TIMING OF ROTAVIRUS VACCINATION AND INCIDENCE OF SEVERE ROTAVIRUS GASTROENTERITIS AMONG INFANTS IN LOW- AND MIDDLE-INCOME COUNTRIES

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ABSTRACT

Joann Frances Gruber: Timing of rotavirus vaccination and incidence of severe rotavirus gastroenteritis among infants in low- and middle-income countries.
(Under the direction of Michele Jonsson Funk)

Rotavirus vaccines effectiveness is much lower in low- and middle-income countries (LMICs) than high-income countries. Some factors associated with decreased immune response, including interference by transplacental antibodies and the microbiota, may be mitigated by altering vaccine schedules. The purpose of this dissertation was to investigate when children in LMICs experienced severe rotavirus gastroenteritis (RVGE) and if the timing of rotavirus vaccine doses was associated with severe RVGE.

We analyzed data from two clinical trials in LMICs. To understand the timing and predictors of severe RVGE, we estimated the rate, cumulative incidence, and age distribution of severe RVGE among unvaccinated infants. Cox proportional hazards models were used to estimate associations between baseline factors and severe RVGE. To estimate the association between rotavirus vaccine dose timing and severe RVGE incidence, we compared different schedules using the complement of the Kaplan-Meier estimator to estimate differences and ratios of cumulative severe RVGE risk at 6, 12, and 18 months of age and also used a Cox proportional hazards model to estimate hazard ratios. To obtain adjusted estimates, we used the associations observed in the placebo group, which should only differ from the null due to confounding, to calibrate the estimates within the rotavirus vaccinated groups.

The cumulative incidence of severe RVGE was 6 – 8 % at 20 months of age. The cumulative incidence increased steadily over the first two years of life and was low at 6 months of age. Antibiotic use was associated with about 1.4 to 2 times the rate of severe RVGE. There was a dose-response relationship between age at first pentavalent vaccine (RV5) dose and severe RVGE. Earlier administration of first RV5 dose was associated with an increased severe RVGE risk and that risk declined with increased age of first dose until approximately 8 – 9 weeks of age. An interval of 4 versus 6 weeks between monovalent vaccine (RV1) doses was associated with increased risk of severe RVGE when RV1 was administered on an approximately 10/14 week schedule. This dissertation, in conjunction with previous scientific literature, indicates severe RVGE episodes may be prevented by altering rotavirus vaccine schedules in LMICs.

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LIST OF ABBREVIATIONS

BCG	Bacillus Calmette–Guérin vaccine
$CCID_{50}$	Median cell culture infective dose
CI	Confidence interval
ELISA	Enzyme-linked immunosorbent assay
EPI	Expanded Program on Immunization
DTP-HB/HIB	Diphtheria, tetanus, pertussis, Hepatitis B, *Haemophilus influenzae* B vaccines
DTaP	Diphtheria, tetanus, acellular pertussis vaccine
DTPw	Diphtheria, tetanus, whole cell pertussis vaccine
GI	Gastrointestinal
GMC	Geometric mean concentration
GNI	Gross national income
HBV	Hepatitis B vaccine
HIC	High-income country
HIV	Human immunodeficiency virus
HR	Hazard ratio
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IPV	Inactivated poliovirus vaccine
IQR	Interquartile range
LMIC	Low- and middle-income country
NA	Not applicable

OPV	Oral poliovirus vaccine
RD	Risk difference
REST	Rotavirus Efficacy and Safety Trial
RR	Risk ratio
RT-PCR	Reverse transcription polymerase chain reaction
RV1	Monovalent rotavirus vaccine
RV5	Pentavalent rotavirus vaccine
RVGE	Rotavirus gastroenteritis
SE	Standard error
SMD	Standardized mean difference
US	United States
WHO	World Health Organization

CHAPTER 1: SPECIFIC AIMS

Prior to the global rollout of rotavirus vaccines, rotavirus was the leading cause of severe diarrhea in children worldwide [1, 2]. Each year there were 140 million cases of rotavirus infection in children under five with 26 million of cases requiring clinic visits or hospitalizations [3]. By the age of 3 – 5 years, every child in the world experienced rotavirus infection [4, 5] and about one in every 260 children died as a result of the infection annually [2]. Despite similar rates of infection, rotavirus-associated mortality disproportionately affected, and continues to affect, children in LMICs, where 80 – 90% of rotavirus-associated deaths occur [1, 6].

As of 2009, the World Health Organization (WHO) recommended rotavirus vaccination for all children [7]. Two live, oral rotavirus vaccines, the two dose monovalent (RV1; Rotarix™, GlaxoSmithKline Biologicals, Rixensart, Belgium) and three dose pentavalent vaccine (RV5; RotaTeq™, Merck & Co., Inc.; Kenilworth, NJ, USA), are available for use across the globe. While high protective one-year efficacy (96 – 98%) against severe RVGE has been reported in high-income countries (HICs) [8-10], the efficacy has been much lower (51 –64%) in trials conducted in African and Asian LMICs [11-13]. This lower vaccine efficacy reported in LMICs is similar for other live oral vaccines including oral poliovirus vaccine (OPV), cholera vaccine, and oral typhoid vaccine [14-16]. Reasons for lower effectiveness in LMICs compared to HICs have been investigated in recent years. While breastfeeding may not greatly affect rotavirus vaccine response [17-19], concomitant vaccination with OPV [20, 21], malnutrition [22, 23], interference by transplacental maternal antibodies [24, 25], and environmental enteropathy and

the infant microbiota [26, 27] all may contribute to the lower vaccine effectiveness observed in LMICs.

Some of the factors shown to decrease rotavirus vaccine performance including interference by transplacentally acquired maternal antibodies [24, 25] and composition of the microbiota [26, 27] may be mitigated by altering rotavirus vaccine schedules. However, even if alterations in vaccine schedules could improve the vaccine performance, changing schedules may not be advantageous if children experience severe RVGE before vaccination. Therefore, it is essential to understand the age at which children are experiencing severe RVGE to ensure vaccination using any schedule will protect the most infants. Although it is well accepted children in LMICs experience rotavirus infection very early in life (median approximate age of 6 – 9 months) [28], it is less well understood exactly when infants experience severe RVGE. Severe RVGE is the most clinically relevant outcome prevented by rotavirus vaccines to reduce hospitalization and death. Also, rotavirus vaccines are highly effective at preventing severe RVGE in HICs, but much less effective at preventing RVGE of any-severity [10, 29]. Therefore, it is important to understand the natural history of severe RVGE to weigh any potential advantages and disadvantages of altering rotavirus vaccination schedules.

The purpose of this dissertation was to investigate when children in LMICs experienced severe RVGE episodes, defined as a Vesikari or modified-Vesikari score of > 11, and if the timing of rotavirus vaccine doses was associated with risk of severe RVGE in these areas. We used data from two clinical trials: one of 4,939 randomized infants in the RV1 trial conducted in South Africa and Malawi (Clinical Trial Number: NCT00241644) and another of 7,504 randomized infants in the RV5 trial conducted in Ghana, Kenya, Mali, Bangladesh, and Vietnam (Clinical Trial Number NCT00362648). Our specific research aims were the following:

Aim 1: To describe the natural history of severe RVGE among infants in the placebo groups of the rotavirus vaccine trials in LMICs.

Aim 1a: To describe the timing of first episode of severe RVGE.

Aim 1b: To estimate the association between incidence of first severe RVGE and baseline factors, including demographic information; breastfeeding and growth status; and concomitant infection, antibiotic use, and vaccination.

Aim 2: To estimate the association between timing of rotavirus vaccine doses and incidence of severe RVGE among vaccinated infants in the rotavirus vaccine trials in LMICs.

The results of this research can help understand the benefits and harms of altering rotavirus vaccine schedules in LMICs. Ultimately, these data can inform administration strategies of rotavirus vaccines in LMICs to prevent the most cases of severe RVGE.

CHAPTER 2: BACKGROUND AND SIGNIFICANCE[1]

Rotavirus Morbidity and Mortality

Rotavirus was a leading cause of severe diarrhea in children worldwide before widespread use of new rotavirus vaccines licensed in countries in 2006. [1, 2]. Worldwide surveillance estimates in 2009 indicated the median prevalence of rotavirus among majority rotavirus unvaccinated children hospitalized for gastroenteritis was 36% (range among countries: 12 – 68%) [30]. The high prevalence of rotavirus among hospitalized children across all countries makes this pathogen an important global health issue.

Despite a similar prevalence of rotavirus worldwide, rotavirus-associated mortality differs greatly between LMICs and HICs. In 2008, 37% of diarrhea-associated deaths and 5% of all deaths in children less than five years of age were attributed to rotavirus [2]. About 1,200 children die of rotavirus each day, and 82% of these rotavirus-associated deaths occur in the world's poorest countries [1]. In particular, five countries accounted for greater than half the rotavirus-associated deaths: the Democratic Republic of the Congo, Ethiopia, India, Nigeria, and Pakistan [2]. In the United States (US) and Europe, there are only about 35 and 230 deaths per year due to rotavirus, respectively [31, 32]. The dramatic differences in rotavirus death rates make rotavirus a different public health issue in LMICs and HICs.

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Rotavirus Virology and Infection

Rotavirus is a segmented, double-stranded RNA, non-enveloped virus with three major protein structures: the outer capsid formed by proteins VP7 and VP4, the intermediate capsid formed by protein VP6, and inner core formed by VP2 and also containing proteins VP3 and VP1 [3, 33]. The VP6 protein largely determines the group and subgroup of the virus while the serotype is determined by the VP4 spike protein and VP7 glycoprotein [3]. The potential for mutation and reassortment of the segmented genome from mixed infections has led to great diversity among rotaviruses [34]. There are seven major groups (A to G) and two major subgroups (I and II) that have been identified [3, 33]. The serotype is defined by the G serotype, or the glycosylated protein VP7, and the P genotype, or the protease-cleaved protein VP4 [3, 33]. At least 10 G serotypes, 11 P genotypes, and 42 P-G combinations have been identified [34].

Human rotavirus infections are primarily caused by group A rotaviruses of either subgroup with a number of serotypes [34, 35]. A review of 124 studies from 52 countries estimated 88% (N = 16,474) of worldwide serotype/genotype strains are P[8]G1 (65%), P[4]G2 (12%), P[8]G4 (8%), and [P8]G3 (3%) [36]. However, the distribution of these serotype/genotypes varies greatly across geographic regions [36]. The relative occurrence of each strain can also change over time, and new strains can emerge with antigenic drift and shift [36].

