions regarding rotavirus vaccines in low-income settings. The aims of the dissertations are as follows:

1) To evaluate the test-negative design as an epidemiologic study design to measure rotavirus vaccine effectiveness in low-income settings.
2) To estimate the relative reduction, or population-level impact, of rotavirus-positive and rotavirus-negative diarrhea incidence after rotavirus vaccine introduction in Matlab, Bangladesh in children <2 years of age.
3) To test the association between null histo-blood group antigen phenotypes (Secretor and Lewis status) and rotavirus diarrhea among children immunized with rotavirus vaccines in The Gambia, Mali, and Kenya.

CHAPTER 2: ROTAVIRUS VACCINE EFFECTIVENESS IN LOW-INCOME SETTINGS: AN EVALUATION OF THE TEST-NEGATIVE DESIGN

2.1 Introduction

Globally, an estimated 200,000 deaths due to rotavirus diarrhea occur annually in children <5 years old, with a majority of the burden in low-income settings [4]. Starting in 2006, two rotavirus vaccines have been introduced worldwide; GlaxoSmithKline's live-attenuated human monovalent vaccine (Rotarix [RV1]) and Merck's live-attenuated pentavalent human-bovine reassortant vaccine (RotaTeq [RV5]). Large multi-site randomized controlled trials (RCTs) of RV1 and RV5 in low-income settings have demonstrated moderate vaccine efficacy against severe rotavirus gastroenteritis in the first year of life (VE: 51-64%) [7–9,83,84]. As of March 2018, 93 countries, including 43 Gavi-eligible countries, have introduced a rotavirus vaccine into its national immunization schedule [12]. However, many high-burden countries have not introduced the vaccine and approximately 70% of the world's infants still do not have access to rotavirus vaccine [14]. Accurate post-introduction monitoring of effectiveness measures is important as results can influence the adoption of rotavirus vaccines in new areas and sustain support in countries where vaccines have been introduced.

Case-control studies are an efficient means to monitor effectiveness and provide confidence in vaccine performance. In low-income settings, identifying community controls, either using a demographic surveillance system or sampling the community in-person, can be impractical and expensive. Hospital controls can be used to minimize bias due to healthcare seeking behavior. However, for rotavirus vaccine studies, careful consideration must be made to use hospital controls without diarrhea or any illness associated with vaccine-preventable diseases. The test-negative design (TND) can theoretically overcome the limitations of both traditionally-used control groups, while also limiting bias due to healthcare seeking behavior [15]. TND rotavirus

vaccine studies enroll cases presenting to a medical facility for acute gastroenteritis and are rotavirus-positive using standard laboratory methods. Controls include those presenting to a medical facility with the same pre-defined case definition of acute gastroenteritis, but are rotavirus-negative. Both traditional case-control and test-negative study designs require rotavirus testing on infants presenting to the clinic with diarrhea to identify cases. The TND is efficient and cost-effective in that those testing-negative for rotavirus serve as the control group, instead of being excluded from the study.

The TND has been used extensively to measure annual influenza vaccine effectiveness [15,85]. Simulation experiments have validated the test-negative design for influenza vaccine under specific core assumptions: 1) vaccine has no effect on the incidence of non-influenza pathogens, 2) a highly sensitive and specific laboratory test is used for pathogen detection, and 3) other sources of bias present in observational studies are minimized [15,85–90]. De Serres et al. validated the TND for influenza vaccine utilizing RCT databases to verify the accuracy and precision of TND estimates and to test the assumption that the vaccines had no effect on non-influenza respiratory illness [16]. RCTs are appropriate to validate this design due to limited selection bias and confounding as a result of randomization and blinding, the use of standardized laboratory testing, and enhanced surveillance. Derived test-negative vaccine effectiveness estimates for influenza vaccines were almost identical to the original RCT vaccine efficacy estimates. Importantly, the vaccine coverage in the test-negative controls represented the vaccine coverage in the underlying study population, upholding the key assumption that the vaccine had no effect on non-influenza illness. Together, these results indicated the TND was a valid epidemiologic study design to measure influenza vaccine effectiveness [16].

The TND is being increasingly used to estimate rotavirus vaccine effectiveness in middle- and low-income settings due to its low cost and feasibility [17–38], but little has been done to assess this epidemiologic study design in the context of rotavirus vaccine effectiveness in low-income settings. In the present analysis, RCT databases for RV1 and RV5 in sub-Saharan Africa and Asia were used to evaluate the TND.

2.2 Methods

2.2.1 Participants and Study Design

Databases from three multi-center, double-blind, individual-randomized, placebo-controlled, trials of rotavirus vaccines in sub-Saharan Africa and Asia were used [7–9,83,84]. Table 2.1 summarizes location, vaccine schedule, per-protocol population size, and surveillance type of the three RCTs.

RV1

This trial was conducted in South Africa and Malawi. Between 2005 and 2007, 4,939 healthy infants aged 5 to 10 weeks were randomly assigned to one of three groups in a 1:1:1 ratio: two doses of RV1, three doses of RV1, or three doses of placebo. Gastroenteritis was defined as three or more loose or watery stools within 24 hours. Clinical characteristics of each diarrheal episode were documented to define severity based on the Vesikari score [91]. Stool samples were tested for rotavirus using enzyme-linked immunosorbent assay (ELISA). The primary outcome was at least one episode of severe rotavirus gastroenteritis (Vesikari score ≥11). Vaccine efficacy was estimated during the period from two weeks after the last dose until the first year of age. Within each study site, a sub-cohort was followed into the second year of life. The mean age at the end of follow-up was 14 months and 19 months for South Africa and Malawi, respectively.

RV5

Two trials of RV5 were conducted in sub-Saharan Africa and Asia between 2007 and 2009. Both trials were conducted under similar protocols; however, the trials were powered and implemented separately. In sub-Saharan Africa, 5,468 healthy infants were enrolled in Ghana, Kenya, and Mali. In Asia, 2,036 healthy infants were enrolled in Bangladesh and Vietnam. Infants aged 4 to 12 weeks were randomly assigned to one of two groups in a 1:1 ratio: three doses of RV5 or three doses of placebo. As in the RV1 trial, severe rotavirus gastroenteritis was defined based on a positive ELISA laboratory result and Vesikari score ≥11. Vaccine efficacy was estimated during the period from two weeks after the last dose until the end of follow-up (March 31, 2009). The mean age at the end of follow-up was 20 months and 19 months for sub-Saharan Africa and Asia, respectively.

For the purposes of this analysis, participants with an episode of severe diarrhea meeting the pre-defined case definition and with an available ELISA test result were categorized as a case if the test was positive for rotavirus or a control if the test was negative for rotavirus. Continuous diarrheal surveillance during the study period allowed for the identification of multiple diarrheal episodes for each participant. A participant was defined as a case if at least one severe rotavirus-positive diarrheal episode occurred during follow-up. A participant was defined as a control if at least one severe rotavirus-negative diarrheal episode occurred during follow-up and the participant had no severe rotavirus-positive episodes.

2.2.2 Statistical Analysis

Logistic regression was used to estimate the relative odds and associated 95% confidence intervals (CI) of severe rotavirus-positive diarrhea compared to severe rotavirus-negative diarrhea by vaccine and placebo status. TND vaccine effectiveness (VE-TND) was defined as (1-

Odds Ratio) X 100. The relative percent difference between VE-TND and the original RCT vaccine efficacy (VE-RCT) was calculated. To estimate the influence of vaccine on rotavirus-negative diarrhea (VE-NEG), the relative risk of severe rotavirus-negative diarrhea in the vaccine group compared to the placebo group with exact 95% CIs were calculated. VE-NEG was defined as (1- Relative Risk) X 100.

The analysis was based on the per-protocol participant populations. The primary analysis of each RCT, combining country-level estimates, was replicated. Additionally, analyses were stratified by country. The RV1 trial was powered to estimate vaccine efficacy for two and three doses separately, therefore analyses were replicated by these dosing combinations. Each trial included diarrheal surveillance on all or a subset of participants into the second year of life. Analyses were conducted for the complete follow-up period (<2 years of age) and separately for diarrheal episodes identified during the first (<1 years of age) and second (1-<2 years of age) years of life. Analyses conducted within the second year of life did not exclude participants with diarrheal episodes during the first year of life. For the RV1 trial, different methods of enrollment into the second year were used for each country; therefore, analyses for the second year of life and complete follow-up were conducted separately for South Africa and Malawi.

In practice, differences in age and time at presentation between cases and test-negative controls are controlled by matching or by adjusting analyses by both month and year of birth and month and year of presentation. Additional analyses were restricted by rotavirus season in Ghana and Mali due to the observed seasonality in these regions. To replicate the primary efficacy results of the RV5 trial, diarrheal episodes identified during the rotavirus seasons in Ghana and Mali were

combined with the year-round diarrheal episodes in Kenya to estimate country-combined estimates.

Analyses were completed using STATA version 14 (Stata Corporation, College Station, TX, USA) and the SAS Clinical Trial Data Transparency software system through the online GSK portal (SAS Institute Inc., Cary, NC, USA).

2.3 Results

Table 2.2 shows the derived test-negative vaccine effectiveness estimates (VE-TND), the original RCT vaccine efficacy estimates (VE-RCT), and the relative percent difference between these estimates in the RV1 trial. During the first year of life (<1 years of age), the country-combined and country-specific efficacy and effectiveness estimates for all doses were similar (two or three doses combined in South Africa and Malawi: VE-TND: 58.2% [95%CI: 35.5-72.9]; VE-RCT: 61.2% [95%CI: 44.0-73.2]; -4.9% relative difference). The sub-cohort in South Africa followed over two rotavirus seasons yielded a low sample of rotavirus-positive cases resulting in less robust estimates during both the second year of life (1-<2 years of age) and using complete follow-up (<2 years of age). The sub-cohort in Malawi followed over two rotavirus seasons resulted in VE-TND and VE-RCT estimates which were not meaningfully different during any periods of follow-up.

VE-TND and VE-RCT estimates for the RV5 trial in sub-Saharan Africa are shown in Table 2.3. Using diarrheal episodes identified in the first year of life (<1 years of age), the VE-TND and the VE-RCT are almost identical, particularly in the country-combined estimate (VE-TND: 66.9% [95%CI: 42.7-80.9]; VE-RCT: 64.2% [95%CI: 40.2-79.4]; 4.2% relative difference). In the second year of life (1-<2 years of age) the VE-TND was greater than the VE-RCT for both

country-combined and country-specific estimates in the second year (combined African study sites: VE-TND: 39.4% [95%CI: 5.0-61.4]; VE-RCT: 19.6% [95%CI: -15.7-44.4]; 101.0% relative difference). Using complete follow-up (<2 years of age), the VE-TND was moderately greater than the VE-RCT in all study settings (combined African study sites: VE-TND: 51.9% [95%CI: 32.1-65.9]; VE-RCT: 39.3% [95%CI: 19.1-54.7]; 32.1% relative difference).

In this trial, the rotavirus season in Ghana occurred between January and March. After restricting the analysis to diarrheal episodes during this time period (Table 2.3), there was no meaningful difference between the VE-TND and VE-RCT estimates (VE-TND: 56.1% [95%CI: -8.3-82.2]; VE-RCT: 55.5% [95%CI: 28.0-73.1]; 1% relative difference). Similarly, after restricting the analysis in Mali to the rotavirus season (October through February), the relative difference between VE-TND and VE-RCT estimates decreased (VE-TND: 9.4% [95%CI: -73.3-52.7]; VE-RCT: 17.6% [95%CI: -22.9-45.0]; -46.6% relative difference). In both rotavirus-season restricted analyses, sample size decreased substantially. Combining diarrheal episodes in Ghana and Mali during their respective rotavirus seasons with year-round diarrheal episodes in Kenya, resulted in a 5.3% relative difference between country-combined VE-TND and VE-RCT estimates.

Table 2.4 shows the VE-TND and VE-RCT estimates for the RV5 trial in Asia. VE-TND and VE-RCT estimates were not meaningfully different in country-combined analyses or during any periods of follow-up (combined Asian study sites using complete follow-up (<2 years of age): VE-TND: 49.8% [95%CI: 14.6-70.5]; VE-RCT: 48.3% [95%CI: 22.3-66.1]; 3.1% relative difference).

Figure 2.1 summarizes the VE-TND and VE-RCT estimates and overlapping 95% CIs for all RCTs during the first year of life and using the complete follow-up (<2 years of age).

Table 2.5 shows both RV1 and RV5 vaccine efficacy against severe rotavirus-negative diarrhea (VE-NEG). In the RV1 trial, the magnitude of VE-NEG was greatest in South Africa during the second year of follow-up with three doses of vaccine (54.0% [95%CI: 7.1-77.2]), while all other age and dose combinations had a low VE-NEG. In the RV5 trial in sub-Saharan Africa, the magnitude of the VE-NEG was greatest in Ghana during the second year of life (1-<2 years of age: -50.5 [95%CI: -170.9-14.9]), and using complete follow-up (<2 years of age: -49.1 [95%CI: -117.9- -2.7]), compared to other settings. The country-combined VE-NEG was statistically significant using complete follow-up. After restricting the analysis to the rotavirus season using complete follow-up, the VE-NEG decreased in all RV5 settings with no statistically significant estimates. In the RV5 trial in Asia, the magnitudes of the country-combined VE-NEG estimates were low (<20%) during all follow-up periods. Due to the low magnitude of each VE-NEG, the ratio of vaccine: placebo test-negative controls replicated the 2:1 or 1:1 randomized vaccine coverage of the underlying study population in most study settings.

2.4 Discussion

Overall, the results from the TND analysis for RV1 and RV5 in sub-Saharan Africa and Asia were similar to primary efficacy results. The heterogeneity of vaccine effectiveness estimates between countries was also observed. In countries with a marked rotavirus season, estimates were not meaningfully different after restricting analyses to these time periods. We also demonstrated that RV1 and RV5 had no effect on severe rotavirus-negative diarrhea, a key assumption of the TND.