Rotaviruses are spread primarily through the fecal-oral route [37]. Infection with rotavirus can occur by contact with contaminated persons, food, water, and surfaces [37] and lead to vomiting and fever followed by non-bloody diarrhea [28, 38-40]. The incubation period is short, usually 24-48 hours [38, 39], and vomiting typically occurs at the start of symptoms and lasts one to two days [37] while duration of other gastrointestinal symptoms is usually four to

seven days [38]. Severe infection, requiring a clinic or hospital visit, has been documented in 7% [41] to 36% [42] of infected children. Treatment for rotavirus is focused on the assessment and subsequent treatment and prevention of dehydration [37, 43]. Rehydration therapy is recommended for children with dehydration [43].

Following infection, the immune response and subsequent immunity is thought to be mediated through localized, mucosal anti-rotavirus immunoglobulin A (IgA) antibodies [4]; however, immune response to wild-type infection or vaccination is not fully understood. Importantly, there is no known correlate of protection between RVGE and anti-rotavirus IgA antibody concentration [44, 45]. However, aggregate anti-rotavirus IgA antibody concentrations have been correlated with rotavirus vaccine efficacy and are often used as a surrogate for clinical endpoints [46]. In addition to IgA, anti-rotavirus immunoglobulin G (IgG) antibodies likely play a role in immune response, because transplacentally acquired anti-rotavirus IgG antibodies seem to largely protect neonates from symptomatic infection [47].

Immunity from natural infection may vary depending on the number of acquired infections and geographic region. First infection is thought to generate a primarily homotypic antibody response whereas later infections are thought to generate a heterotypic response, conferring more broad protection [48]. In a birth cohort of Mexican infants (N = 200), two infections (symptomatic or asymptomatic) were shown to confer 100%, 75% (95% CI: 45%, 89%), and 62% (95% CI: 34%, 79%) immunity to subsequent moderate-to-severe diarrhea, mild diarrhea, and asymptomatic infection, respectively [49]. In a birth cohort in India (N= 373), two infections with moderate to severe diarrhea, mild diarrhea or asymptomatic infection conferred 57% (95% CI: 6%, 80%), 72% (95% CI: 58%, 81%), and 33% (95% CI: 16%, 46%) protection against moderate to severe diarrhea, mild diarrhea or asymptomatic infection, respectively [50].

In Guinea-Bissau, a single rotavirus infection conferred 70% (95% CI: 29%, 87%) and 52% (95% CI: 16%, 73%) against rotavirus diarrhea and infection, respectively [51].

Rotavirus Vaccines

Due to the high burden of RVGE and the potential immunizing effect of infection, the development of safe and effective vaccines has been a global priority. First generation vaccines used naturally attenuated animal strains of rotavirus and were generally unsuccessful at consistently preventing severe RVGE [52-54]. Second generation vaccines use either attenuated human or human-animal reassorted strains of rotavirus and have been much more successful than first generation vaccines [53, 55].

In 1998, a promising second generation, tetravalent rhesus-human reassortant rotavirus vaccine was licensed in the US (RotaShield®, Wyeth Lederle Vaccines) [53, 56-58]. This vaccine was withdrawn from the market after one year when increased risk of intussusception (intestinal obstruction when part of the intestine telescopes into an adjacent part) [59], was found to be associated with vaccination [60].

Currently, there are two live, oral rotavirus vaccines available broadly across the globe, RV1 and RV5. In addition, there are a few other vaccines licensed for use in national markets including: ROTAVAC® manufactured by Bharat Biotech International Limited and licensed for use in India in 2014, Rotavin-M1^{TM} manufactured by the Center for Research and Production of Vaccines and licensed for use in Vietnam in 2007, and Lanzhou Lamb Rotavirus Vaccine (first generation vaccine) manufactured by Lanzhou Institute of Biological Products and licensed for use in China in 2000. Development of new vaccines is also taking place in Brazil, China, India, Indonesia, and the US.

RV5 is an oral pentavalent rotavirus vaccine that was first licensed for use in 2006 for infants 6 to 32 weeks of age [55]. Each 2 mL vaccine dose contains 5 live human-bovine reassortant rotaviruses (G1, G2, G3, G4, and P1A[8]) at a concentration of 2.0 – 2.8 x 10^6 infectious units per dose, depending on the genotype. Vaccination is a three part series beginning at 6 to 12 weeks of age with subsequent doses given at 4 to 10 week intervals with the final dose administered before 32 weeks. The vaccine is contraindicated for those with history of intussusception, severe combined immunodeficiency disease, and hypersensitivity to the vaccine or its components.

RV1 is an oral monovalent rotavirus vaccine that was first licensed for use in 2008 for infants 6 to 24 weeks of age [61]. Each 1mL vaccine dose contains live-attenuated human G1P[8] rotavirus at a median concentration of at least 10^6 Cell Culture Infective Dose. The two dose vaccine series begins at 6 to 20 weeks of age with a subsequent dose at least 4 weeks after the first dose and prior to 24 weeks of age. This vaccine is contraindicated for infants with history of intussusception, severe combined immunodeficiency disease, hypersensitivity to the vaccine or its components, and uncorrected congenital malformation of the gastrointestinal tract that would predispose the infant to intussusception.

Use, Efficacy, and Effectiveness of Rotavirus Vaccines

As of 2009, the WHO recommended rotavirus vaccination for all infants [7], and as of May 2016, 81 countries have introduced rotavirus vaccines into their national immunization schedules, including 38 Gavi-eligible countries [62]. Eight countries (Austria, Brazil, El Salvador, Luxembourg, Nicaragua, Panama, the US, and Venezuela), including one Gavi-eligible country (Nicaragua), were the first to introduce rotavirus vaccines in 2006. Of the 81 countries

who have introduced rotavirus vaccines, 21 (26%) are low income (≤ $1,045 per capita gross national income (GNI), 23 (28%) are lower middle income ($1,045 – $4,125 GNI), 19 (23%) are upper middle income ($4,125 – $12,746 GNI), and 18 (22%) are high income (≥ $12,476 GNI). Of these 81 countries, 59 (73%) use RV1, 18 (22%) use RV5, and 4 (5%) use both RV1 and RV5. Among the 38 Gavi-eligible countries, 33 (87%) use RV1 and the remaining 5 (13%) use RV5.

The one-year efficacies of RV1 and RV5 at preventing severe RVGE have been high in clinical trials in HICs (96 – 98%) [8-10], but lower (51 – 64%) in LMICs [11-13]. These estimates come from some of the first rotavirus vaccine trials conducted for RV1 and RV5 in these areas. The Rotavirus Efficacy and Safety Trial (REST) for RV5 was conducted in 11 countries from 2001 to 2004 among about 70,000 infants of which a subset of about 5,700 were followed for efficacy [10]. Similarly, the clinical trial for RV1 took place from 2004 to 2006 in 6 European countries and efficacy against wild-type rotavirus was determined for about 4,000 infants who received 2 doses of vaccine/placebo [63]. A trial of RV5 was conducted in 5,225 infants in Africa (Ghana, Kenya, Mali) [12] and 1,969 infants in Asia (Bangladesh, Vietnam) from 2007 to 2009 [13]. The trial of the RV1 was conducted from 2005 to 2007 in 4,939 infants living in South Africa and Malawi [11]. Other trials and studies have been conducted, but these were the first large scale trials for their respective vaccines/regions. The efficacies estimated in these trials are summarized in Table 2.1.

Rotavirus vaccines have lower efficacy in LMICs when compared to HICs. The pooled efficacy of rotavirus vaccines in LMICs is 51% whereas in HICs, it is 85% [64]. The same pattern has continued in post-introduction effectiveness studies. Despite the success of these

vaccines at reducing health care encounters and hospitalizations [65-67], rotavirus vaccine effectiveness is lower in LMICs as compared to HICs [68, 69].

Several rotavirus vaccine experts have hypothesized and investigated reasons for lower rotavirus vaccine efficacy based on differences between rotavirus presentation in LMICs and HICs. There are three main factors that could influence the effectiveness of rotavirus vaccines: 1) rotavirus epidemiology, 2) vaccine availability after entry into the host (i.e., the ability to reach the epithelial cells of the gastrointestinal tract), and 3) the immune response of the infant [15].

The epidemiology of rotavirus is different between LMICs and HICs. There is a large amount of diversity among the rotaviruses that are found in LMICs as compared to HICs [36]. In addition, rotaviruses tend to circulate year round in many LMICs, because there is not a strong seasonal pattern of rotavirus infections in tropical areas as compared to the winter peaks seen in temperate climates of most HICs [3, 4, 42, 70]. Also, the age of first severe RVGE is thought to be earlier in children in LMICs (median 6 – 9 months) compared to HICs (median 9 – 15 months) [28].

Factors that may affect vaccine availability are transplacental antibodies, breast milk, and stomach pH [15]. There are high levels of circulating anti-rotavirus antibodies in adults living in LMICs compared to HICs [71], and some studies have reported high correlations (0.57 to 0.86) between anti-rotavirus IgG antibody levels in mothers and infants in LMICs [71, 72]. One study in India demonstrated the ability of pre-existing anti-rotavirus IgG antibodies in infant blood to neutralize the effect of a new rotavirus vaccine (ORV-116E) [73]. There is also some evidence that anti-rotavirus IgG antibodies inhibit the immune response in vaccinated infants. In Ghana, a trial investigating the impact of alternative RV1 vaccines schedules on seroconversion found seroconversion was higher among infants with the lowest compared to highest quartile of pre-

vaccination IgG levels [25]. A similar result was also found in Pakistan and India [17, 24]. Although transplacentally acquired antibodies decay exponentially with a half-life of around 35 to 40 days, there is considerable individual variability in the rate of clearance [74]. One study has examined the clearance rate of passively acquired anti-rotavirus antibodies in 54 Mexican infants and found a gradual decline until four months of age and then an increase, likely due to incident rotavirus infections [75]. In addition, the critical level at which maternally acquired antibody concentrations are low enough to elicit a robust immune response in infants is unknown. Consequently, these data suggest it is possible that passively acquired antibodies could interfere, or even entirely negate, the immunologic response to rotavirus vaccines given to infants at the same age or earlier in LMICs compared to HICs. Similarly, breast milk contains anti-rotavirus IgA antibodies as well as neutralizing immune factors that can inhibit vaccine availability [71, 76] and these immune factors have been shown to be in higher concentration in women from LMICs compared with women from HICs [77-79]. However, a randomized trial of breastfeeding in South Africa (N = 204) found that withholding breastfeeding one hour before and after each dose of rotavirus vaccine had no effect on one-month IgA seroconversion status compared to unrestricted breastfeeding before/after vaccination [18]. Similarly, a randomized trial of withholding breastfeeding 30 minutes before/after each dose of vaccine compared to encouragement not to withhold breastfeeding did not have a significant impact on one-month IgA seroconversion status in Indian infants (N = 391) [17]. In addition, high levels of stomach acid can inactivate live vaccines [80]. Although the vaccine is administered with a buffer solution, stomach acid levels in children in LMICs could be higher resulting in a lower immunogenic dose of vaccine.

Factors that may affect the immune response to rotavirus vaccination are breastfeeding, malnutrition, co-infection and environmental enteropathy, the gut microbiota, and co-administration of vaccines or medications [15]. While breast milk may provide factors that inhibit vaccine uptake, it can also provide components that result in a more robust infant immune system [81]. For example, exclusive breastfeeding in Bangladeshi infants was associated with higher antibody titers after OPV administration [82]. In addition, adequate nutrition and specific micronutrients are critical for proper immune function [83]. Consequently, malnutrition could be a reason for hyporesponsiveness to rotavirus vaccines in LMICs with high levels of malnutrition in mother and infants. A recent study in Bangladesh found children with wasting had significantly lower antibody titers following OPV administration compared to children without wasting [82]. Furthermore, infants in LMICs are exposed to high levels of co-infections (e.g., human immunodeficiency virus (HIV), malaria, diarrheal diseases, etc.) that may affect vaccine response [84]. In addition, the combination of undernourishment and repeated exposure to enteric pathogens can lead to chronic environmental enteropathy which can lead to altered enteric immunity [26]. Environmental enteropathy has been associated with lower rotavirus vaccine response in Bangladeshi infants [26]. Similarly, the composition of the microbiota itself is constantly evolving early in life and may influence the vaccine response. A randomized trial in India reported higher seroconversion rates among infants receiving RV1 with zinc or probiotics compared to the RV1 alone [85]. A case control study of 6 week old Ghanaian infants found RV1 vaccine responders (post-vaccination IgA antibody levels $\geq$ 20 IU/mL) had microbiotas more closely resembling Dutch infants than Ghanaian non-responders (post-vaccination IgA antibody levels $\geq$ 20 IU/mL) [27]. Additionally, infants in LMICs are administered OPV rather than the inactivated poliovirus vaccine (IPV) that is given in HICs, and OPV can interfere with

the immune response to rotavirus vaccines, particularly with the first doses of rotavirus vaccines [20, 21]. Finally, concomitant antibiotic or other vaccine use may also influence immune response to rotavirus vaccines [86].

Timing of Rotavirus Vaccine Doses and Immunologic Response

Some of the factors shown to decrease rotavirus vaccine performance may be mitigated by altering rotavirus vaccine schedules. For example, interference by transplacental maternal IgG anti-bodies could potentially be reduced by increasing the age the first dose of rotavirus vaccine is given. Also, delaying the timing of first dose, and consequently subsequent doses, could provide more time for the infant immune system and microbiota to develop, which could result in a more robust immune response to vaccination. Therefore, alterations in rotavirus vaccine schedules could result in stronger immune responses to vaccines and ultimately prevent more episodes of severe RVGE among children in LMICs.

There have been eight clinical trials that have assessed different RV1 vaccine schedules in LMICs (Table 2.2). There have been no randomized or observational studies assessing the effect of vaccine schedule on the efficacy/effectiveness of the RV5 in LMICs, nor have there been observational studies assessing different dosing schedules RV1. The one-month seroconversion proportions and geometric mean titer by trial, country, and schedule are summarized in Table 2.3.

Differences in seroconversion proportions comparing 6/10 versus 10/14 week schedules and 6/10/14 versus 10/14 week schedules are presented in Figure 2.1. Generally, seroconversion proportions for the 6/10 week schedule were lower than for the 10/14 week schedule. By contrast, seroconversion proportions for the 6/10/14 week schedule were similar or slightly

higher than seroconversion proportions for the 10/14 week schedule. Differences in the less commonly used schedules are presented in Table 2.4. There was no difference in seroconversion proportions between the 6/10/14 and 6/10/14/18/22 week schedules in India [87]. However, there was a lower seroconversion proportion for the 8.8/13.2 week schedule compared to the 8.6/17.4 week schedule in Vietnam [88]. The seroconversion proportion for the 6.5/15.1 week schedule in the Philippines was lower than the 10.6/15.2 week schedule [88].

The ratios of geometric mean concentration (GMC) levels comparing the 6/10 and 6/10/14 week schedules to the 10/14 week schedule are presented in Figure 2.2. These ratios followed a similar pattern to the differences in seroconversion proportions. Generally, the GMCs were lower for the 6/10 versus 10/14 week schedules. Similar to the results for difference in seroconversion proportion, the GMCs were similar or slightly higher for 6/10/14 versus 10/14 week schedules. For less commonly used schedules, the GMC was significantly lower for 8.8/13.2 week schedule compared to the 8.6/17.4 week schedule in Vietnam [88]. The responses were similar or slightly higher for the 6/10/14 week schedule compared to the 6/10/14/18/22 week schedule in India [87]. The responses were also similar or slightly higher for the 6.5/15.1 week schedule compared to the 10.6/15.2 week schedule [88].

One trial, (NCT00241644 [11, 89-91]), conducted in South Africa and Malawi, has reported vaccine efficacies by schedule using clinical outcomes (Table 2.5). In South Africa, the 6/10/14 week schedule had slightly higher efficacy when compared to the 10/14 week schedule. However, these efficacy estimates, particularly for the second year and cumulative two year efficacy, had large variability. In Malawi, the efficacy for the 6/10/14 week schedule was largely indistinguishable from the 10/14 week schedule for the first year efficacy, but the 6/10/14 week

schedule had a numerically higher efficacy compared to the 10/14 week schedule during the second year, though the estimates were very imprecise.

Other than the trial conducted in Malawi and South Africa, the studies conducted have been limited to analysis of immunologic response, of which there is no known correlate of protection [44, 45]. This means despite compelling evidence that the 6/10/14 or 10/14 week schedule may be more immunogenic than the 6/10 week schedule, this may not relate to protection against severe RVGE. Further research is needed to determine if dose timing, particularly for RV5 and only two doses of RV1, which is as it is used in routine immunization, impacts the incidence of severe RVGE among children in LMICs.

Dosing Schedule and Timing of Severe RVGE

When considering the potential alteration of rotavirus vaccine schedules, it is important to consider when children are experiencing severe RVGE. This is because the vaccine must be given early enough to prevent severe RVGE and to avoid vaccination during the natural peak in incidence of intussusception in children, but late enough to elicit a robust immune response from infants. Understanding the timing of severe RVGE events is critical to weigh the potential benefits and harms of altering vaccine schedules.

Although it is well accepted that infants in LMICs are experiencing early exposure to rotavirus (median age of infection in LMICs at 6 – 9 months of age compared to 9 – 15 months HICs), it is less understood exactly when infants experience their first episode of severe RVGE. Severe RVGE is the most critical outcome prevented by rotavirus vaccines to reduce hospitalization and death. Therefore, it is important to understand the natural history of severe RVGE to weigh the potential advantages and disadvantages of altering vaccination schedules.

In LMICs, there have been a number of longitudinal, birth cohort studies describing the natural history of RVGE infections in young children. However, many of these studies have several limitations for determining when children experience their first episode of severe RVGE. First, several studies summarizing timing of infection do not differentiate between asymptomatic and symptomatic infections [49, 50] or severe or non-severe symptomatic infections [51, 92-97]. Second, many studies provide the number or proportion of symptomatic rotavirus infections rather than the rate of infection by age groups less than one year of age [49, 92-96, 98, 99]. Since there can be variable lengths of follow-up among cohort members, particularly in low-resource settings where implementation of research studies can be challenging, it is important to consider the length of follow-up in these age groups. Third, many studies do not provide descriptive statistics describing the distribution of timing of first RVGE [49, 51, 93-96, 98]. These data are important to better understanding the distribution and variability of timing of severe RVGE in LMICs. Ultimately, these limitations make it extremely difficult to use existing data to understand when children in LMICs are experiencing severe RVGE and how that may relate to potential alterations of rotavirus vaccine schedules.

Summary

Rotavirus is historically an important cause of severe diarrhea in children across the globe. Although rotavirus vaccines have been highly effective at preventing severe RVGE in HICs, these vaccines have not performed as well in LMICs, where the majority of rotavirus-associated mortality occurs. Concomitant vaccination with OPV, malnutrition, interference by transplacental maternal antibodies, environmental enteropathy, and the infant microbiota may all contribute to the lower vaccine effectiveness observed in LMICs. Importantly, some of the

factors shown to decrease rotavirus vaccine performance, including interference by transplacentally acquired maternal antibodies and composition of the microbiota, may be mitigated by altering rotavirus vaccine schedules. However, even if alterations in vaccine schedules may improve the vaccine performance, changing schedules may not be advantageous if children experience severe RVGE before vaccination. Therefore, in addition to understanding if timing of rotavirus vaccine doses affects the incidence of severe RVGE, it is also important to understand the timing of severe RVGE in children in LMICs to weigh any potential advantages and disadvantages of altering vaccination schedules.

Table 2.1 Rotavirus vaccine efficacies against severe RVGE from select clinical trials.

Vaccine	Countries	N	Efficacy (%) (95% Confidence Interval (CI))	
			First Year	Second Year
RV5	US, Finland	4,512	98.0 (88.3, 100)	88.0 (49.4, 98.7)
RV1	Czech Republic, Finland, France, Germany, Italy, Spain	3,848	95.8 (89.6, 98.7)	85.6 (75.8, 91.9)
RV5	Ghana, Kenya, Mali	5,225	64.2 (40.2, 79.4)	19.6 (-15.7, 44.4)
RV5	Bangladesh, Vietnam	1,969	51.0 (12.8, 73.3)	45.5 (1.2, 70.7)
RV1	South Africa, Malawi	2,939	58.7 (35.7, 74.0)	

RVGE, rotavirus gastroenteritis

Table 2.2 Characteristics of trials and trial populations that have investigated alternative RV1 schedules.