VE-TND during the first year of life was comparable to VE-RCT for all three trials. The control group accurately represented the vaccine coverage of the underlying study population. For

example, in the RV1 trial the ratio of vaccinated (two doses) controls to placebo controls is nearly 1:1 (93:91), the randomized vaccine: placebo ratio. In all trials, VE-TND estimates during the second year of life were largely limited by the low number of diarrheal episodes and rotavirus-positive cases identified. Primary VE-RCTs for the second year of life were not statistically significant and this was replicated in the VE-TND results. The sustained effect of rotavirus vaccine during the second year of life remains unclear [83,92].

In the analysis using complete follow-up (<2 years of age), the VE-TND was similar to the VE-RCT in RV1 and RV5- Asia. Differences between the estimates were demonstrated in the RV5 trial in sub-Saharan Africa for country-combined and country-specific estimates. Ghana and Mali have distinct rotavirus seasons. Analyses were restricted to the rotavirus season in order to obtain time-matched controls. This strategy better emulates the practice of incidence density sampling and provides results that are closer to the relative risk (and in turn VE=1-Relative Risk) derived from an RCT. These results support the importance of temporally matched controls or accounting for timing of birth and case presentation in the TND to obtain accurate vaccine effectiveness estimates.

Generally, less robust VE-TND and VE-RCT estimates in Mali are in part due to changes in surveillance after the first year of the study, which initially missed most of the rotavirus season and yielded a low number of rotavirus cases. Surveillance and community engagement increased during the second year of the trial to increase case capture [8]. Notably, during the first year the ratio of vaccinated controls to placebo controls is almost exactly 1:1 (48:49), indicating the surveillance appropriately identified the underlying vaccine coverage in test-negative controls during this time.

Further evidence of the accuracy of the test-negative control group is demonstrated in rotavirus vaccine case-control studies using multiple control groups in sub-Saharan Africa and Latin America [18,20,21,24,93]. In Malawi, RV1 effectiveness for severe rotavirus-positive diarrhea during the first year of life was similar using test-negative controls and community controls (VE-TND: 68%, VE-Community controls: 68%) [18]. In South Africa, RV1 effectiveness for hospital admission with rotavirus-positive diarrhea in children <2 years old was comparable using test-negative controls and hospitalized controls (VE-TND: 57%, VE-Hospital controls: 63%) [21]. In Bolivia, RV1 effectiveness for severe rotavirus-positive diarrhea during the first year of life was moderately different using test-negative controls and hospitalized controls (VE-TND: 66%, VE-Hospital controls: 78%) [93]. In Nicaragua, RV5 effectiveness for severe rotavirus-positive diarrhea during the first year of life was slightly different using test-negative controls and a combined group of hospitalized and community controls (VE-TND: 70%, VE-Hospital/Community controls: 83%). In children >1 years old, the difference in estimates was more substantial (VE-TND: 33%, VE-Hospital/Community controls:70%) [20]. Authors suggest the combined control group differed from the test-negative and test-positive participants, likely due to healthcare seeking behaviors. In Guatemala RV1 and RV5 effectiveness against a rotavirus-positive diarrheal episode resulting in a hospital visit was moderately different using test-negative controls and hospitalized controls (VE-TND: 52%, VE-Hospital controls: 74%) [24]. All studies observed vaccine effectiveness results similar to efficacy estimates of RCTs conducted in Latin America.

Importantly, while the TND can be valuable, this study design is susceptible to biases present in all observational studies including selection bias, confounding, and misclassification of vaccine

and rotavirus status [90]. In these RCTs we are highly confident of accurate vaccine ascertainment, but this can be problematic in the field with missing documentation, unreliable parental recall, and the added complexities of multiple doses [94]. Additionally, the sensitivity and specificity of the test to identify the etiologic pathogen is especially important when using the TND. A test with low specificity influences results more substantially than a test with low sensitivity [86]. All RCTs and case-control studies used ELISA for rotavirus detection. While RT-PCR is the gold standard, ELISA has high sensitivity (75-82%) and specificity (100%) to identify rotavirus [95]. A simulation study comparing true and estimated TND vaccine effectiveness results based on varying rotavirus test characteristics and attack rates demonstrated minimal bias with the currently used ELISA [96].

This analysis evaluated the use of the TND to estimate rotavirus vaccine effectiveness in low-income settings. Three separate rotavirus vaccine trials, testing two vaccines in seven countries, showed TND vaccine effectiveness estimates were nearly identical to the primary efficacy estimates of the original RCTs. The key assumption of the TND, the vaccine has no impact on rotavirus-negative diarrhea, was also upheld. This study supports the test-negative design as an appropriate method to measure rotavirus vaccine effectiveness in low-income settings.

2.5 Tables and Figures

Table 2.1: Summary of Rotavirus Vaccine Clinical Trials in Low-Income Settings

Vaccine	Dosing Schedule	Surveillance Type	Study Site	Age during follow-up	Primary per-protocol population (Vaccine/Placebo)	Country specific per-protocol population (Vaccine/Placebo)	Reference
Rotarix (RV1)	6, 10, 14 weeks or 10,14 weeks	Active: Scheduled weekly home visits and clinic visits	South Africa	<1 Years	2974/1443	1944/960	[7]
			Malawi			1030/483	
			South Africa	1-<2 Years	*	686/332	[83]
			Malawi			814/380	[84]
RotaTeq (RV5)	6, 10, 14 weeks	Passive: clinic visits	Ghana	<2 Years	2404/2385	940/930	[8]
			Kenya			573/577	
			Mali			891/878	
RotaTeq (RV5)	6, 10, 14 weeks	Passive: clinic visits	Bangladesh	<2 Years	995/988	557/561	[9]
			Vietnam			438/427	

* Vaccine efficacy was estimated separately in South Africa and Malawi for the second year of this study.

Table 2.2: RV1 Test-Negative Results

Study Site	Age (Years)	Doses	Cases*	Controls*	VE-TND (95%CI)		VE-RCT (95%CI)		% Relative Difference**
South Africa and Malawi	<1	2 or 3 doses	56/70	174/91	58.2	(35.5-72.9)	61.2	(44.0-73.2)	-4.9
		2 doses	30/70	93/91	58.1	(29.7-75.0)	58.7	(35.7-74.0)	-1.0
		3 doses	26/70	81/91	58.3	(28.3-75.7)	63.7	(42.4-77.8)	-8.5
South Africa	<1	2 or 3 doses	15/32	46/25	74.5	(44.3-88.4)	76.9	(56.0-88.4)	-3.1
		2 doses	9/32	24/25	70.7	(25.9-88.4)	72.2	(40.4-88.3)	-2.1
		3 doses	6/32	22/25	78.7	(39.5-92.5)	81.5	(55.1-93.7)	-3.4
Malawi	<1	2 or 3 doses	41/38	128/66	44.4	(5.3-67.3)	49.4	(19.2-68.3)	-10.1
		2 doses	21/38	69/66	47.1	(0.7-71.9)	49.2	(11.1-71.7)	-4.3
		3 doses	20/38	59/66	41.1	(-12.3-69.1)	49.7	(11.3-72.7)	-17.3
South Africa	1-<2	2 or 3 doses	5/4	32/23	10.2	(-271.6-78.3)	40.0	(-204.0-87.0)	-74.5
		2 doses	4/4	21/23	-9.5	(-394.2-75.7)	3.0	(-43.0-82.0)	-416.7
		3 doses	¼	11/23	47.4	(-424.6-94.8)	76.0	(-143.0-100.0)	-37.6
Malawi	1-<2	2 or 3 doses	30/17	89/45	10.8	(-78.7-55.5)	17.6	(-59.2-56.0)	-38.6
		2 doses	18/17	44/45	-8.3	(-136-50.5)	2.6	(-101.2-52.6)	-419.2
		3 doses	12/17	45/45	29.4	(-64.6-68.7)	33.1	(-48.6-70.9)	-11.2
South Africa	<2	2 or 3 doses	11/13	58/32	53.3	(-16.2-81.2)	59.0	(1.0-83.0)	-9.7
		2 doses	9/13	38/32	41.7	(-54.0-77.9)	32.0	(-71.0-75.0)	30.3
		3 doses	2/13	20/32	75.4	(-20.7-95.0)	85.0	(35.0-98.0)	-11.3
Malawi	<2	2 or 3 doses	69/53	183/90	36.0	(0.8-58.7)	38.1	(9.8-57.3)	-5.5
		2 doses	38/53	96/90	32.8	(-11.5-59.5)	34.0	(-2.0-57.7)	-3.5
		3 doses	31/53	87/90	39.5	(-3.0-64.5)	42.3	(8.8-64.0)	-6.6

*No. Vaccine/No. Placebo

**VE-TND compared to VE-RCT

Cases: severe (Vesikari ≥11) rotavirus-positive diarrhea

Controls: severe rotavirus-negative diarrhea

VE-TND: Vaccine effectiveness against severe rotavirus diarrhea using the test-negative design

VE-RCT: Vaccine efficacy against severe rotavirus diarrhea- original randomized control trial estimates

Table 2.3: RV5 Test-Negative Results in sub-Saharan Africa

Study Site	Age (Years)	Cases*	Controls*	VE-TND (95%CI)		VE-RCT (95%CI)		% Relative Difference**
African Study Sites	<1	23/61	115/101	66.9	(42.7-80.9)	64.2	(40.2-79.4)	4.2
Ghana		16/42	44/36	68.8	(35.6-84.9)	65.0	(35.5-81.9)	5.8
Kenya		2/14	23/16	90.1	(50.1-98.0)	83.4	(25.5-98.2)	8.0
Mali		5/5	48/49	-2.1	(-275.3-72.2)	1.0	(-431.7-81.6)	-310.0
African Study Sites	1-<2	57/71	110/83	39.4	(5.0-61.4)	19.6	(-15.7-44.4)	101.0
Ghana		11/15	33/22	51.1	(-26.0-81.0)	29.4	(-64.6-70.7)	73.8
Kenya		3/2	8/11	-106.3	(-1435.7-72.3)	-54.7	(-1752.7-82.3)	94.3
Mali		43/54	69/50	42.3	(0.9-66.4)	19.2	(-23.1-47.3)	120.3
African Study Sites	<2	80/132	208/165	51.9	(32.1-65.9)	39.3	(19.1-54.7)	32.1
Ghana		27/57	74/50	68.0	(42.7-82.1)	55.5	(28.0-73.1)	22.5
Kenya		5/16	27/25	71.1	(9.3-90.8)	63.9	(-5.9-89.8)	11.3
Mali		48/59	107/90	31.6	(-9.8-57.4)	17.6	(-22.9-45.0)	79.5
Restricted to Rotavirus Season								
African Study Sites[a]	<2	69/123	68/71	41.4	(6.4-63.3)	39.3	(19.1-54.7)	5.3
Ghana[a]		22/54	13/14	56.1	(-8.3-82.2)	55.5	(28.0-73.1)	1.1
Kenya		5/16	27/25	71.1	(9.3-90.8)	63.9	(-5.9-89.8)	11.3
Mali[a]		42/53	28/32	9.4	(-73.3-52.7)	17.6	(-22.9-45.0)	-46.6

*No. Vaccine/No. Placebo

**VE-TND compared to VE-RCT

Cases: severe (Vesikari ≥11) rotavirus-positive diarrhea

Controls: severe rotavirus-negative diarrhea

VE-TND: Vaccine effectiveness against severe rotavirus diarrhea using the test-negative design

VE-RCT: Vaccine efficacy against severe rotavirus diarrhea- original randomized control trial estimates

[a] Cases/controls restricted to rotavirus season in Ghana (January –March) and Mali (October-February), but year-round in Kenya

Table 2.4: RV5 Test-Negative Results in Asia

Study Site	Age (Years)	Cases*	Controls*	VE-TND (95%CI)		VE-RCT (95%CI)		% Relative Difference**
Asian Study Sites		19/38	36/40	44.4	(-13.2-72.7)	51.0	(12.8-73.3)	-12.9
Bangladesh	<1	17/31	31/38	32.8	(-43.5-68.5)	45.7	(-1.2-71.8)	-28.2
Vietnam		2/7	5/2	88.6	(-10.8-98.8)	72.3	(-45.2-97.2)	22.5
Asian Study Sites		19/34	27/23	52.4	(-4.9-78.4)	45.5	(1.2-70.7)	15.2
Bangladesh	1-<2	16/25	23/18	42.3	(0.9-66.4)	39.3	(-18.3-69.7)	7.6
Vietnam		3/9	4/5	58.3	(-166.0-93.5)	64.6	(-47.7-93.9)	-9.8
Asian Study Sites		38/72	62/59	49.8	(14.6-70.5)	48.3	(22.3-66.1)	3.1
Bangladesh	<2	33/56	53/52	42.2	(-2.8-67.5)	42.7	(10.4-63.9)	-1.2
Vietnam		5/16	9/7	75.7	(0.6-94.1)	63.9	(7.6-90.9)	18.5

*No. Vaccine/No. Placebo

**VE-TND compared to VE-RCT

Cases: severe (Vesikari ≥11) rotavirus-positive diarrhea

Controls: severe rotavirus-negative diarrhea

VE-TND: Vaccine effectiveness against severe rotavirus diarrhea using the test-negative design