Trial Number	Phase	Location	Inclusion	Exclusion	Study Notes
NCT00346892 [100]	II	South Africa	• Healthy infants • 5 – 10 weeks at first dose • HIV-confirmed negative mother*	• History of 1) allergic disease, 2) clinically significant chronic gastrointestinal (GI) disease, 3) serious medical condition • Confirmed or suspected immunosuppressive or immunodeficient condition • Receipt of treatment prohibited by protocol	• Lyophilized formulation • Viral concentration: $1x10^{5.6}$ $CCID_{50}$ • EPI vaccines given • 100% concomitantly received OPV (group 1) • 100% concomitantly received IPV (group 2) • Breast feeding not restricted
NCT00383903 [101]	II	South Africa	• Healthy infants • 5 – 10 weeks at first dose • ≥ 36 weeks gestation at birth • HIV-confirmed negative mother	• History of 1) allergic disease, 2) clinically significant chronic GI disease, 3) serious medical condition, 4) polio disease • Confirmed or suspected immunosuppressive or immunodeficient condition (including HIV) • Receipt of treatment prohibited by protocol	• Lyophilized formulation • Viral concentration: $1x10^{6.0}$ $CCID_{50}$ • EPI vaccines given • 100% concomitantly received OPV • Breast feeding not restricted
NCT00345956 [88]	II	Vietnam	•Healthy infants •6 – 10 weeks • Birth weight > 2,000g	• History of allergic disease or suspected reaction • Chronic administration (since birth) of immunosuppressants or immune-modifying drugs • Planned vaccinations outside of protocol except DTPw, HBV and OPV vaccines within 14 days of each dose • Concurrent participation in another clinical study where an investigational product is used < 30 days of first dose or where any pharmaceutical product is used in study	• Liquid formulation • Viral concentration: $1x10^{6.0}$ $CCID_{50}$ • EPI vaccines given • 100% concomitantly received OPV • Breast feeding not mentioned
NCT00432380 [88]	II	Philippines	• Healthy infants • 5 – 10 weeks at first dose •Birth weight > 2,000g	• History of allergic disease or suspected reaction • Chronic administration of immunosuppressants or immune-modifying drugs six months prior to first dose; confirmed or suspected immunosuppressive or immunodeficient condition; receipt of immunoglobulins or blood products since birth or planned use in study • Planned vaccinations outside of protocol except DTPw, HBV and OPV vaccines within 14 days of each dose and BCG at birth according to local EPI guidelines • Concurrent participation in another clinical study; use of investigational product < 30 days of first dose or planned use in study; Acute disease at enrollment	• Liquid formulation • Viral concentration: $1x10^{6.0}$ $CCID_{50}$ • EPI vaccines given • 100% concomitantly received OPV • Breast feeding not mentioned

Trial Number	Phase	Location	Inclusion	Exclusion	Study Notes
NCT00241644 [11, 89-91]	III	South Africa Malawi	• Healthy infants • 5 – 10 weeks at first dose • > 36 weeks gestation at birth† • Birth weight > 2,000g or unknown† • Parents/guardians who investigators believe will comply with study requirements	• History of 1) allergic disease, 2) clinically significant chronic GI disease, 3) serious medical condition, 4) neurologic disorders or seizures, 5) confirmed RVGE, 6) acute or chronic pulmonary, cardiovascular, hepatic or renal function abnormalities • Confirmed or suspected immunosuppressive or immunodeficient condition; receipt of immunosuppressants for > 14 days since birth; family history of congenital or hereditary immunodeficiency; receipt of immunoglobulins or blood products since birth or planned use in study • Previous routine vaccination except BCG, HBV, OPV at birth; planned receipt of vaccines not described in protocol within 14 days of each dose • Acute disease at enrollment or gastroenteritis within 7 day preceding first dose • History of experimental rotavirus vaccine use; concurrent participation in another clinical study where an investigational product is used < 30 days of first dose or planned use in study	• Lyophilized formulation • Viral concentration: $1x10^{6.0}$ $CCID_{50}$ • EPI vaccines given • ≥99% concomitantly received OPV • Breast feeding not restricted
NCT01199874 [24]	IV	Pakistan	• Healthy infants • 6 weeks 0 days to 6 weeks 6 days at enrollment	• History of 1) intussusception, 2) abdominal surgery, 3) hypersensitivity to vaccine components • Use of immunosuppressants; receipt of immunoglobulins or blood products since birth or planned use in study • Concurrent participation in another trial; use of investigational product < 30 days of first dose or planned use in study • Birth weight < 1,500g or, if birth weight unknown, weight < 2,000g by 28 days of age	• Lyophilized formulation • Viral concentration: $1x10^{6.}$ $CCID_{50}$ • EPI vaccines given • ≥99% concomitantly received OPV • Breast feeding not restricted
CTRI-2012-02-002454 [87]	IV	India	• Infants • < 7 weeks of age attending Well Baby Clinic for routine EPI immunization • 36 – 42 weeks gestation at birth	• History of 1) intussusception, 2) abdominal surgery, 3) congenital abdominal pain, 4) confirmed RVGE, 5) chronic diarrhea, 6) failure to thrive, 7) hypersensitivity to vaccine components • Confirmed or suspected impairment of immunological function; receipt of any intramuscular, oral, or intravenous corticosteroid treatments in past 30 days • Active gastroenteritis or fever (≥ 38.1°C)	• Lyophilized formulation • Viral concentration: $1x10^{6.0}$ $CCID_{50}$ • EPI vaccines given • % concomitant OPV not reported‡ • Breast feeding not mentioned

Trial Number	Phase	Location	Inclusion	Exclusion	Study Notes
			• Birth weight ≥ 2,000g • Guardians available for follow-up	• Prior rotavirus vaccination • Exclusion from routine EPI immunization	
NCT01575197 [25]	IV	Ghana	• Healthy infants • 42 – 55 days at enrollment • Guardians able to follow study procedures	• History of intussusception or abdominal surgery • Receipt of immunoglobulins or blood products since birth or planned use in study • Concurrent participation in another intervention trial or use of investigational product in study • Birth weight < 2,000g or < 36 weeks gestation at birth, if available • Prior rotavirus vaccination • Planned relocation prior to study completion • Another child living in same compound is already enrolled in study until vaccine is introduced in EPI system in Navrongo at which point a child in the same compound who is < 16 weeks of age can be enrolled	• Lyophilized formulation • Viral concentration: $1x10^{6.0}$ $CCID_{50}$ • EPI vaccines given • 100% concomitantly received OPV • Breast feeding not mentioned

$CCID_{50}$, Median Cell Culture Infective Dose

*HIV status was only confirmed after 2002 rotavirus season

†South Africa only

‡ Exact percentage not reported; the recommended poliovirus vaccine schedule from the Indian Academy of Pediatrics Committee on Immunization is OPV (birth, 6 months and 9 months) and IPV (6, 10, 14 weeks).

Table 2.3 One-month seroconversion percent and GMC by trial and country for different schedules of RV1 administered concomitantly with routine vaccines, including OPV unless otherwise indicated.

Trial Number Trial Month/Year	Location	Schedule	N	Seroconversion[*] % (95% CI)	GMC (U/ml) (95% CI)
NCT00346892[†]	South Africa	6/10	64[§]	36 (23, 50)	28.1 (18.2, 43.2)
Nov/2001 - Oct/2003		10/14	63[§]	61 (43, 76)	48.6 (29.9, 78.9)
		6/10[‡]	41[§]	43 (29, 58)	32.6 (20.7, 51.3)
		10/14[‡]	42[§]	55 (39, 70)	56.7 (32.5, 98.9)
NCT00383903	South Africa	6/10/14	133	44.4 (35.8, 53.2)[¶]	30.7 (24.0, 39.3)[¶]
Sept/2003 - Feb/2004		10/14	131	44.3 (35.6, 53.2)[¶]	29.3 (23.0, 37.3)[¶]
NCT00345956[\\]	Vietnam	8.8/13.2	130	56.2 (47.2, 64.8)	48.7 (36.1, 65.8)
Sept/2006 - Mar/2007		8.6/17.4	119	81.5 (73.4, 88.0)	176.3 (123.8, 251.1)
NCT00432380[\\]	Philippines	6.5/15.1	120	59.2 (49.8, 68.0)	75.6 (52.5, 109.0)
Mar/2007 - Sept/2007		10.6/15.2	120	70.0 (61.0, 78.0)	68.0 (50.1, 92.1)
NCT00241644	South Africa	6/10/14	66	66.7 (54.0, 77.8)	94.3 (56.5, 157.4)
Oct/2005 - July/2007		10/14	70	57.1 (44.7, 68.9)	59.4 (37.5, 93.9)
	Malawi	6/10/14	83[§]	57.1 (42.2, 71.2)	51.2 (26, 102)
		10/14	68[§]	47.2 (30.4, 64.5)	63.0 (36, 109)
NCT01199874	Pakistan	6/10	46	29.7 (23.1, 37.3)	19.7 (16.2, 23.9)
Apr/2011 - Sept/2012		6/10/14	62	36.7 (29.8, 44.2)	25.8 (20.5, 32.5)
		10/14	60	38.5 (31.2, 46.3)	24.4 (19.5, 30.6)
CTRI-2012-02-002454[‡]	India	6/10/14	15	46.7 (21.3, 73.4)[**]	72.9 (30.9, 172.3)
Mar/2012 - Dec/2012		6/10/14/18/22	22	45.5 (24.4, 67.8)[**]	60 (35.3, 102.2)
NCT01575197	Ghana	6/10	142	28.9 (22.1, 36.8)	22.5 (17.4, 28.2)
Sept/2012 - Feb/2013		6/10/14	143	43.4 (35.5, 51.6)	32.6 (24.7, 43.2)
		10/14	139	37.4 (29.8, 45.7)	26.5 (20.7, 34.0)

[*] Percent of participants with post-anti-rotavirus IgA antibody concentrations of ≥ 20 U/ml
[†] Vaccine with viral concentration of $1x10^{5.6}$ $CCID_{50}$
[‡] Concomitant IPV
[§] Exact sample size not reported; sample size estimated
[¶] Two-month seroconversion proportion/GMC
[\\] Liquid formulation of vaccine
[**] Exact 95% CI estimated

Table 2.4 Seroconversion proportion difference and ratio of GMC by trial and country of RV1 for vaccine schedules less commonly reported.