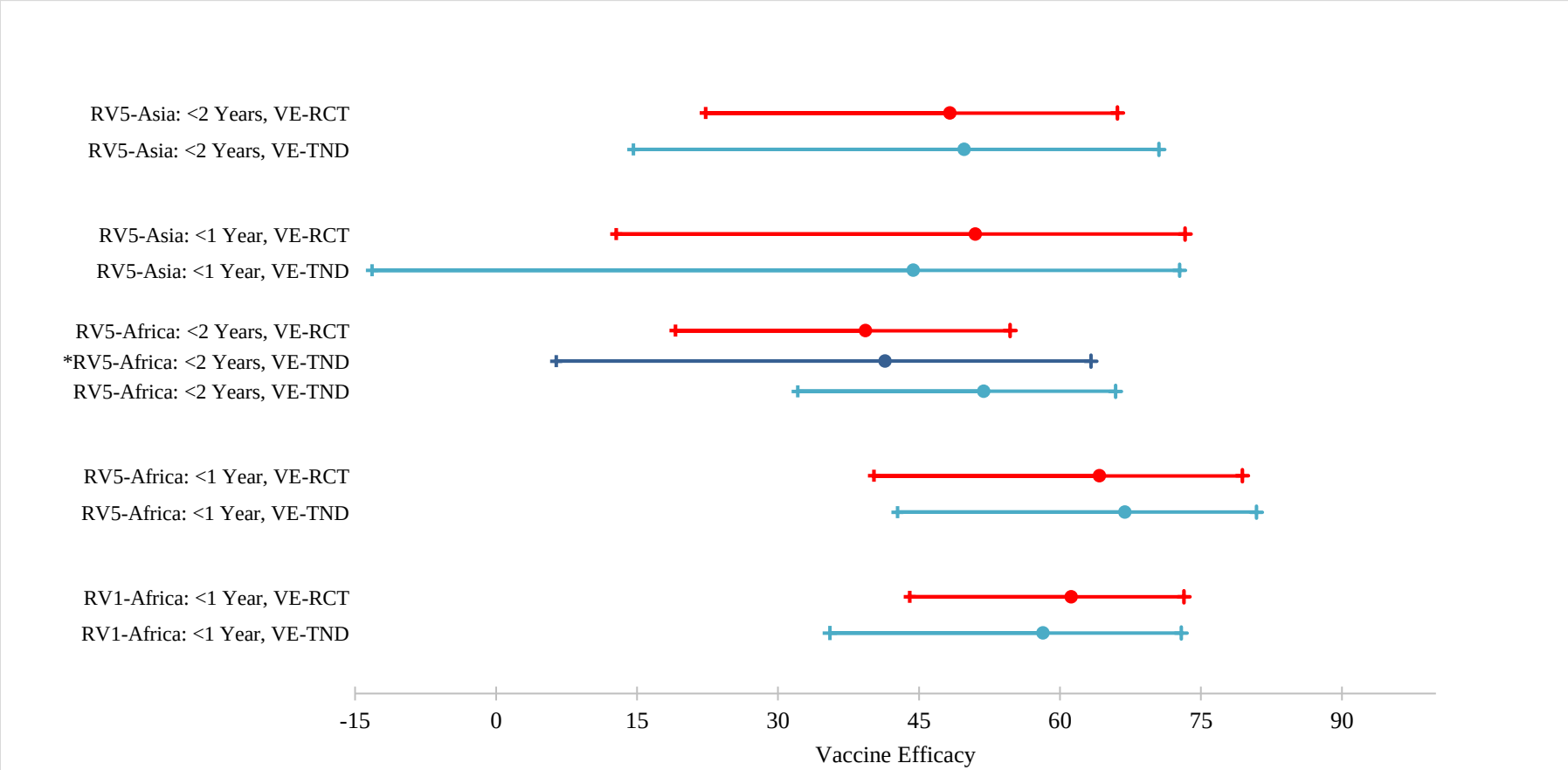
VE-RCT: Vaccine efficacy against severe rotavirus diarrhea- original randomized control trial estimates

Table 2.5: RV1 and RV5 Vaccine Efficacy Against Severe Rotavirus-Negative Diarrhea Results

Vaccine	Study Site	Age (Years)	Doses	VE-NEG (95%CI)	
RV1	South Africa and Malawi	<1	2 or 3 doses	7.2	(-18.6-27.4)
			2 doses	1.4	(-30.4-25.5)
			3 doses	13.1	(-16.2-35.0)
	South Africa	<1	2 or 3 doses	9.1	(-47.0-43.8)
			2 doses	5.1	(-65.0-45.4)
			3 doses	13.2	(-52.9-50.7)
	Malawi	<1	2 or 3 doses	9.1	(-19.9-31.0)
			2 doses	3.8	(-31.7-29.7)
			3 doses	14.5	(-18.7-38.4)
	South Africa	1-<2	2 or 3 doses	32.7	(-13.2-60.0)
			2 doses	11.1	(-57.5-49.8)
			3 doses	54.0	(7.1-77.2)
	Malawi	1-<2	2 or 3 doses	7.7	(-29.4-34.1)
			2 doses	10.0	(-33.1-39.2)
			3 doses	5.2	(-39.8-35.8)
	South Africa	<2	2 or 3 doses	12.3	(-32.9-42.1)
			2 doses	-15.9	(-81.8-26.1)
			3 doses	40.0	(-3.2-65.1)
	Malawi	<2	2 or 3 doses	4.7	(-19.8-24.1)
			2 doses	1.9	(-27.2-24.3)
			3 doses	7.5	(-20.7-29.2)
RV5	African Study Sites	<1	3 doses	-13.3	(-49.6-14.0)
	Ghana			-21.4	(-94.1-23.6)
	Kenya			-46.4	(-196.6-25.9)
	Mali			3.7	(-46.5-36.7)
	African Study Sites	1-<2	3 doses	-33.1	(-79.1-0.8)
	Ghana			-50.5	(-170.9-14.9)
	Kenya			27.3	(-98.5-74.6)
	Mali			-38.9	(-104.1-4.9)
	African Study Sites	<2	3 doses	-26.5	(-56.1- -2.6)
	Ghana			-49.1	(-117.9- -2.7)
	Kenya			-9.2	(-96.1-39.0)
	Mali			-18.4	(-58.5-11.4)
RV5: ***Restricted to Rotavirus Season***[a]	African Study Sites	<2	3 doses	5.1	(-34.3-32.9)
	Ghana			8.1	(-110.7-60.2)
	Kenya			-9.2	(-96.1-39.0)
	Mali			-13.9	(-47.5-50.1)
RV5	Asian Study Sites	<1	3 doses	10.8	(-43.6-44.7)
	Bangladesh			18.5	(-34.6-50.9)
	Vietnam			-145.3	(-2375.8-59.8)
	Asian Study Sites	1-<2	3 doses	-17.2	(-113.9-35.3)
	Bangladesh			-38.9	(-104.1-4.9)
	Vietnam			22.0	(-262.4-84.5)
	Asian Study Sites	<2	3 doses	-4.8	(-52.3-27.8)
	Bangladesh			-2.8	(-53.7-31.2)
	Vietnam			-26.0	(-298.3-58.2)

[a]Cases/controls restricted to rotavirus season in Ghana (January –March) and Mali (October-February), year-round in Kenya

Figure 2.1: VE-TND and VE-RCT estimates and 95% confidence intervals for each rotavirus vaccine RCT



*VE-TND is restricted to rotavirus season in Ghana (January-March) and Mali (October-February), but year-round in Kenya

CHAPTER 3: POPULATION-LEVEL IMPACT OF ROUTINE ROTAVIRUS VACCINE USE IN CHILDREN LESS THAN 2 YEARS OF AGE IN RURAL MATLAB, BANGLADESH

3.1 Introduction

Globally, an estimated 200,000 deaths due to rotavirus diarrhea occur annually in children <5 years of age, with most of the burden in sub-Saharan Africa and Asia [4]. While diarrhea associated mortality rates have decreased worldwide in the last decade, the burden of rotavirus diarrhea remains substantial in low-income settings [3]. Starting in 2006, two rotavirus vaccines were introduced worldwide: GlaxoSmithKline's live-attenuated human monovalent vaccine (Rotarix [RV1]) and Merck's live-attenuated pentavalent human-bovine reassortant vaccine (RotaTeq [RV5]). Large multi-site randomized controlled trials (RCTs) of both vaccines in Africa demonstrated vaccine efficacy (VE) against severe rotavirus diarrhea during the first year of life (RV5 [VE: 64.2, 95% confidence interval (CI): 40.2-79.4)], RV1 [VE: 61.2, 95%CI: 44.0-73.2]) [7,8]. As of March 2018, 93 countries, of which 43 are Gavi-eligible, have introduced rotavirus vaccines into their regional or national immunization programs [12]. In sub-Saharan Africa, 32 of 47 countries have introduced rotavirus vaccination. In this region, studies have shown significant rotavirus vaccine effectiveness and population-level impact against all-cause and rotavirus diarrhea in children <5 years of age within 2-3 years of initiation of routine use [23,39–46].

Despite the WHO recommendation for rotavirus vaccine use worldwide, only 2 of 11 countries in the WHO South East Asian region have introduced a rotavirus vaccine. Thailand introduced RV1 sub-nationally in September 2012 [47]. In 2016, India began a phased introduction of Rotavac, a locally developed oral rotavirus vaccine [48]. Limited data on vaccine effectiveness and population impact may have slowed the introduction of rotavirus vaccines in Asia [49]. The only multi-site RCT of RV5 in Asia demonstrated moderate vaccine efficacy against severe

rotavirus gastroenteritis in the first two years of life (Bangladesh VE: 42.7%, 95% CI: 10.4-63.9, Vietnam VE: 63.9%, 95% CI: 7.6-90.9, Combined VE: 51.0, 95% CI: 12.8-73.3) [9]. Similarly, RCTs in India of two new rotavirus vaccines, Rotavac and ROTASIIL, showed significant vaccine efficacy against severe rotavirus gastroenteritis during the first year of life (Rotavac VE: 53.6%, 95%CI: 35.0-66.9, ROTASIILVE:34.1%, 95%CI: 6.3-53.6) [48,50].

To evaluate the effectiveness of RV1 on rotavirus diarrhea in Asia, a two-year cluster-randomized trial (CRT) was conducted in Matlab, Bangladesh, beginning in 2008 [51]. Overall effectiveness, which assesses the overall reduction in incidence of acute rotavirus diarrhea regardless of vaccination status, was 29.0% (95%CI: 11.3-43.1) in children <2 years of age. This study provided initial evidence of the potential population impact of routine rotavirus vaccine use in Bangladesh. After the CRT, rotavirus vaccine was provided for routine use among infants in all Matlab villages between March 2011 and September 2014.

To evaluate the population-level impact of RV1 in Matlab, Bangladesh, during 3.5 years of routine use following the CRT, we examined rotavirus-positive (RV+) and rotavirus-negative (RV-) diarrhea incidence rate trends between February 2000 and September 2014.

3.2 Methods

3.2.1 Study Setting

The study utilized diarrheal surveillance data collected among children <2 years of age residing in villages of the Matlab Health and Demographic Surveillance System (Matlab HDSS), administered by the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), and presenting to Matlab Hospital. The Matlab HDSS covers a population of about 25,000 children <5 years of age in 142 villages. The HDSS was established in 1966 to accurately count

the population at risk during cholera vaccine trials [97]. The HDSS is divided into the icddr,b service area (ISA) (67 villages) and the government service area (GSA) (75 villages). icddr,b provides ISA villages with child and maternal health intervention programs and the Bangladesh Ministry of Health and Family Welfare provides GSA villages with the government standard of care. The HDSS maintains a census, registration of vital events, internal and external migration, and immunization records.

3.2.2 Diarrheal Surveillance

Matlab Hospital is the central diarrhea treatment facility for the Matlab HDSS population. Annually, free treatment is provided to about 30,000 diarrheal patients from the HDSS area of whom 60% are children <5 years old [98]. The present study includes children <2 years of age due to the high rotavirus incidence rate in this age group. Incidence for presentations to Matlab Hospital of all-cause diarrhea among children from GSA villages is about half of the incidence for presentations from ISA villages, likely due to location of the hospital (one section of the GSA is quite far from Matlab Hospital) and because GSA families have much less interaction with icddr,b staff and less familiarity with provided services. Patients seeking care from many GSA villages must travel a greater distance or cross the river [51].

All patients presenting with diarrhea (three or more loose stools per 24 hours) to Matlab hospital are included in the Diarrhoeal Disease Surveillance System (DDSS). As part of the DDSS, hospital staff systematically record clinical and demographic data from diarrheal patients. Stool specimens or rectal swabs are collected from all children. Samples are tested for group A rotavirus VP6 antigen using a solid phase sandwich type enzyme immunoassay (EIA) (Prospect™, Oxoid Diagnostics Ltd, Hampshire, United Kingdom).

3.2.3 Rotavirus Vaccine Coverage

Timing of rotavirus vaccine coverage ascertained through the HDSS database is shown in Figure 3.1. Rotavirus vaccine was not available in Matlab between February 2000 and March 2007. As part of a larger individually randomized controlled trial (RCT) in Bangladesh and Vietnam, between April 2007 and March 2009, 568 infants in only ISA villages were randomized to receive three doses of RV5 at 6, 10, and 14 weeks of age and 568 infants were randomized to placebo [9]. In a stratified RV1 CRT in both ISA and GSA areas, villages were randomized for introduction of infant vaccination with two doses of RV1 at 6 and 10 weeks of age or randomized as observed control-only villages [51]. In the GSA villages, the CRT started November 2008, before the end of the RCT in the ISA villages. In the ISA villages, the study started April 2009 after the RV5 RCT. During the RV1 CRT, icddr,b research staff consented and collected baseline demographic data prior to rotavirus vaccination in both ISA and GSA villages, but local health staff of icddr,b and the Government of Bangladesh administered vaccines in their respective areas. Follow-up and vaccination during the CRT occurred in both ISA and GSA villages through March 2011. Through a donation of vaccine post-CRT, RV1 was then provided routinely starting April 2011. After September 2014, rotavirus vaccine was unavailable, as the donation was exhausted and the Government of Bangladesh had not yet introduced rotavirus vaccination in its immunization program.