Trial Number Trial Month/Year	Location	Schedule	Seroconversion proportion difference (95% CI)	Ratio of GMCs (95% CI)
CTRI-2012-02-002454[*] Mar/2012 - Dec/2012	India	6/10/14 6/10/14/18/22	0.01 (-0.32, 0.34) (reference)	1.2 (0.4, 3.3) (reference)
NCT00345956[†] Sept/2006 - Mar/2007	Vietnam	8.8/13.2 8.6/17.4	-0.25 (-0.36, -0.14) (reference)	0.3 (0.2, 0.4) (reference)
NCT00432380[†] Mar/2007 - Sept/2007	Philippines	6.5/15.1 10.6/15.2	-0.11 (-0.23, 0.01) (reference)	1.1 (0.7, 1.8) (reference)

[*] Concomitant IPV

[†] Liquid formulation of vaccine

Table 2.5 Rotavirus vaccine efficacy against severe RVGE comparing different schedules from trial NCT00241644.

Location	Schedule	N*	One Year Efficacy (95% CI)	Second Year Efficacy (95% CI)	Cumulative Two Year Efficacy (95% CI)
South Africa	10/14	971	72.2 (40.4, 88.3)	3 (-43, 82)	32 (-71, 75)
	6/10/14	973	81.5 (55.1, 93.7)	76 (-143, 100)	85 (35, 98)
Malawi	10/14	525	49.2 (11.1, 71.7)	2.6 (-101.2, 52.6)	34.0 (-2, 57.7)
	6/10/14	505	49.7 (11.3, 72.2)	33.1 (-48.6, 70.9)	42.3 (8.8, 64.0)

* Sample size in each arm for one year efficacy analysis.

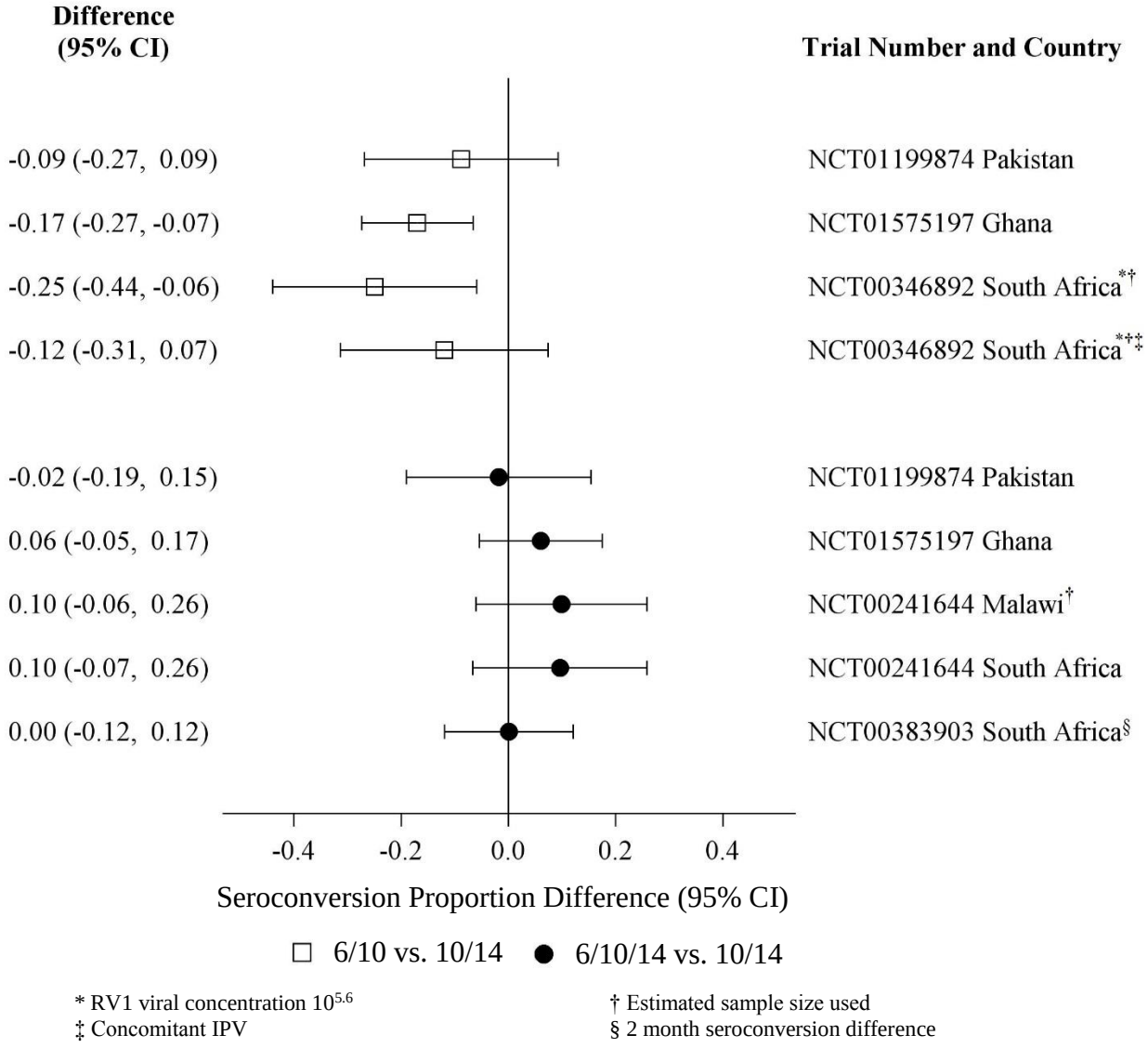
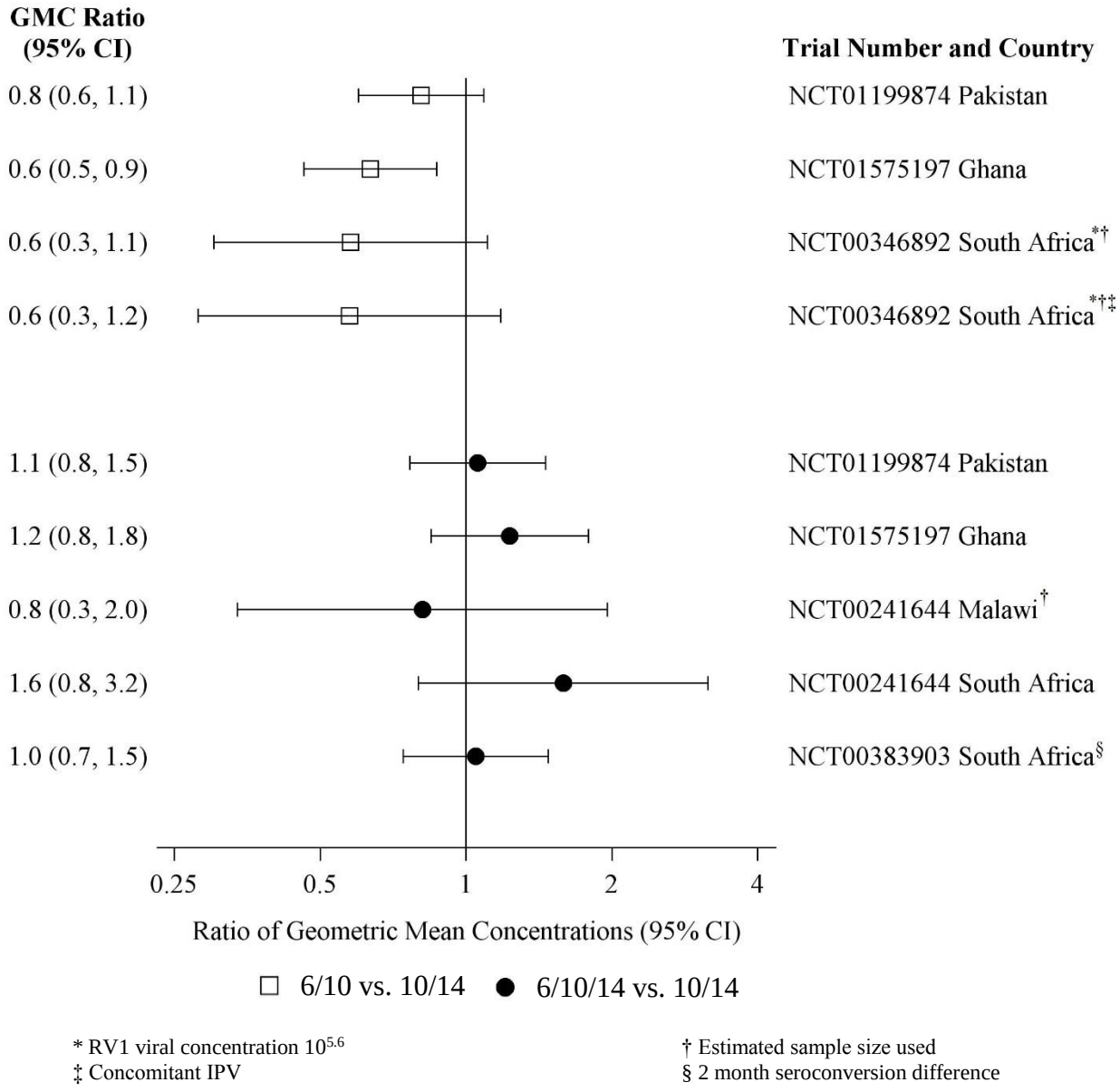


Figure 2.1 One-month seroconversion proportion differences comparing different RV1 schedules from five trials conducted in LMICs with concomitantly administered OPV unless otherwise indicated.



* RV1 viral concentration $10^{5.6}$
† Estimated sample size used
‡ Concomitant IPV
§ 2 month seroconversion difference

Figure 2.2 One-month ratios of GMCs comparing different RV1 schedules from five trials conducted in LMICs with concomitantly administered OPV unless otherwise indicated.

CHAPTER 3: RESEARCH METHODS

Research Aims

Aim 1: To describe the natural history of severe RVGE among infants in the placebo groups of the rotavirus vaccine trials in LMICs.

Aim 1a: To describe the timing of first episode of severe RVGE.

Aim 1b: To estimate the association between incidence of first severe RVGE and baseline factors, including demographic information; breastfeeding and growth status; and concomitant infection, antibiotic use, and vaccination.

Aim 2: To estimate the association between timing of rotavirus vaccine doses and incidence of severe RVGE among vaccinated infants in the rotavirus vaccine trials in LMICs.

Study Data & Design of Parent Studies

This was an analysis of two randomized control trials of RV1 and RV5 in LMICs (Clinical Trial Number NCT00241644 (RV1) and NCT00362648 (RV5)).

RV1 Trial in Malawi and South Africa

The RV1 data came from a Phase III, double-blind, placebo-controlled, multicenter randomized trial conducted in order to determine the efficacy of two or three doses of RV1 against severe RVGE [11, 90, 91]. The study was conducted from October 2005 through January 2009 in South Africa and Malawi.

Healthy infants, as determined by medical history and clinical examination, with parents or guardians of legal age who provided written informed consent were eligible to participate in the trial. Infants were excluded if 1) parents or guardian could not or would not comply with the protocol requirements; 2) birth weight of South African infants was ≤ 2,000 grams or if the weight was unknown if the gestation age of the child at delivery was ≤ 36 weeks; 3) any investigational or non-registered product was used within 30 days preceding the first dose or during the study period; 4) a vaccine not foreseen by the study protocol was administered within 14 days before or after each vaccine dose; 5) chronic (> 14 days) immunosuppressants were administered since birth; 6) there was a history of experimental rotavirus vaccine use; 7) previous routine vaccines were used, excluding BCG, HBV, and OPV at birth; 8) there was a clinically significant history of chronic gastrointestinal disease; 9) there was confirmed or suspected immunosuppressive or immunodeficient condition; 10) there was a history of allergic disease or reaction likely to be exacerbated by the vaccine; 11) there was acute disease at the time of enrollment; 12) there was gastroenteritis within 7 days preceding first vaccine dose; 13) there was previously confirmed occurrence of RVGE; 14) there was a family history of congenital or hereditary immunodeficiency; 15) immunoglobulins or blood product were administered since birth or there was planned administration during the study period; 16) there was a history of any neurologic disorders or seizures; 17) there was acute or chronic, clinically significant pulmonary, cardiovascular, hepatic or renal functional abnormality, as determined by physical examination or laboratory screening tests.

Infants 5 – 10 weeks of age were randomly assigned to receive one of three treatment regimens: one dose of placebo at 6 weeks of age and two doses of RV1 at 10 and 14 weeks of age, three doses of vaccine at 6, 10, and 14 weeks of age, or three doses of placebo at 6, 10, and

14 weeks of age. Infants recruited after 6 weeks of age were given the treatment doses at the time of enrollment with approximately 4 weeks between all subsequent doses. There were 1,647 randomized to receive two doses of RV1, 1,651 randomized to receive three doses of RV, and 1,641 infants randomized to receive three doses of placebo. There were no restrictions on timing of breast feeding or administration of other pediatric vaccines. Each vaccine dose contained live human G1P[8] rotavirus at a median concentration of at least 10^6 Cell Culture Infective Dose. The placebo contained all elements except the viral antigens.

There was active surveillance beginning with study enrollment for occurrence of gastroenteritis through weekly visits to parents or guardians to collect diary cards and through visits to health clinics that served the populations. Gastroenteritis was defined as three or more, looser than normal stools within a 24 hour period. Stool samples were collected during any episode of gastroenteritis occurring from the first vaccine dose to one year of age. A subset of infants was followed until two years of age. Stool samples were analyzed for the presence of rotavirus antigens using an enzyme-linked immunosorbent assay (ELISA) (Rotaclone, Meridian Bioscience). Reverse transcription polymerase chain reaction (RT-PCR) followed by reverse hybridization assay was used to confirm rotavirus and to determine rotavirus P and G genotypes.

RV5 Trial in Ghana, Kenya, Mali, Bangladesh, and Vietnam

The RV5 data came from a Phase III, double-blind, placebo-controlled, multicenter randomized trial conducted in order to determine the efficacy of three doses of RV5 against severe RVGE [12, 13]. The study was conducted from March 2007 through March 2009 in five sites in countries eligible for assistance from Gavi: medical facilities in rural Kassena-Nankana

district, Ghana; rural Karemo division, Siaya district, Nyanza province, western Kenya; urban Bamako, Mali; rural Matlab, Bangladesh, and urban and periurban Nha Trang, Vietnam.

Infants were enrolled between 4 – 12 weeks of age. Infants were excluded if the infant had symptoms of active gastrointestinal disease, if the parents were unable to understand study procedures and give consent, or if they were currently or expected to participate in a study of investigational products during the 6 weeks after the final treatment dose. There were no restrictions on potential infections, including HIV, or on the administration of other pediatric vaccines. Enrolled infants were randomly assigned to receive three doses of RV5 or placebo at approximately 4 week intervals after enrollment (target schedule 6, 10, and 14 weeks of age). There were 3,751 infants randomized to receive three doses of RV5 and 3,753 infants randomized to receive three doses of placebo. Each vaccine dose contained approximately $2x10^7$ infectious units per reassortant rotavirus with the WC3 bovine strain as backbone and viral surface proteins from human rotavirus serotype G1, G2, G3, G4, and P1A[8] in 2ml of buffered liquid. The placebo contained all elements except the viral antigens. Vaccines were stored and transported according to the standard operating procedure.

There was active surveillance at local clinics and hospitals for any occurrence of gastroenteritis occurring after study entry. Gastroenteritis was defined as three or more watery or looser than normal stools within a 24 hour period or forceful vomiting. Stool samples and patient histories were collected from infants presenting with symptoms of gastroenteritis. Parents or guardians of participants were visited monthly to remind them to bring their child to a medical facility if the infant developed any symptoms of gastroenteritis. Infants were followed for approximately two years following vaccination. Stool samples were analyzed for the presence of

enteric pathogens including rotavirus antigens using enzyme immunoassays. RT-PCR was used to confirm wild-type rotavirus and to determine rotavirus P and G genotypes.

Aim 1 Methods

Aim 1: To describe the natural history of severe RVGE among infants in the placebo groups of the rotavirus vaccine trials in LMICs.

Aim 1a: To describe the timing of first episode of severe RVGE.

Aim 1b: To estimate the association between incidence of first severe RVGE and baseline factors, including demographic information; breastfeeding and growth status; and concomitant infection, antibiotic use, and vaccination.

Study Data

In this analysis, the objective was to describe the timing and predictors of severe RVGE among those not receiving the rotavirus vaccine; therefore, only the placebo groups of each trial were analyzed. There were 1,641 and 3,753 infants randomized to receive only the placebo treatment in the RV1 trial and the RV5 trial, respectively. From here onward, cohort 1 is used to describe the placebo group from the RV1 trial, and cohort 2 is used to describe the placebo group from the RV5 trial. In cohort 1, infants were excluded if they were not randomized, their randomization code was broken at the investigator site, the study vaccine dose was not administered according to the protocol, or they did not have at least one day of follow-up. In cohort 2, infants were excluded if they received any doses of RV5. Each cohort was analyzed separately, but the results are presented in parallel.

Statistical Analysis

Prior to analysis, we categorized variables measured at enrollment including demographic information, breastfeeding and growth status, history of or current infection, history of or current antibiotic use, routine vaccinations, severe RVGE, and anti-rotavirus IgA seropositivity. Breastfeeding status was classified as exclusive versus non-exclusive. We classified relevant nutrition indicators by using underweight, stunted, and wasting cutoffs specified by the WHO [102]. Length of infants was not recorded in Bangladesh; therefore, stunted and wasting status were not determined for Bangladeshi infants. We also classified current or prior infections and antibiotic use using data collected in medical histories taken at baseline (e.g., enrollment). Topical antibiotics were not included in current or prior antibiotic use. Routine vaccines were also classified to determine the number of doses received prior to or at enrollment for all vaccines except Bacillus Calmette–Guérin vaccine (BCG), which was classified based on receipt before enrollment. Severe RVGE was defined as a Vesikari or modified-Vesikari score of > 11. For cohort 1, we summarized anti-rotavirus IgA antibody measures and considered participants seropositive if anti-rotavirus IgA antibody concentration was ≥ 20 U/ml. Antibody data were not available for cohort 2. We also determined the age at first severe RVGE episode. For cohort 1, age of enrollment was provided as weeks completed; therefore, age of severe RVGE may not be exact but within 6 days of the actual age the event occurred.

To describe the timing of first severe RVGE episode among children in LMICs, we estimated the incidence rates, cumulative incidence, and age distribution of severe RVGE for overall and for each country. Specifically, rates and exact 95% CIs [103] were estimated as the number of first severe RVGE episodes from enrollment through 1 or 2 years of follow-up

divided by the person-time accumulated. To estimate the cumulative incidence and 95% CI of first severe RVGE episode, we obtained the complement of the extended Kaplan-Meier survival curve overall and stratified by country. Use of the extended Kaplan-Meier survival curve allowed for late entry on an age-specific time scale [104]. In cohort 1 and 2, follow-up began at 6 weeks of age and continued until the event occurred or infants were censored. Any infant who was recruited into either study before 6 weeks of age began accumulating person-time at 6 weeks of age. Follow-up time in cohort 1 was within 6 days of exact number of days followed from 6 weeks of age, because age at enrollment was provided in weeks completed and follow-up was provided as days from enrollment. Finally, among those experiencing a severe RVGE episode, we described the age distribution of first episodes overall and by country.

We estimated the association between baseline factors and rates of first severe RVGE using a Cox proportional hazards model with the exact method to analyze tied events. Baseline factors considered in both cohorts were sex (female/male), underweight status (yes/no), current or prior infection (yes/no) at enrollment, current or prior antibiotic use (yes/no) at enrollment, and timely routine vaccination [BCG receipt prior to enrollment (0 vs. $\geq$ 1), DTP-HB/HIB or DTaP and HBV receipt prior or at enrollment (0 vs. $\geq$ 1), and OPV receipt prior or at enrollment ($\leq$ 1 dose vs. 2)]. Stunted (yes/no) and wasting (yes/no) were considered as potential predictors in cohort 1. Exclusive breastfeeding (yes/no) was considered as a potential predictor in cohort 2. Due to the low number of severe RVGE cases, we analyzed each cohort separately adjusted for country in the multivariable model rather fit individual models for each country in the cohorts. To be included as a potential predictor of first severe RVGE episode, there had to have been at least 10 severe RVGE events within each strata of each factor. To examine the proportional hazards assumption, we inspected the plot of log(time) and log(-log(Survival)) for each variable.