3.2.4 Statistical Analysis

Interrupted time series using segmented regression models were used to estimate the impact of rotavirus vaccine introduction in Matlab, Bangladesh, among children <2 years of age [99]. Due to varied rotavirus vaccine coverage and baseline diarrheal incidence, primary analyses were conducted within all villages in each service area (ISA villages, GSA villages) and secondary

models were assessed within ISA and GSA villages randomized as control-only during the CRT. Monthly incidence of RV+ and RV- diarrhea were examined separately by age group (0-<12 months, 12-<24 months, and combined, 0-<24 months). Incidence rates were calculated for RV+ and RV- diarrhea with the number of events presenting to Matlab Hospital per month as the numerator and the monthly population at-risk using HDSS census estimates as the denominator.

Among the ISA villages, the following time periods were defined as: pre-vaccine (February 2000-February 2007), RCT (March 2007-March 2009), CRT (April 2009-March 2011), RV1 introduction (April 2011-September 2014). Among the GSA villages, the following periods were defined as: pre-vaccine (February 2000-October 2008), CRT (November 2008 – March 2011), and RV1 introduction (April 2011-September 2014).

Two models (Model 1, Model 2) were used to estimate the impact of RV1 use on RV+ and RV- diarrhea incidence rates. Model 1 and model 2 differ by the baseline pre-vaccine period used as the referent category (explained below). In both models, a generalized linear model was fit to the time-series data assuming a negative-binomial distribution due to over-dispersion of the data [100]. Calendar month was included in each model to account for seasonality and a sequential monthly term for every month over the entire time period was included to account for secular trends. The natural log of the monthly population at risk was included in the model as the offset term. Based on the Breusch-Godfrey test, 95% CIs were estimated using Newey-West heteroskedastic- and autocorrelation-consistent variance estimators with a lag of 2 [99,101]. Both models included indicator variables for each time period, as defined previously, following the pre-vaccine time period. Yearly RV1 impact was estimated with indicators for each post-

introduction year. The estimates of the coefficients for each time period were exponentiated to estimate incidence rate ratios compared to the referent category.

In model 1, within the ISA and GSA areas separately, the corresponding pre-vaccine time period was used as the referent category. To estimate the incidence rate ratio and corresponding 95% CIs, the time periods corresponding to the RCT, CRT, and each of the 3.5 years of routine RV1 use were modeled with separate indicator variables. This is a conservative model which directly compares incidence rates in February 2000 - February 2007 (ISA villages) and February 2000 - October 2008 (GSA villages) to the years of routine RV1 use starting in April 2011 in all ISA and GSA villages, while accounting for secular trends and seasonality.

In the secondary analysis (model 2), within ISA and GSA villages randomized as control-only villages during the CRT, the pre-vaccine and CRT time periods were combined in the referent category. The time period corresponding to the RCT was excluded due to potential changes in reported diarrhea incidence. To estimate the incidence rate ratio and corresponding 95% CIs, each of the 3.5 years of routine RV1 use were modeled with indicator variables. This approach directly compares incidence rates in February 2000 - March 2011, excluding the RCT time period, to the years of routine RV1 use starting in April 2011 in ISA and GSA villages randomized as controls, while accounting for secular trends and seasonality.

Monthly vaccine coverage was estimated as the proportion of children 6-<52 weeks receiving each RV1 dose within regions of Matlab, Bangladesh. Analyses were completed using STATA version 14 (Stata Corporation, College Station, TX, USA). This study was approved by the ethical review committee of icddr,b in Bangladesh and the Fred Hutchinson Cancer Research Center.

3.3 Results

Between February 2000 and February 2007, among those <2 years of age reporting to Matlab Hospital, the RV+ incidence rate was on average 34 per 1,000 person-years in ISA villages and 20 per 1,000 person-years in GSA villages. The proportion of gastroenteritis due to rotavirus was 37% in ISA villages and 45% in GSA villages. Tables 3.1a -3.1b show RV+ and RV- counts and average incidence rates for each time period within the ISA and GSA areas. In both areas, the largest rotavirus incidence rate occurred in the younger age group (0-<12 months). Prior to vaccine introduction, rotavirus transmission showed a strong seasonality with two seasonal peaks in December-February and July-September (Figure 3.2).

3.3.1 Rotavirus Vaccine Coverage and Timing

Figure 1 shows the changing rotavirus vaccine coverage levels over time within ISA and GSA areas during the CRT and during routine RV1 use among children <1 year of age. During the CRT, both ISA and GSA villages showed similar vaccine coverage levels, with about 70% coverage of dose one and 60% coverage of dose two of RV1 in villages randomized to vaccine. Following the CRT, vaccine coverage decreased in RV1-randomized ISA and GSA villages, while coverage increased in control-only villages. At the end of the first year of routine RV1 use in March 2012, among age-eligible infants, ISA villages had 65% coverage of dose one and 48% of dose two, while GSA villages had 50% coverage of dose one and 35% of dose two. After the second year of routine vaccine use in March 2013, the coverage for ISA villages increased further to 79% for dose one and 52% for dose two, while coverage in the GSA started to decrease. During the last year and a half, dose one coverage was maintained at 78% in ISA villages, but decreased to 42% in the GSA villages. In the ISA villages, the average age at first

dose was 7.6 weeks (range 5.9-23.4 weeks) and second dose was 12.0 weeks (10-27.9 weeks). In GSA villages, the average age at first dose was 9 weeks (1.9-23.9 weeks) and second dose was 13.6 (range 5.9-37.9 weeks).

3.3.2 ISA Villages

Using Model 1, with the pre-vaccine time period as the referent category, RV+ diarrhea increased during the RCT period and the CRT period in both age groups in ISA villages (Table 3.2a, Figure 3.3). During periods of routine RV1 use there was a non-statistically significant downward trend in RV+ diarrhea incidence after each additional year of vaccine use. During the entire 3.5 years of routine use there was no statistically significant decrease in RV+ diarrhea in 0-<12 months olds (IRR: 0.72, 95% CI:0.39-1.33) or 12-<24 month olds (IRR: 0.91, 95% CI: 0.46-1.83). Using Model 2, combining the pre-vaccine time period and the CRT time period in control-only villages in the referent category, there was a downward trend in RV+ diarrhea incidence after each additional year of routine RV1 use in both age-groups (Table 3.2b, Figure 3.3). During 3.5 years of routine RV1 use there was a significant 41% decrease in RV+ diarrhea in 0-<12 month olds (IRR: 0.59, 95%CI: 0.43-0.80), a 35% decrease in 12-<24 month olds (IRR: 0.65, 95%CI: 0.42-1.02), and a significant 39% decrease in children 0-<24 months of age (IRR: 0.61, 95%CI: 0.45-0.82).

In Model 1, RV- diarrhea increased significantly during the RCT period and the CRT period in both age groups. During periods of routine RV1 use there was an increased risk of RV- diarrhea in 0-<12 months olds (IRR: 1.59, 95% CI: 1.09-2.31) and no significant change in 12-<24 month olds. In Model 2, there was no significant change in RV- diarrhea during periods of RV1 routine use.

3.3.3 GSA Villages

Using Model 1, with the pre-vaccine time period as the referent category, RV+ diarrhea increased during the CRT period in 0-<12 month olds, but did not meaningfully change in 12-<24 month olds (Table 3.3a, Figure 3.4). During periods of routine RV1 use there was an upward trend in RV+ diarrhea incidence after each additional year of vaccine use in 0-<12 month olds, but no clear trends in 12-<24 month olds. During 3.5 years of routine use there was no significant change in RV+ diarrhea in 0-<12 months olds (IRR: 1.25, 95% CI:0.78-2.01) or in 12-<24 month olds (IRR: 1.00, 95% CI: 0.52-1.92). Using Model 2, there was a downward trend in RV+ diarrhea incidence after each additional year of routine RV1 use in both age-groups (Table 3.3b, Figure 3.4). However, during 3.5 years of routine RV1 use there was no significant change in RV+ diarrhea in either age-group. In Models 1 and 2, there was no significant change in RV- diarrhea during periods of RV1 routine use.

3.4 Discussion

Our study demonstrates a decreasing trend in RV+ diarrhea incidence among children <2 years of age from ISA villages presenting to Matlab Hospital during 3.5 years of routine RV1 use. Using a conservative model to estimate pre-vaccination rotavirus diarrhea trends (model 1), results were not statistically significant. However, by restricting the analysis to control-only villages, we gained an additional two years of pre-vaccine time to model baseline trends (model 2), and found a statistically significant 39% reduction in RV+ diarrhea in children 0-<24 months of age. Significant impact of RV1 on RV+ diarrhea incidence among children from GSA villages was not observed using either model. Differences in population-level impact between ISA and

GSA villages are likely due to lower RV1 coverage and lower reported diarrhea incidence in GSA areas compare to ISA villages.

Our study also examined changes in RV- diarrhea as a control outcome with the assumption that RV1 introduction should have no significant impact on RV- diarrhea [102]. In model 1, using only the pre-vaccine period in the referent category, we observed an increasing trend in both RV+ and RV- diarrhea in children 0-<24 months of age in ISA villages during the RCT and CRT time periods. While other interventions or unmeasured biases may have influenced all-cause gastroenteritis incidence, we believe this increase was due to changes in healthcare seeking behaviors due to the RCT. During the RCT, field staff visited the homes of infants enrolled in the study to remind parents to bring their child to the hospital for episodes of diarrhea [9]. A change in community healthcare seeking behavior is the most likely explanation as there was no significant change in all-cause diarrhea in the corresponding time period in the GSA villages where no RCT took place (Figure 3.2). The most conservative model to estimate RV1 impact (model 1) modelled the RCT and CRT time periods separately and directly compared the pre-vaccine time period to the years of routine RV1 use in ISA and GSA villages. However, if increased healthcare seeking behaviors were sustained, results from model 1 would underestimate the population-level impact of RV1. There was some evidence of this with increasing RV- diarrhea during periods of routine RV1 use.

In the secondary analysis (model 2), both to increase power and to include relevant healthcare seeking behaviors to estimate baseline incidence, we restricted the analysis to villages randomized as control-only during the CRT period and assessed the impact of routine RV1 use on diarrhea over time. The referent category combined the pre-vaccine time period and the CRT

time period. These models showed a significant impact of routine RV1 use on RV+ diarrhea in 0-<24 month olds in ISA villages, but not in GSA villages. RV- diarrhea did not significantly change over time using this model. Notably, both models showed a decreasing trend in RV+ diarrhea in ISA villages during sustained RV1 coverage. This analysis demonstrates the importance of using the appropriate baseline incidence and underlying trends in time-series analyses.

Despite the potential differences in healthcare seeking behavior over time, our results are similar to the RCT and CRT conducted in Matlab, Bangladesh, with the biggest impact of rotavirus vaccine on children 0-<12 months of age. To our knowledge no other population-level impact analyses have been reported in Asia, though a cohort study in Thailand in 2012 showed RV1 to be 88% (95% CI: 76%-94%) effective in preventing rotavirus hospitalization in the first 18 months of life [47]. An estimated 40% reduction in RV+ diarrhea incidence observed in ISA villages is similar to rotavirus vaccine impact studies in regions of sub-Saharan Africa with high vaccine coverage. After 2-3 years of rotavirus vaccine use a 49% (95% CI: 32%-63%) decrease in rotavirus diarrhea in <5 year olds was observed in Ghana [23], a 54% (95% CI: 33%-69%) decrease in rotavirus diarrhea in <1 year olds was observed in Malawi [44], a 33% (95% CI: 25%-41%) reduction in rotavirus diarrhea was seen in <5 year olds in Botswana [46], and a 38% reduction in rotavirus positivity among children with diarrhea was seen in Zambia in <5 year olds [43]. Importantly, in these studies >90% vaccine coverage for one or two doses of rotavirus vaccine was reported within one year of vaccine introduction. In our study, the maximum two-dose RV1 coverage of 68% was attained in the ISA villages during the second year of routine use.

Our study has limitations. As in any time-series analysis, our study may have been confounded by other interventions or other unmeasured factors associated with RV+ diarrhea and the timing of vaccine introduction that impacted the results. However, our confidence in the impact of RV1 is increased because no meaningful changes in RV- diarrhea were observed. Additionally, while the Matlab HDSS database shows lower vaccine coverage in GSA areas, coverage may be underestimated due to the lack of recording on health cards in this region.

Research continues to understand the role of co-enteric pathogens, maternal antibody interference, concomitant oral polio vaccination, and the gut microbiome in the moderate efficacy of rotavirus vaccines in Bangladesh [68,69]. However, this study provides evidence of the population-level impact of rotavirus vaccines in children <2 years of age in regions of high vaccine coverage in Matlab, Bangladesh. Pecenka et al. (2017) estimated that with a Gavi subsidy in Bangladesh, the averted cost/disability adjusted life year (DALY) ratio ranged between $58/DALY and $142/DALY indicating a highly cost-effective vaccine [103]. In our study, during the pre-vaccine period, rotavirus was detected in 34.5% of diarrhea cases in children <5 years of age presenting to Matlab Hospital. Other regions of Bangladesh show an average of 64% of diarrhea due to rotavirus in children <5 years of age [104]. With sustained vaccine coverage and a significant nationwide burden of rotavirus diarrhea, larger impacts of RV1 on rotavirus gastroenteritis are likely to be observed long-term in Bangladesh.

This was the first study to conduct time-series analyses estimating rotavirus vaccine impact in Asia. Bangladesh plans to introduce rotavirus vaccine into its national immunization schedule in 2018. Continued diarrheal surveillance and nationwide population-level impact analyses will be

important to understand the role of rotavirus vaccine coverage and to influence other countries in the region to introduce rotavirus vaccine.

3.5 Tables and Figures

Table 3.1a: Rotavirus-positive and rotavirus-negative trends by period in icddrb service area (ISA)

ISA	Feb 2000-Feb 2007 (pre-vaccine)	March 2007-March 2009 (RCT)	April 2009 - March 2011 (CRT)	April 2011 - March 2012 (YR1)	April 2012 - March 2013 (YR2)	April 2013 - March 2014 (YR3)	April 2014 - September 2014 (YR3.5)	April 2011 - September 2014 (Vaccine Years)
0-12 months of age								
Population, person- years	18,281	5,153	4,936	2,494	2,683	2,586	1,286	9,048
RV+, count	738	265	216	64	81	54	24	223
RV-, count	1,258	823	380	179	208	166	79	632
RV+ Incidence	40	51	44	26	30	21	19	25
RV- Incidence	69	160	77	72	78	64	61	70
12-24 months of age								
Population, person- years	18,363	5,124	5,008	2,437	2,493	2,649	1,275	8,853
RV+, count	502	200	145	43	41	49	9	142
RV-, count	844	432	185	87	90	89	44	310
RV+ Incidence	27	39	29	18	16	19	7	16
RV- Incidence	46	84	37	36	36	34	35	35
0-24 months of age								
Population, person- years	36,644	10,276	9,945	4,930	5,176	5,235	2,561	17,901
RV+, count	1,240	465	361	107	122	103	33	365
RV-, count	2,102	1,255	565	266	298	255	123	942
RV+ Incidence	34	45	36	22	24	20	13	20
RV- Incidence	57	122	57	54	58	49	48	53

Rotavirus-positive (RV+)
Rotavirus-negative (RV-)

Table 3.1b: Rotavirus-positive and rotavirus-negative trends by period in government service area (GSA)

GSA	Feb 2000-Oct 2008 (pre-vaccine)	Nov 2008-March 2011 (CRT)	April 2011 - March 2012 (YR1)	April 2012 - March 2013 (YR2)	April 2013 - March 2014 (YR3)	April 2014 - September 2014 (YR3.5)	April 2011 - September 2014 (Vaccine Years)
0-12 months of age							
Population, person- years	22,777	5,359	2,317	2,306	2,201	1,162	7,987
RV+, count	542	144	35	51	37	15	138
RV-, count	671	142	59	80	64	26	229
RV+ Incidence	24	27	15	22	17	13	17
RV- Incidence	29	26	25	35	29	22	29
12-24 months of age							
Population, person- years	23,168	5,641	2,224	2,312	2,305	1,111	7,951
RV+, count	365	90	46	28	20	8	102
RV-, count	459	94	40	44	24	13	121
RV+ Incidence	16	16	21	12	9	7	13
RV- Incidence	20	17	18	19	10	12	15
0-24 months of age							
Population, person- years	45,945	10,999	4,541	4,618	4,506	2,273	15,938
RV+, count	907	234	81	79	57	23	240
RV-, count	1,130	236	99	124	88	39	350
RV+ Incidence	20	21	18	17	13	10	15
RV- Incidence	25	21	22	27	20	17	22

Rotavirus-positive (RV+)
Rotavirus-negative (RV-)

Table 3.2a: Rotavirus-positive and rotavirus-negative diarrhea trends, icddr,b service area (ISA) region (Model 1)

ISA	Feb 2000-Feb 2007 (Pre)	March 2007-March 2009 (RCT)			April 2009 - March 2011 (CRT)			April 2011 - March 2012 (YR1)			April 2012 - March 2013 (YR2)			April 2013 - March 2014 (YR3)			April 2014 - September 2014 (YR3.5)			April 2011 - September 2014 (Vaccine Years)		
		IRR	95% CI		IRR	95% CI		IRR	95% CI		IRR	95% CI		IRR	95% CI		IRR	95% CI		IRR	95% CI	
0-12 months of age																						
RV+	REF	1.32	0.91	1.92	1.16	0.72	1.88	0.67	0.37	1.21	0.85	0.41	1.75	0.57	0.27	1.20	0.63	0.26	1.49	0.72	0.39	1.33
RV-	REF	2.93	2.03	4.24	1.51	1.14	2.00	1.49	1.01	2.19	1.71	1.18	2.49	1.46	0.97	2.19	1.25	0.79	1.96	1.59	1.09	2.31
12-24 months of age																						
RV+	REF	1.84	1.19	2.84	1.45	0.86	2.46	0.86	0.41	1.80	0.94	0.40	2.17	1.08	0.47	2.45	0.62	0.25	1.56	0.91	0.46	1.83
RV-	REF	1.95	1.42	2.69	0.86	0.60	1.22	0.85	0.54	1.34	0.91	0.52	1.58	0.83	0.45	1.54	0.67	0.35	1.30	0.88	0.56	1.38
0-24 months of age																						
RV+	REF	1.50	1.03	2.19	1.26	0.80	2.00	0.72	0.40	1.31	0.90	0.44	1.84	0.74	0.36	1.52	0.66	0.29	1.51	0.79	0.43	1.43
RV-	REF	2.55	1.91	3.41	1.24	0.98	1.55	1.23	0.89	1.70	1.40	0.99	1.97	1.19	0.82	1.73	1.01	0.68	1.49	1.31	0.95	1.79

Rotavirus-positive (RV+)
Rotavirus-negative (RV-)
IRR: Incidence rate ratio

Table 3.2b: Rotavirus-positive and rotavirus-negative diarrhea trends, icddr,b service area (ISA) region (Model 2)

ISA	Feb 2000-March 2011 (Pre-vaccine and CRT)	April 2011 - March 2012 (YR1)			April 2012 - March 2013 (YR2)			April 2013 - March 2014 (YR3)			April 2014 - September 2014 (YR3.5)			April 2011 - September 2014 (Vaccine Years)		
		IRR	95% CI		IRR	95% CI		IRR	95% CI		IRR	95% CI		IRR	95% CI	
0-12 months of age																
RV+	REF	0.61	0.43	0.86	0.68	0.46	1.00	0.46	0.29	0.72	0.51	0.29	0.90	0.59	0.43	0.80
RV-	REF	1.07	0.74	1.52	1.33	1.05	1.69	1.14	0.87	1.49	0.89	0.62	1.28	1.15	0.91	1.47
12-24 months of age																
RV+	REF	0.72	0.44	1.17	0.58	0.29	1.12	0.74	0.41	1.33	0.33	0.11	0.99	0.65	0.42	1.02
RV-	REF	1.00	0.72	1.39	1.05	0.62	1.79	1.10	0.66	1.84	0.86	0.56	1.32	1.03	0.74	1.43
0-24 months of age																
RV+	REF	0.64	0.46	0.89	0.66	0.44	0.98	0.55	0.35	0.85	0.48	0.25	0.91	0.61	0.45	0.82
RV-	REF	1.05	0.80	1.38	1.27	0.98	1.64	1.12	0.85	1.46	0.89	0.70	1.13	1.12	0.91	1.37

Rotavirus-positive (RV+)
Rotavirus-negative (RV-)
IRR: Incidence rate ratio

Table 3.3a: Rotavirus-positive and rotavirus-negative diarrhea trends, government service area (GSA) region (Model 1)

GSA	Feb 2000-Oct 2008 (Pre-vaccine)	Nov 2008 - March 2011 (CRT)			April 2011 - March 2012 (YR1)			April 2012 - March 2013 (YR2)			April 2013 - March 2014 (YR3)			April 2014 - September 2014 (YR3.5)			April 2011 - September 2014 (Vaccine Years)		
		IRR	95% CI		IRR	95% CI		IRR	95% CI		IRR	95% CI		IRR	95% CI		IRR	95% CI	
0-12 months of age																			
RV+	REF	1.46	1.02	2.09	0.99	0.63	1.54	1.58	0.90	2.77	1.27	0.75	2.16	1.55	0.66	3.61	1.25	0.78	2.01
RV-	REF	1.01	0.75	1.37	0.96	0.62	1.47	1.33	0.87	2.04	1.13	0.70	1.82	0.79	0.43	1.43	1.10	0.73	1.65
12-24 months of age																			
RV+	REF	0.98	0.63	1.53	1.31	0.73	2.33	0.82	0.38	1.77	0.60	0.28	1.25	0.89	0.36	2.22	1.00	0.52	1.92
RV-	REF	0.97	0.72	1.32	1.06	0.70	1.62	1.16	0.76	1.75	0.65	0.37	1.13	0.67	0.40	1.14	0.98	0.64	1.50
0-24 months of age																			
RV+	REF	1.26	0.89	1.77	1.18	0.76	1.85	1.24	0.72	2.12	0.96	0.57	1.63	1.34	0.61	2.95	1.16	0.73	1.85
RV-	REF	0.98	0.77	1.26	0.99	0.71	1.38	1.25	0.88	1.78	0.92	0.61	1.40	0.75	0.47	1.19	1.04	0.74	1.47

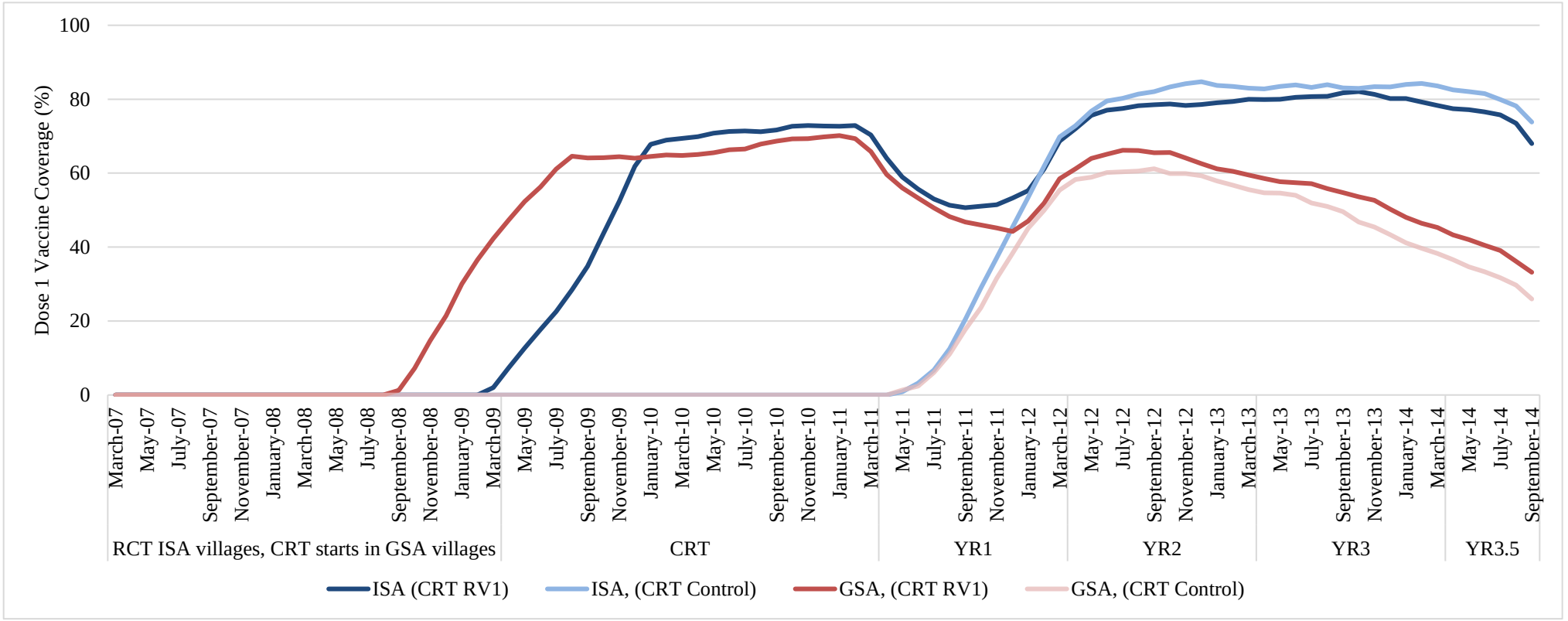
Rotavirus-positive (RV+)
Rotavirus-negative (RV-)
IRR: Incidence rate ratio

Table 3.3b: Rotavirus-positive and rotavirus-negative diarrhea trends, government service area (GSA) region (Model 2)

GSA	Feb 2000- March 2011 (Pre-vaccine and CRT)	April 2011 - March 2012 (YR1)			April 2012 - March 2013 (YR2)			April 2013 - March 2014 (YR3)			April 2014 - September 2014 (YR3.5)			April 2011 - September 2014 (Vaccine Years)		
		IRR	95% CI		IRR	95% CI		IRR	95% CI		IRR	95% CI		IRR	95% CI	
0-12 months of age																
RV+	REF	0.91	0.58	1.43	0.79	0.39	1.61	0.62	0.34	1.14	0.65	0.29	1.46	0.78	0.48	1.27
RV-	REF	1.38	0.93	2.06	1.66	1.00	2.75	1.36	0.85	2.17	1.06	0.60	1.85	1.43	0.97	2.12
12-24 months of age																
RV+	REF	1.38	0.91	2.07	0.63	0.34	1.16	0.61	0.36	1.03	0.23	0.03	1.53	0.87	0.53	1.43
RV-	REF	0.98	0.56	1.72	0.72	0.43	1.22	0.72	0.40	1.30	0.61	0.33	1.10	0.79	0.51	1.23
0-24 months of age																
RV+	REF	1.12	0.80	1.55	0.72	0.48	1.08	0.62	0.41	0.94	0.50	0.24	1.06	0.82	0.57	1.19
RV-	REF	1.20	0.87	1.67	1.24	0.84	1.84	1.06	0.71	1.58	0.86	0.56	1.31	1.15	0.86	1.53