Similar to the methods described above, follow-up began at 6 weeks of age for both cohorts with late entry for those enrolled after 6 weeks of age. Crude and adjusted hazard ratios (HR) and 95% CIs were estimated and were considered statistically significant at a cutoff of $\alpha = 0.05$. As a sensitivity analysis, we determined the crude and adjusted HRs for cohort 2 excluding Mali and Kenya, because there were problems with gastroenteritis surveillance in those countries [105].

If more than 10% of participants in either cohort discontinued follow-up, we examined the potential for differential dropout (right censoring) within each level of each predictor to determine if censoring could be informative.

Aim 2 Methods

Aim 2: To estimate the association between timing of rotavirus vaccine doses and incidence of severe RVGE among vaccinated infants in the rotavirus vaccine trials in LMICs.

Study Data

We analyzed data from the placebo and vaccinated arms of the RV1 and RV5 trials. In the RV1 trial, we include infants randomized to receive three doses of placebo or one dose of placebo and two doses of RV1, respectively. Timing of doses in the RV1 trial referred to the timing of RV1 doses, meaning the first dose of the vaccine administered at 10 weeks, and not the placebo dose administered at approximately 6 weeks, was considered the first dose. Infants randomized to the arm to receive three doses of RV1 were excluded, because RV1 is administered as a two dose series, and we were interested in analyzing the association between of timing as the vaccine is administered and severe RVGE. Infants in the RV1 trial were excluded if the infant was not randomized, the randomization code was broken at the investigator site, the

study vaccine dose was not administered according to the protocol, or infants had less than one day of follow-up after 12 weeks of age. In the RV5 trial, we included infants randomized to receive three doses of placebo or three doses of RV5. We excluded infants who received a mixed series of placebo and RV5 doses or infants with less than one day of follow-up after 12 weeks of age. Each trial was analyzed separately, but the results are presented in parallel.

Overview of Study Design

We used a novel study design to understand if timing of rotavirus vaccine doses affected risk of severe RVGE. We began by defining five different aspects of vaccine timing, specified below. For a specific aspect of timing (e.g., timing of first dose), we compared two or more predefined schedules related to that aspect of timing (e.g., first dose given at < 10 versus ≥ 10 weeks). We compared the schedules by assessing the cumulative risk of severe RVGE on an age specific time scale beginning at the latest time any infant enrolled in either trial (i.e., 12 weeks of age). Using an age-specific time scale was essential, because we compared different timing of doses and needed to account for events that occurred early in age. Specifically, we did this by partitioning the follow-up time of infants such that infants could contribute person-time and severe RVGE events to more than one of the predefined schedules of interest to assess an aspect of dose timing. This meant that schedules with delayed vaccine doses had early events included in the estimates. Person-time for each infant was included in each schedule until that infant deviated from the predefined timing requirements allowed for each dose of that particular schedule. At that point, the infant was censored from that schedule. Figure 3.1 shows an example of five hypothetical infants and how the events and person-time for that infant were assigned for one aspect of timing. Once we had appropriately partitioned follow-up time and events from 12

weeks of age, we estimated the association between each schedule and incidence of severe RVGE.

To adjust the estimates, since timing was not randomized, we used the estimated associations observed in the in the placebo group, which should only differ from the null due to confounding, to calibrate the estimates within the rotavirus vaccinated group to obtain adjusted estimates. To verify that imbalances in covariates were similar between the rotavirus vaccinated and placebo groups, we calculated standardized mean differences to verify the assumption among measured covariates before calibrating estimates. Additional details of the approach are stated below.

Defining Schedules for Aspects of Timing

We classified the timing of doses *a priori* using five main aspects of dose timing: 1) whole schedules focused on timing of first dose holding interval(s) between doses constant; 2) timing of first dose; 3) timing of last dose; 4) length of interval(s) between doses; and 5) number of doses received at ≥ 10 weeks of age. For each aspect of timing, we developed two or more schedules to compare based that aspect of dose timing. Each of these schedule comparisons required infants to receive a complete series of RV1 or RV5 except for the last comparison, which was comparing the number of doses received at ≥ 10 weeks of age. We conducted a sensitivity analysis by excluding those without a full RV5 series from the analysis to ensure estimates were not due to receiving fewer vaccine doses. All predefined schedules were developed based on biologic plausibility, the potential for realistic interventions (e.g., alterations in rotavirus schedules that would fit at times routine vaccines are given as part of the Expanded Program on Immunization (EPI)), and the nature of the data. The schedules for each aspect of

timing are specified in Table 3.1. Due to the number of associations assessed, we *a priori* chose our primary aspect of timing to be the whole schedule focused on timing of the first dose holding interval(s) between doses constant at 4 – 6 weeks. Weeks of age completed were used for all schedule definitions (e.g., 6 weeks and 5 days of age was categorized as 6 weeks of age).

Outcome

We classified the outcome, first episode of severe RVGE, as infants experiencing RVGE with a Vesikari or modified-Vesikari score of > 11. For all analyses, we analyzed data on an age-specific time scale with follow-up beginning at the latest time any infant enrolled in either trial (i.e., 12 weeks of age). As mentioned above, we partitioned the follow-up time of infants such that infants could contribute person-time and severe RVGE events to more than one schedule for each aspect of timing. This approach allowed us to account for any early severe RVGE events that occurred. Specifically, infants contributed person-time to each schedule for each aspect of timing from 12 weeks of age until the earliest of any of the following instances occurred: 1) the infant was lost to follow-up, 2) the infant experienced a severe RVGE event, or 3) the timing of the infant's actual doses deviated from the predefined timing requirements allowed for that specific schedule. Appendix Table 1 includes detailed information on inclusion and censoring from each schedule, and Appendix Table 2 illustrates detailed examples of three hypothetical infants in each trial and the person-time contributed for each schedule for each aspect of timing.

Covariates

We categorized covariate data on demographic information; breastfeeding and growth status; and concomitant infection, antibiotic use, and vaccination. Demographic data were

measured at enrollment. Breastfeeding status was measured at enrollment and the two subsequent study visits in the RV5 trial. Infants were classified as exclusively or non-exclusively breastfed. Growth status was classified as underweight, stunted, and wasting status at enrollment using the WHO cutoffs [102]. Stunted and wasting status could not be determined for Bangladeshi infants in the RV5 trial, because length of infants was not recorded. Current or prior infections and antibiotic use were classified using medical histories taken at enrollment and the two subsequent study visits in both the RV1 and RV5 trials. Antibiotic use did not include topical antibiotics. Concomitant vaccination was determined using the data collected at enrollment and the two subsequent study visits in both trials.

Statistical Analysis

To estimate the association between timing of rotavirus vaccine doses and incidence of severe RVGE, we estimated risk differences (RDs) and risk ratios (RRs) between different schedules for each aspect of timing at 6, 12, and 18 months of age using the difference or ratio of cumulative risk estimates obtained from the complement of the Kaplan-Meier function curve at those time points [106]. We *a priori* chose to focus on RDs and RRs at 12 months of age as our primary time point of interest. We did not estimate RDs and RRs at specific time points if any schedule had less than five severe RVGE events at that time point. We also estimated HRs using a Cox proportional hazard model with the exact method to account for tied events.

There was potential for bias when estimating the association between dose timing and severe RVGE, because of informative censoring from specific schedules and confounding. We used data from the placebo groups to account for both potential sources of bias under the assumption any factors influencing the timing of receipt of doses in the placebo groups were the

same factors as those in the rotavirus vaccinated groups. Specifically, we accounted for bias by calibrating the estimates obtained among vaccinated infants with the estimates obtained among unvaccinated infants. Since the placebo doses should not influence the incidence of severe RVGE, any association of the timing of placebo doses on the incidence of severe RVGE was due to bias. Consequently, the association between the timing of placebo doses and incidence of severe RVGE provided a quantitative estimate of amount of bias for each comparison made within the vaccinated groups. These data were used to calibrate (i.e., adjust) the estimates among those in the vaccinated groups.

Before calibrating estimates, we empirically verified that any imbalances in measured covariates between schedules were similar in the placebo and vaccinated groups. We did this by calculating the standardized mean differences (SMDs) between covariates in each schedule for each aspect of timing for the placebo and vaccinated groups. The SMD was calculated as $(p1 - p2)/\text{sqrt}((p1(1 - p1) + p2(1 - p2))/2)$, where p1 was the proportion (or mean) of the binary covariate for a specific schedule (e.g., first dose at < 6 weeks) and p2 was the proportion in a different schedule (e.g., first dose at ≥ 6 weeks). We obtained a SMD for each covariate in the placebo and vaccinated groups and compared the SMDs between the estimates for the placebo and vaccinated groups. If the imbalance in covariates was similar between the placebo and vaccinated groups, we assumed calibration of the estimates in the vaccinated group would yield adjusted marginal estimates.

To estimate the adjusted association of rotavirus vaccine dose timing and severe RVGE, we obtained difference and ratio measures comparing schedules for each aspect of timing, as described above, for both the placebo and vaccinated groups of each trial. We then calibrated the estimates obtained among those vaccinated with the estimates obtained among those in the

placebo groups by subtracting the difference measures and dividing the ratio measures (i.e., $RD_{RV} - RD_{Placebo}$, $\frac{RR_{RV}}{RR_{Placebo}}$, and $\frac{HR_{RV}}{HR_{Placebo}}$). A nonparametric bootstrap with 2,000 sample draws with replacement was used to obtain the point estimates and 95% empirical CIs [107]. The median of the distribution of calibrated estimates was reported for the difference and ratio estimates and the 2.5^{th} and 97.5^{th} percentiles of the distribution were reported for the lower and upper bounds of the 95% empirical CIs.