Rotavirus-positive (RV+)
Rotavirus-negative (RV-)
IRR: Incidence rate ratio

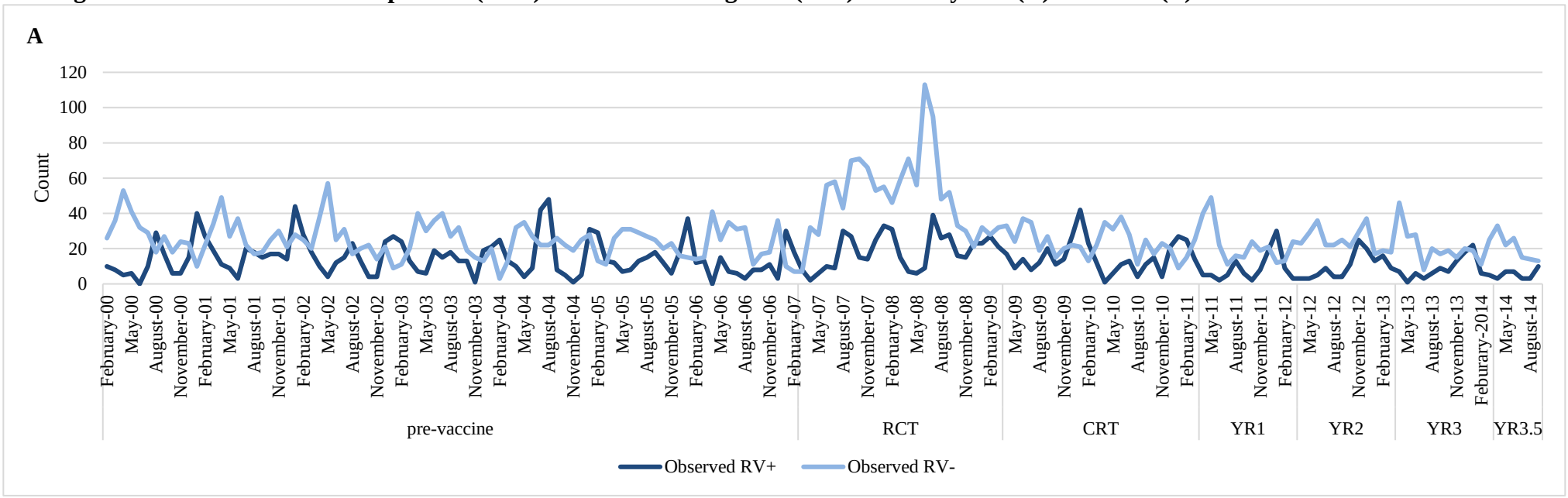
Figure 3.1: Timing of RV1 coverage (dose 1) over time by ISA and GSA villages randomized to RV1 or control-only in <1 year olds

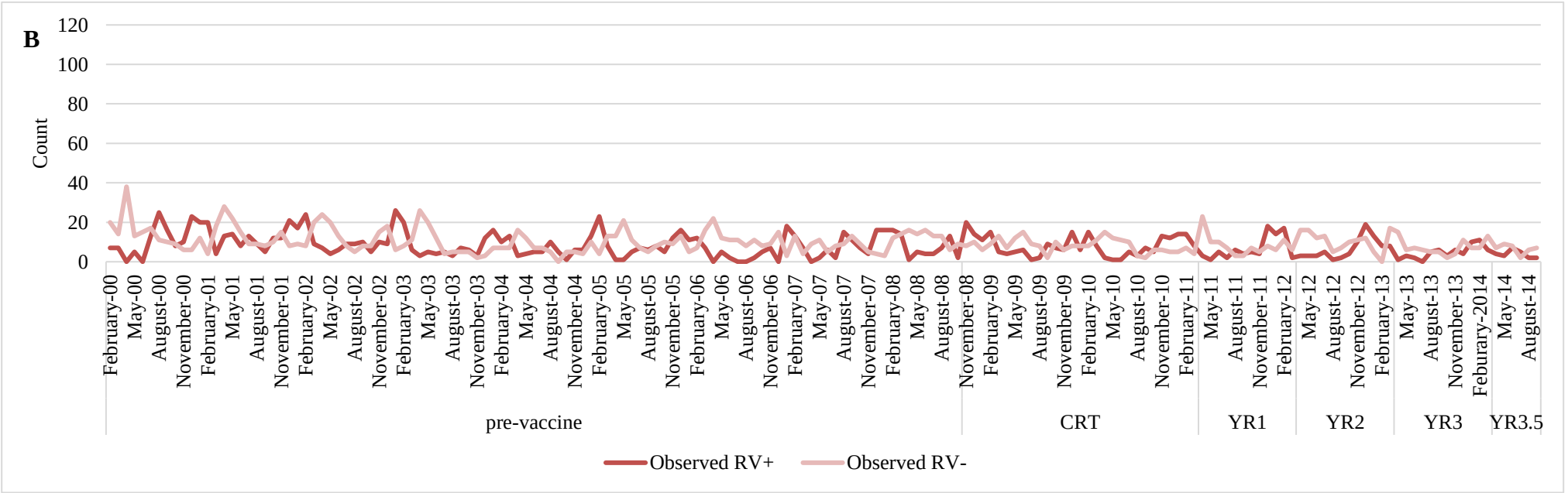


-ISA, RV1: icddr,b service areas randomized to RV1 during the CRT
-ISA, Control: icddr,b service areas randomized as control-only villages during the CRT
-GSA, RV1: Government service areas randomized to RV1 during the CRT
-GSA, Control: icddr,b service areas randomized as control-only villages during the CRT

*23 children were vaccinated in GSA villages in September-October 2008 before the start of the cluster-randomized trial (CRT). This time period is still considered pre-vaccine due to the small number of children vaccinated.

Figure 3.2: Observed rotavirus-positive (RV+) and rotavirus-negative (RV-) counts by ISA (A) and GSA (B) areas





ISA: icddr,b service areas
GSA: Government service areas

Figure 3.3: Observed incidence and incidence rate ratios (IRR) of RV+ and RV- diarrhea in icddr,b service area (ISA) villages using Models 1 & 2 in 0-<12 month olds (A) and 12-<24 month olds (B)

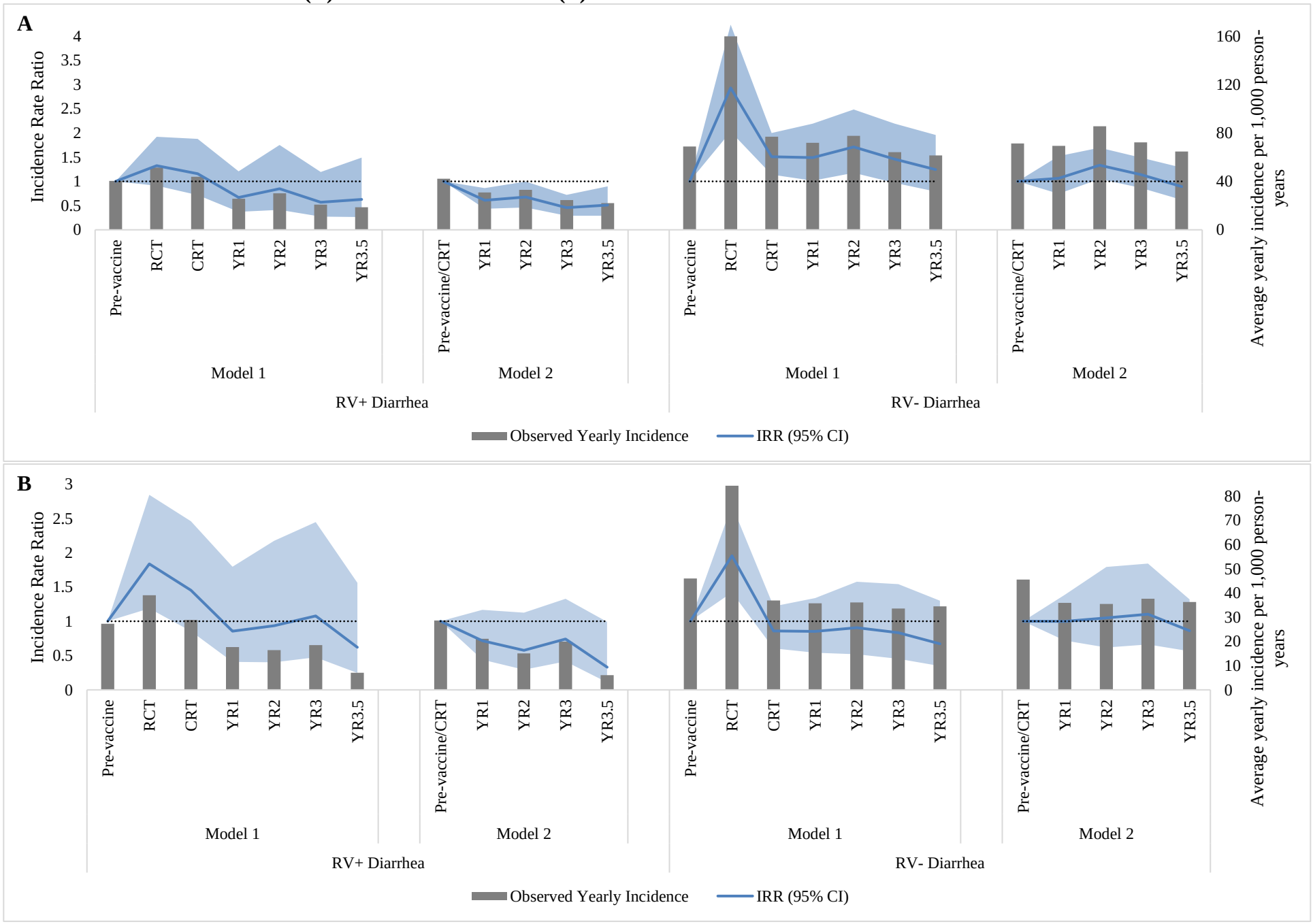
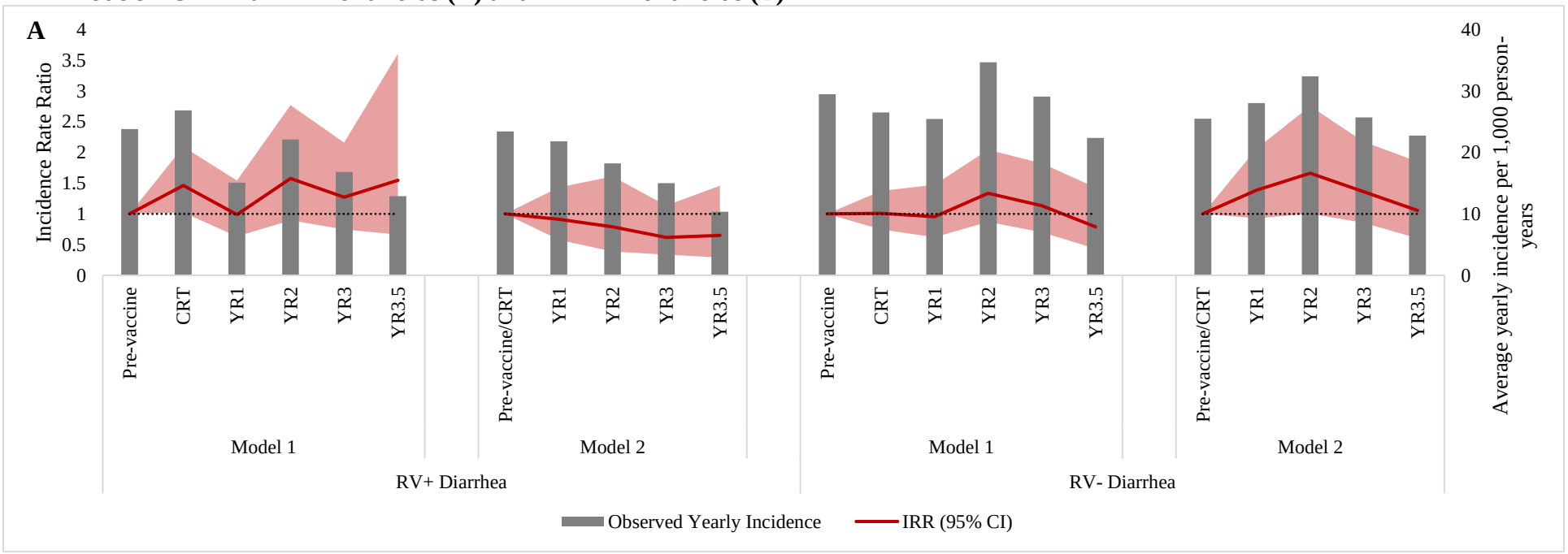
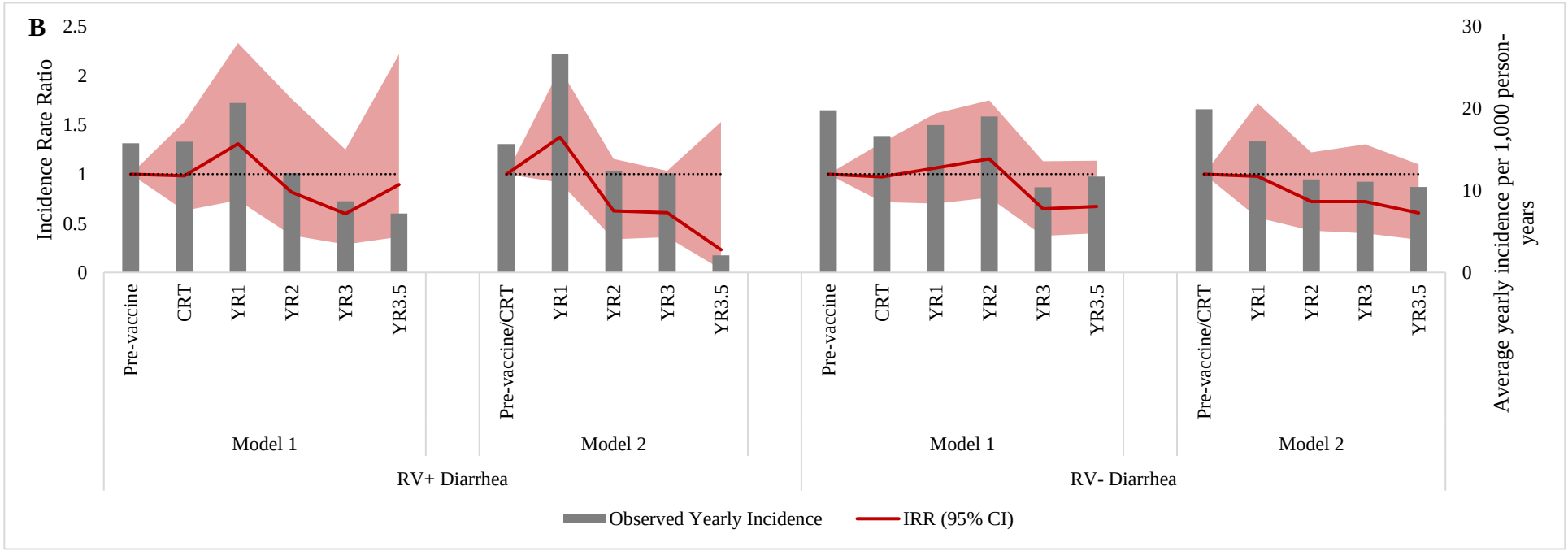


Figure 3.4: Observed incidence and incidence rate ratios (IRR) of RV+ and RV- diarrhea in government service area (GSA) villages using Models 1 & 2 in 0-<12 month olds (A) and 12-<24 month olds (B)





CHAPTER 4: HOST GENETIC DETERMINANTS OF ROTAVIRUS VACCINE FAILURE IN SUB-SAHARAN AFRICA: A PRELIMINARY ANALYSIS

4.1 Introduction

Globally, an estimated 200,000 deaths due to rotavirus diarrhea occur each year among children <5 years of age, with most of the burden in low-income settings [4]. Despite the significant reduction of rotavirus diarrhea and associated number of deaths averted in the past decade with the introduction of GlaxoSmithKline's live-attenuated human monovalent vaccine (Rotarix [RV1]) and Merck's live-attenuated pentavalent human-bovine reassortant vaccine (RotaTeq [RV5]) [3], clinical trials and observational studies in sub-Saharan Africa and Asia have consistently estimated moderate rotavirus vaccine effectiveness (40%-60%) [7–9] compared to similar studies in high-resource settings (90%) [10]. It is a highly problematic issue that the efficacy of these vaccines is lowest in the countries with the greatest diarrheal burden. High rates of rotavirus vaccine failure, that is developing rotavirus diarrhea after a full course of immunizations, decreases vaccine efficacy. Previously-studied risk factors for rotavirus vaccine failure include high maternal rotavirus antibodies during pregnancy and breastfeeding [52–59], concomitant OPV administration [60–65] malnutrition [66,67], a high burden of co-enteric pathogens [68–70], and the microbiome [71,72]. However, these factors alone do not fully explain the reduced rotavirus vaccine efficacy in low-income settings. One novel risk factor is the potential role of genetically determined susceptibility to rotavirus and rotavirus vaccine failure.

Histo-blood group antigens (HBGAs) are expressed in the gut and are thought to act as receptors for many enteric pathogens. Rotavirus is classified by two surface proteins; glycoprotein (G) determines genotype and protease sensitive binding protein (P) determines serotype. Rotavirus binds to HBGA receptors on gut epithelial cells with the P binding protein. The relationship

between HBGAs and infectious disease susceptibility has been observed for cholera [105,106], enterotoxigenic *E. coli* [107], *H. pylori* [108–113], norovirus [76,81,82,114–129], and most recently, some rotavirus serotypes [73–77,130]. HBGAs are synthesized by sequential additions of monosaccharides encoded by three gene families; ABO, Lewis, and Secretor. Genetic polymorphisms in Secretor (*FUT2*) and Lewis (*FUT3*) gene families cause loss of function mutations, leading to null phenotypes (non-secretor and Lewis-negative). Expression of Lewis A, Lewis B, ABO antigens, and subsequent infectious disease receptors, is dependent on the phenotypes of these gene families [130].

Among individuals with null HBGA phenotypes, pathogens that normally bind to HBGA receptors cannot attach to the host cell membrane, and infection is prevented. *In vitro* studies show that resistance to rotavirus due to null HBGA phenotypes is P serotype-dependent [73–75]. Significantly lower neutralizing antibody titers against rotavirus serotype P[8] have been observed both in non-secretors compared to secretors, and in Lewis-negative samples compared to Lewis-positive samples. Antibody titers against rotavirus serotype P[6] were similar in secretors, non-secretors, Lewis-positive and Lewis-negatives samples [131].

Epidemiologic studies have also demonstrated significant associations between HBGA phenotypes and rotavirus diarrhea. Studies in France, Vietnam, and the US, where P[8] was the dominant infecting serotype, consistently observed 0% of rotavirus positive cases were non-secretors while 20-46% of rotavirus negative or healthy controls were non-secretors [74,76,77]. All children who were non-secretors (null *FUT2* phenotype) were resistant to P[8] rotavirus infections. Non-P[8] rotavirus infections in these studies were either nonexistent or limited in number for additional analyses. Associations between Lewis (*FUT3*) phenotypes and rotavirus infection were not evaluated in these studies due to a low prevalence (~5%) of Lewis-negative

individuals in these settings [82]. In sub-Saharan Africa, the Lewis-negative phenotype is more common [81] and there is a greater diversity of circulating rotavirus serotypes [78–80,132]. A case-control study in Burkina Faso confirmed previous findings that non-secretors and Lewis-negative children were not susceptible to P[8] rotavirus infection [130]. Additionally, Lewis-negative children were more susceptible to P[6] infection compared to Lewis-positive children.

Based on this evidence, HBGA phenotype may be a risk factor for rotavirus vaccine failure in sub-Saharan Africa. Existing oral rotavirus vaccines are effective by mimicking natural infection. Vaccine virus infects host cells in the gut epithelium and induces an immune response that will later target circulating serotypes of natural rotavirus infection. While current rotavirus vaccines differ in their included genotypes (Rotarix=G[1], Rotateq=G[1],G[2],G[3],G[4]), both include P[8] as the sole P component. Both vaccines demonstrate cross-protection, with efficacy against heterotypic serotypes, strains not included in the vaccine, at the same level of homotypic strains [133]. Individuals resistant to P[8] infection (non-secretors and Lewis-negative children) may not be able to mount an immune response to existing vaccines and are therefore fully susceptible to non-P[8] circulating serotypes. This has less impact on vaccine efficacy in countries where P[8] is the prevalent circulating serotype. While vaccine response is not induced in non-secretor and Lewis-negative individuals, these individuals have innate resistance to the most prevalent rotavirus serotype. In sub-Saharan Africa, with greater strain diversity and a greater frequency of null HBGA phenotypes, there could be an increased rate of rotavirus vaccine failure and subsequent low vaccine efficacy.

To explore the role of host genetic determinants in rotavirus vaccine failure, we conducted a substudy among children 3-<24 months of age enrolled in the Vaccine Impact on Diarrhea in

Africa (VIDA) study, a case-control study assessing diarrheal etiologies in The Gambia, Mali, and Kenya after rotavirus vaccine introduction.

4.2 Methods

4.2.1 Participants and Study Design

The current substudy was nested within the VIDA study, a case-control study of moderate-to-severe diarrhea (MSD) in The Gambia, Mali, and Kenya conducted between 2015 and 2018. The VIDA study assessed diarrheal etiologies and rotavirus vaccine effectiveness and impact following rotavirus vaccine introduction in children <5 years of age. Participants were enrolled at three African study sites (Medical Research Council (MRC) Basse, The Gambia, Center for Vaccine Development (CVD), Bamako, Mali and CDC Kenya Medical Research Institute (KEMRI) Siaya County, Kenya). All three countries introduced rotavirus vaccine between 2013-2014. Mali and The Gambia introduced Rotateq while Kenya introduced Rotarix. The Gambia recently converted to Rotarix.

Methods in VIDA are similar to those used in GEMS [134]. Cases were evaluated for MSD and enrolled at sentinel health care centers. MSD cases had to fulfill more than one of the following criteria 1) sunken eyes, 2) loss of skin turgor, 3) intravenous hydration prescribed, 4) hospitalized, or 5) dysentery. Cases were enrolled within three age groups (0-11 months, 12-23 months, 24-59 months). Within each age group, 9 MSD cases were enrolled for a given two-week period, though during rotavirus season all MSD cases seeking care were enrolled. One to two healthy matched controls, defined as having no diarrhea within seven days of enrollment, were identified through the Demographic Surveillance System (DSS) and enrolled at their home. Controls were matched by residence (same or nearby village or community as case), sex, and age

group and enrolled within 14 days of the case enrollment date. This substudy includes only MSD cases and matched controls 3-<24 months of age.

4.2.2 Data Collection and Procedures

After informed consent was obtained in the local language, the interviewer administered a standardized questionnaire to the parent/primary caretaker of cases and controls to collect demographic, epidemiologic, and clinical data. Anthropometric measurements were also documented. Interviewers recorded the dates that a child received rotavirus vaccine from the vaccination card. In situations where a vaccination card was not available, data was obtained from the administration center. A single, fresh, whole stool sample was collected at enrollment from cases and matched controls within 12 hours.

For this substudy, saliva was collected at the end of the interview using SalivaBio Infant's Swab Method kit. Mothers were asked to refrain from breastfeeding during the 20-minute interview. The child's mouth was washed with oral rehydration solution or clean water if breastfeeding occurred during the previous 30 minutes. Saliva and stool samples were put on ice packs or refrigerated until taken to the site-specific laboratory. Stool samples were processed on-site while saliva samples were frozen at -80°C. If insufficient saliva was collected during the enrollment visit, saliva was collected again during a follow-up visit.

4.2.3 Rotavirus infection and genotypes

Rotavirus VP6 antigen was detected by the ProSpecT ELISA rotavirus kit [135]. Preliminary results used genotypes ascertained using a custom TaqMan Array Card (Thermo Fisher, Carlsblad, CA, USA) [136,137]. Due to the recent recognition that the TaqMan Array card may

not use appropriate probes for rotavirus genotypes, traditional PCR may be used for the final data results.

4.2.4 Secretor and Lewis Phenotypes

HBGA phenotype was determined by testing for the presence of H-type 1, Type A, Type B, Lewis A, and Lewis B antigens in saliva using enzyme-linked immunosorbent assays (ELISAs). After thawing frozen samples, samples were boiled and 1:500 dilutions were created using PBS. 100 µl of each diluted sample was transferred into 96-well microtiter plates and incubated at room temperature for four hours. 10% NFDM was coated onto the plate and incubated at 4°C overnight. The plates were washed 3 times with 0.05% Tween-20/PBS. Anti-Lewis A, Anti-Lewis B, Anti-A, and Anti-B primary antibodies were transferred into columns for the plate and incubated at 37ºC for one hour. Plates were washed with 0.05% Tween-20/PBS. Conjugated antibodies IgG-HRP and UEA-1 lectin-HRP were then added to the wells and incubated at 37ºC for one hour. Plates were washed again with 0.05% Tween-20/PBS. The signals were developed with TMB Peroxidase Substrate and Peroxidase Substrate Solution B. Positive and negative quality controls were also used on the plate for each antibody and conjugated antibody. An OD cutoff of 0.2 was used for all measures.

Secretor status was defined as positive if Type A, Type B, Lewis B, or the UEA-1 lectin assay was positive. Secretor status was defined as negative if all of these assays were negative. Lewis status was defined as positive if Lewis A or Lewis B assays were positive. Lewis status was defined as negative if both Lewis A and Lewis B assays were negative.

4.2.5 Statistical Analysis

The present analysis was restricted to rotavirus-positive MSD cases and their matched healthy control(s). Both case and controls had sufficient saliva to ascertain HBGA phenotype and documentation of at least one dose of rotavirus vaccine. In this preliminary analysis, separate conditional logistic regression models were used to estimate the relative odds and associated 95% confidence intervals (CIs) for the association between rotavirus diarrhea and Secretor status (secretor, non-secretor) or Lewis status (Lewis-positive, Lewis-negative). Sensitivity analyses excluded partially vaccinated children and stunted children, an anthropometric measure of long-term growth, defined as height-for-age z-score (HAZ) <-2 standard deviations. The final analysis will also adjust for a wealth index, which is currently unavailable. Future analyses will be stratified by infecting rotavirus serotype (P[8], P[6], and P[4]). For this preliminary analysis only summary measures were described due to the low proportion of non-P[8] serotypes.

Analyses were completed using STATA version 15 (Stata Corporation, College Station, TX, USA). This study was approved by the ethical review committees at the University of Washington, the University of Maryland, The Medical Research Council in Basse, The Gambia, the Center for Vaccine Development in Bamako, Mali and the Kenya Medical Research Institute in Siaya County, Kenya.

4.3 Results

Saliva Collection

Saliva collection started in June, August, and November 2016 in Bamako, Mali, Kisumu, Kenya, and Basse, the Gambia, respectively. As of April 2018, saliva was collected in a total of 1,842 MSD cases and 2,584 matched healthy controls (Figure 1). After excluding rotavirus-negative MSD cases, cases with no rotavirus results, non-vaccinated children, and those without a matched case or control, 181 MSD rotavirus-positive cases with 235 matched controls were

identified for saliva testing. Of these, HBGA phenotype results were available for 132 MSD rotavirus-positive cases and 174 matched controls. In the preliminary analysis, 43% (n=57), 28% (n=37), and 29% (n=38) of MSD rotavirus-positive cases were from The Gambia, Mali, and Kenya, respectively. A similar distribution of controls from each country was observed. Table 4.1 shows the demographic characteristics of cases and controls by site and overall. As expected, cases and controls had a similar median age (11 months) and sex distribution. All sites had a high proportion of fully immunized children, with the Gambia showing the largest proportion of partially immunized children. Stunting occurred more frequently in Kenya and was slightly more common in controls compared to cases.

Table 4.2 shows the distribution of HBGA phenotypes at each site and across all sites among cases and controls. Across all sites, about 3% of cases and 9% of controls were defined as non-secretors. Across all sites, 11% of cases and 25% of controls were defined as Lewis-negative. When examining the crude association between secretor status and rotavirus diarrhea, regardless of serotype, non-secretors were 73% less likely to be a rotavirus-positive MSD case (matched Odds Ratio (mOR): 0.27, 95% CI: 0.08-0.95) (Table 4.3). After excluding stunted and partially vaccinated children, the odds of being a rotavirus-positive MSD case did not change meaningfully, though the estimate was not statistically significant. When examining the crude association between Lewis status and rotavirus diarrhea, regardless of serotype, Lewis-negative children were 66% less likely to be a rotavirus-positive case (mOR: 0.34, 95%CI: 0.17-0.67). These estimates did not change meaningfully after excluding stunted and partially vaccinated children.

Table 4.4 shows the distribution of rotavirus serotypes among the MSD cases. Of the 132 rotavirus MSD cases, serotype data is currently available for 49% of cases. In this set of data, 83% of rotavirus infections were P[8]. Non-P[8] infections were mostly P[4] infections from Kenya. Table 4.5 shows the distribution of HBGA phenotypes by rotavirus serotype. Among P[8] infections, there are zero non-secretors and a small proportion of Lewis-negative children. Among non-P[8] infections, zero cases are non-secretors and zero cases are Lewis-negative.

4.4 Discussion

In this preliminary analysis examining the association between Secretor and Lewis phenotypes and rotavirus vaccine failure, we demonstrated both null phenotypes reduced the risk of rotavirus vaccine failure in a population with mostly P[8] infections. These result are expected given the the hypothesis that non-secretors are innately protected from P[8] rotavirus infections, with or without vaccine. The current sample was limited in both the proportion of children with null HBGA phenotypes and cases with non-P[8] infections. Conclusions regarding the role of null HBGA phenotypes in rotavirus vaccine failure for non-P[8] infections cannot be made in this preliminary analysis.

The next steps of the study include identifying additional rotavirus-positive MSD cases and matched controls as the study finishes in July 2018 and testing the final samples to determine HBGA phenotype. We will also further investigate the low proportion of non-secretors in this sample, which was unexpected given the results of other studies worldwide. Studies in both low- and high-resource settings have consistently shown samples of about 20% non-secretors [77,81,126,138,139].

The current sample yielded more than 80% of P[8] infections using the TaqMan array card. Recent evidence suggests traditional PCR methods provide more accurate results to determine rotavirus serotypes (*communication with C. Kirkwood*). In the VIDA study traditional serotyping using PCR was conducted in Mali and 84% were P[8] infections. Rotavirus serotype distribution changes annually and it is possible the year of saliva collection was mostly P[8] infections.

Results from the final analysis of this study will supplement recent studies conducted in low-income settings examining the association between null HBGA phenotypes and rotavirus vaccine effectiveness. A study in Pakistan assessed immunogenicity of Rotarix by Secretor and Lewis phenotypes [138]. In this study 30% of individuals were non-secretors. Blood group O secretors were 2.8 (95%CI: 1.5-5.2) times more likely to seroconvert compared to non-secretors and 1.7 (95%CI: 1.1-2.7) times more likely to seroconvert compared to non-blood group O secretors. There was no statistically significant association for Lewis-negative children.

Within an RCT in Bangladesh, the association between HBGA null phenotypes and rotavirus diarrhea was examined in both vaccinated and unvaccinated groups [139]. In this study about 33% of children were non-secretors and 15% were Lewis-negative. Among unvaccinated children, non-secretors had a significantly reduced risk of rotavirus diarrhea and were resistant to P[4] infections, with no difference in P[8] or P[6] infections. Among vaccinated children, there was no association between Secretor or Lewis phenotype and rotavirus diarrhea. These results differ from our preliminary analysis, likely due to differences in severity of rotavirus diarrhea and serotype distribution.

To our knowledge this is the first multi-site study in sub-Saharan Africa to assess the role of host genetics and rotavirus vaccine failure. If conclusions for this preliminary analysis are confirmed

in the final analysis, it may be important to account for HBGA phenotype in the development of future global rotavirus vaccines. Currently both ABO blood type and secretor status are included as eligibility criteria for cholera and norovirus vaccine trials [105,140,141]. Additional studies are needed to explore this relationship in regions of the world with a high burden of rotavirus diarrhea and where effective vaccines will provide significant public health impact.

4.5 Tables and Figures

Table 4.1: Summary of rotavirus-positive MSD cases and matched controls

Characteristic, n (%)	The Gambia		Mali		Kenya		All-sites	
	Rotavirus cases	Healthy controls	Rotavirus cases	Healthy controls	Rotavirus cases	Healthy controls	Rotavirus cases	Healthy controls
	n=57	n=79	n=37	n=39	n=38	n=56	n=132	n=174
Age in months (median)	11	11	10	11	10.5	9.5	10.5	10
Sex								
Male	31 (54)	45 (57)	22 (59)	22 (56)	20 (53)	35 (63)	73 (55)	102 (59)
Female	26 (46)	34 (43)	15 (41)	17 (44)	18 (47)	21 (37)	59 (45)	72 (41)
Rotavirus Vaccine*								
Partially Immunized	7 (12)	9 (11)	1 (3)	3 (8)	1 (3)	3 (5)	9 (7)	15 (9)
Fully Immunized	50 (88)	70 (89)	36 (97)	36 (92)	37 (97)	53 (95)	123 (93)	159 (91)
Stunting								
HAZ <-2	6 (11)	13 (16)	3 (8)	3 (8)	6 (16)	9 (16)	15 (11)	25 (14)
HAZ ≥-2	52 (89)	66 (84)	34 (92)	36 (92)	32 (84)	47 (84)	117 (89)	149 (86)

*Partially immunized is less than 2 doses in Kenya and less than 3 doses in the Gambia and Mali
HAZ: Height-for-age z-score

Table 4.2: Summary of Secretor and Lewis phenotypes for rotavirus-positive MSD cases and matched controls

HBGA Phenotype, n (%)	The Gambia		Mali		Kenya		All-sites	
	Rotavirus cases	Healthy controls	Rotavirus cases	Healthy controls	Rotavirus cases	Healthy controls	Rotavirus cases	Healthy controls
	n=57	n=79	n=37	n=39	n=38	n=56	n=132	n=174
Secretor								
Non-secretor	2 (4)	7 (9)	0 (0)	5 (13)	2 (5)	4 (7)	4 (3)	16 (9)
Secretor	55 (96)	72 (91)	37 (100)	34 (88)	36 (95)	52 (93)	128 (97)	158 (91)
Lewis								
Lewis-negative	3 (5)	19 (24)	5 (13)	7 (18)	6 (16)	17 (30)	14 (11)	43 (25)
Lewis-positive	54 (95)	60 (76)	32 (87)	32 (82)	32 (84)	39 (70)	118 (89)	131 (75)

Table 4.3: Association between Secretor and Lewis phenotype and rotavirus-positive MSD

Models	Crude	Exclude children with stunting	Exclude children with stunting and partially vaccinated
Characteristic	mOR (95% CI)	mOR (95% CI)	mOR (95% CI)
Secretor Status			
Secretor	REF	REF	REF
Non-Secretor	0.27 (0.08-0.95)	0.30 (0.08-1.05)	0.32 (0.09-1.16)
Lewis Status			
Lewis-positive	REF	REF	REF
Lewis-negative	0.34 (0.17-0.67)	0.34 (0.16-0.72)	0.36 (0.16-0.79)

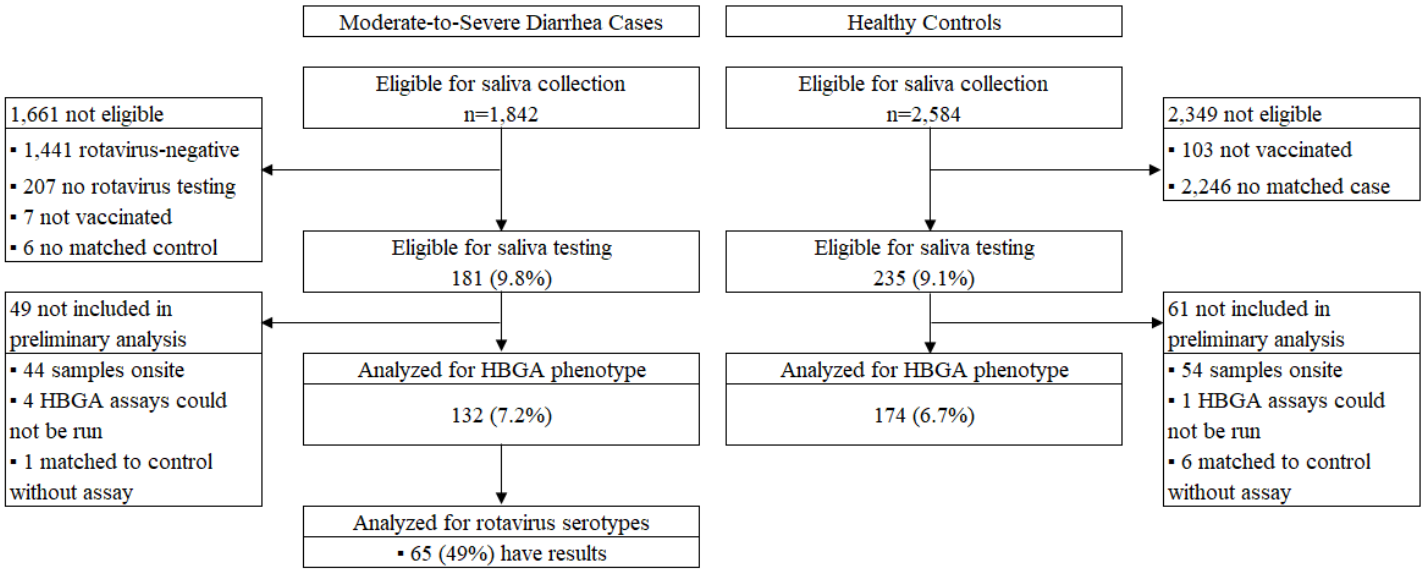
Table 4.4: Summary of rotavirus serotypes, using TaqMan

Serotype, n (%)	The Gambia	Mali	Kenya	All-sites
	n=26	n=26	n=13	n=65
P[4]	0 (0)	0 (0)	10 (77)	10 (15)
P[6]	0 (0)	0 (0)	1 (8)	1 (2)
P[8]	26 (100)	26 (100)	2 (15)	54 (85)

Table 4.5: Summary of Secretor and Lewis phenotypes for rotavirus-positive MSD cases and matched controls, by rotavirus serotype

HBGA Phenotype	P[8]	P[6]	P[4]	P[6] or P[4]
	cases/controls	cases/controls	cases/controls	cases/controls
Secretor Status				
Secretor	54/51	1/1	10/9	11/10
Non-Secretor	0/7	0/0	0/1	0/1
Lewis Status				
Lewis-positive	51/45	1/1	10/6	11/7
Lewis-negative	3/13	0/0	0/4	0/4

Figure 4.1 Saliva collection in VIDA



CHAPTER 5: CONCLUSION

The results from this dissertation highlight the importance of evaluating epidemiologic study designs to understand the accuracy of rotavirus vaccine effectiveness results, assessing the population-level impact of rotavirus vaccine after introduction in Bangladesh, and understanding the role of genetic determinants of rotavirus vaccine susceptibility and rotavirus vaccine failure in sub-Saharan Africa. Overall, these studies show the remarkable public health impact of rotavirus vaccine introduction worldwide.

As observed in Chapter 2, using a randomized clinical trial database, the results from the TND analysis for RV1 and RV5 in sub-Saharan Africa and Asia were similar to primary efficacy results and upheld key assumptions of the TND. After accounting for known biases in observational studies, the TND can provide accurate rotavirus vaccine effectiveness results. As new vaccines are introduced into immunization programs, researchers may use the TND to assess performance of these vaccines due to its low-cost and efficiency. It will be important to evaluate and test the assumptions of the TND for these pathogens and under various conditions in low-income settings.

Chapter 3 provides evidence of the population-level impact of rotavirus vaccine in children <2 years of age in regions of high vaccine coverage in Matlab, Bangladesh during 3.5 years of routine use. We used two models to attempt to minimize the influence of the RCT during our study period and its impact on healthcare seeking behavior. This analysis demonstrates the importance of using the appropriate baseline incidence and underlying trends in time-series analyses. To our knowledge no other population-level impact analyses have been reported in Asia. Our estimates were similar to those observed in sub-Saharan Africa, though in these studies >90% vaccine coverage for one or two doses of rotavirus vaccine was reported within one year

of vaccine introduction. With sustained vaccine coverage and a significant nationwide burden of rotavirus diarrhea, larger impacts of RV1 on rotavirus gastroenteritis are likely to be observed long-term in Bangladesh. Future studies may also be able to measure the direct and indirect effects of rotavirus vaccine in young and older age groups. Continued diarrheal surveillance and nationwide population-level impact analyses will be critical to influence other countries in Asia to introduce rotavirus vaccine.

Chapter 4 examined the preliminary results of the largest case-control study in sub-Saharan Africa assessing the role of host genetic determinants and rotavirus vaccine failure. The study replicated results from other studies showing resistance to P[8] infections in children with null HBGA phenotypes. Additional data on non-P[8] infections will be critical to understand null HBGA phenotypes as a risk factor for rotavirus vaccine failure and inform future rotavirus vaccine development.

The results from this dissertation provide critical information related to appropriate study designs to measure rotavirus vaccine effectiveness, providing further evidence of rotavirus vaccine impact in Asia, and understanding of a novel risk factor for low rotavirus vaccine efficacy in sub-Saharan Africa. Continued research assessing rotavirus vaccines in low-income settings is essential to improving the performance of rotavirus vaccines in regions with the greatest burden.

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VITA

Lauren Michelle Schwartz was born in Detroit, Michigan and grew up in Franklin, Michigan. She received a BS in Neuroscience and Medical Anthropology from the University of Michigan in Ann Arbor, Michigan in 2009 and an MSPH in Global Disease Epidemiology and Control from the Johns Hopkins Bloomberg School of Public Health in 2011. Lauren completed her PhD in Epidemiology at the University of Washington in 2018.