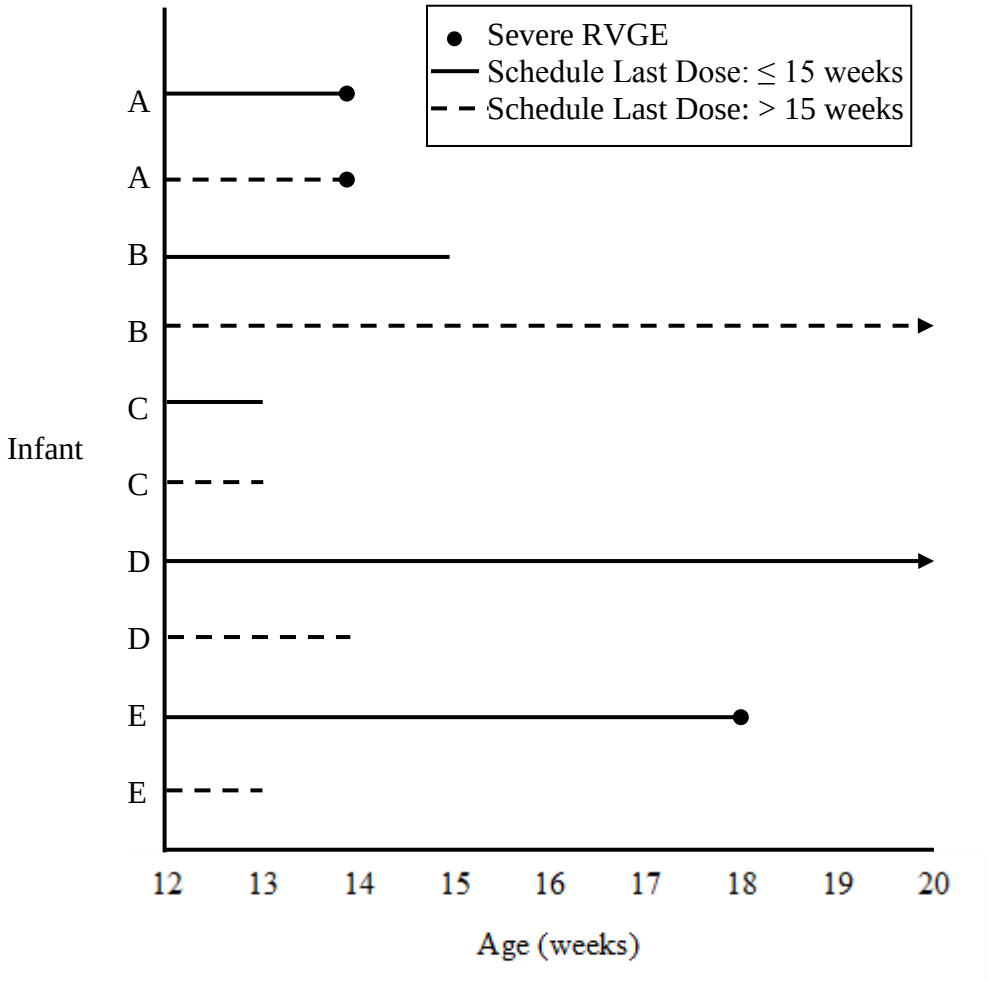
Table 3.1 Description of predefined rotavirus vaccine schedules developed for each aspect of dose timing. Timing is in weeks of age unless otherwise indicated. The primary comparison of interest is bolded.

Aspect of Timing	RV Type	Dose 1	Dose 2	Dose 3
Whole Schedules	**RV5**	**3 – 6**	**4 – 6 wks after 1st dose**	**4 – 6 wks after 2nd dose**
	RV5	**7 – 9**	**4 – 6 wks after 1st dose**	**4 – 6 wks after 2nd dose**
	RV5	**10 – 12**	**4 – 6 wks after 1st dose**	**4 – 6 wks after 2nd dose**
	RV1	**10 – 12**	**4 – 6 wks after 1st dose**	**NA**
	RV1	**13 – 16**	**4 – 6 wks after 1st dose**	**NA**
First Dose	RV5	< 6	≤ 10 wks after 1st dose	≤ 10 wks after 2nd dose
	RV5	≥ 6	≤ 10 wks after 1st dose	≤ 10 wks after 2nd dose
	RV5	< 10	≤ 10 wks after 1st dose	≤ 10 wks after 2nd dose
	RV5	≥ 10	≤ 10 wks after 1st dose	≤ 10 wks after 2nd dose
Last Dose	RV5	≤ 7	≤ 11	≤ 15
	RV5	≤ 12	≤ 10 wks after 1st dose	> 15 & ≤ 10 wks after 2nd dose
	RV1	≤ 11	≤ 15	NA
	RV1	≤ 16	> 15 & ≤ 10 wks after 2nd dose	NA
Interval between Doses	RV5	≤ 12	4 wks after 1st dose	4 wks after 2nd dose
	RV5	≤ 12	4 or 5 wks after 1st dose	4 or 5 wks after 2nd dose
	RV5	≤ 12	4, 5 or 6 wks after 1st dose	4, 5, or 6 wks after 2nd dose
	RV1	≤ 16	4 wks after 1st dose	NA
	RV1	≤ 16	5 wks after 1st dose	NA
	RV1	≤ 16	6 wks after 1st dose	NA
Number of Doses ≥ 10 Weeks of Age	RV5	< 10	< 10	< 10
	RV5	< 10	< 10	≥ 10 & ≤ 32
	RV5	< 10	≥ 10 & ≤ 32	≥ 10 & ≤ 32
	RV5	≥ 10	≥ 10 & ≤ 32	≥ 10 & ≤ 32

NA, not applicable; wks, weeks

‡ At least one interval between doses must be 5 weeks; § At least one interval between doses must be 6 weeks.

ǀ Other timing of doses resulting in the same number of doses received ≥ 10 are possible and are included in Appendix Table 1.



A: event at 14 weeks (counted in both groups).
B: no event before 20 weeks; infant vaccinated with final dose > 15 weeks.
C: no event before 13 weeks; infant dropped out at 13 weeks.
D: no event before 20 weeks; infant vaccinated with final dose at 14 weeks.
E: event at 18 weeks; infant vaccinated with final dose at 13 weeks.

Figure 3.1 Example of five infants and how events and person-time are assigned for the schedules for timing of the last dose.

CHAPTER 4: RESULTS AIM 1

Background

Prior to global roll-out of rotavirus vaccines, rotavirus was the leading cause of severe diarrhea in infants and children [1, 2]. Global surveillance estimates from 2009 indicated the median prevalence of rotavirus among children hospitalized for gastroenteritis was 36% (range among countries: 12 – 68%) [30]. In the pre-vaccine era, almost every child in the world was thought to experience rotavirus infection [4, 5] and about one in every 260 children would die as a result of the infection [2]. Although incidence of RVGE is similar in high-, middle-, and low-income countries, 80 – 90% of rotavirus-associated deaths occur in the world's poorest countries [1].

As of 2009, the WHO recommended rotavirus vaccination for all infants [7]. There are two live, oral rotavirus vaccines used broadly across the globe: RV1 and RV5. The one-year efficacies of the RV1 and RV5 at preventing severe RVGE were high in clinical trials in HICs (96 – 98%) [10, 29, 108], but were much lower (51 – 64%) in LMICs [11-13]. The disparity in vaccine efficacy between HICs and LMICs is similar to results recorded for other live oral vaccines [109-111]. It remains unclear what are the most important causes of low efficacy of rotavirus vaccines in LMICs.

One potential intervention that may increase the effectiveness of rotavirus vaccines in LMICs is altering the vaccine schedule (number or timing) of rotavirus vaccines doses. Recent research suggests delaying the start of the rotavirus vaccine series may result in some gains in vaccine efficacy [11, 23, 25], possibly due to less interference from transplacental antibodies or

further development of the immune system. However, first RVGE episode is thought to occur early in children in LMICs (median 6 – 9 months)[28]. Consequently, delaying vaccination could result in severe RVGE occurring prior to administration of the vaccine.

Although it is well accepted that infants in LMICs experience early exposure to rotavirus, it is less understood exactly when infants experience severe RVGE. Severe RVGE is the most clinically relevant outcome prevented by rotavirus vaccines to reduce hospitalization and death. Also, rotavirus vaccines are highly effective at preventing severe RVGE in HICs, but much less effective at preventing RVGE of any-severity [10, 29]. Therefore, it is important to understand the age when severe RVGE occurs to weigh the potential advantages and disadvantages of altering vaccination schedules. Although there have been studies investigating the natural history of rotavirus [49-51, 93, 94], these studies have not provided data on the cumulative incidence of severe RVGE over the first few years of life. Consequently, it is still unknown when in life children are experiencing severe RVGE episodes that could be prevented through vaccination.

In this study, we analyzed the placebo groups of two large rotavirus vaccine trials conducted in LMICs to better understand the timing of first, severe RVGE and predictors of severe RVGE among unvaccinated children in LMICs.

Methods

Parent Study Data

This was a secondary data analysis of the Phase III, placebo-controlled, multicenter randomized trials of RV1 and RV5 in LMICs (Clinical Trial Numbers: NCT00241644 (RV1) and NCT00362648 (RV5)). Each trial has been described previously [11, 12, 91]. A brief overview of each trial is below.

The RV1 trial was conducted from October 2005 through January 2009 in South Africa and Malawi. Infants, 5-10 weeks of age, were randomly assigned to receive doses of vaccine or placebo at approximately 6, 10, and 14 weeks of age. Infants recruited after 6 weeks of age were given the treatment doses at the time of enrollment with approximately 4 weeks between all subsequent doses. The placebo contained all elements of the vaccine except the viral antigens. Infants were excluded if they were not healthy. Baseline demographic and health information was collected for each participant. Beginning at enrollment, there was active surveillance of any gastroenteritis through weekly visits to parents or guardians to collect diary cards and through visits to health clinics that served the populations. Gastroenteritis was defined as three or more looser than normal stools within a 24 hour period. Stool samples were collected during any episode of gastroenteritis occurring from enrollment to 1 year of age, with a subset of infants followed to up to two years of age. Stool samples were analyzed for the presence of rotavirus antigens using an ELISA (Rotaclone, Meridian Bioscience). RT-PCR was used to confirm rotavirus infection and determine rotavirus P and G genotypes. Severity was defined using the 20-point Vesikari clinical score for PCR-confirmed RVGE cases [112]. In addition, serum samples were collected from a random subset of infants at enrollment. These samples were analyzed for anti-rotavirus IgA concentrations (U/ml) using an ELISA (GlaxoSmithKline Biologicals) with a cutoff of 20 U/ml used to indicate seroconversion.

The RV5 trial was conducted from March 2007 to March 2009 in medical facilities in rural Kassena-Nankana district, Ghana; rural Karemo division, Siaya district, Nyanza province, western Kenya; urban Bamako, Mali; rural Matlab, Bangladesh; and urban and periurban Nhas Trang, Vietnam. Enrolled infants, 4 – 12 weeks of age, were randomly assigned to receive either three doses of RV5 or placebo at approximately 6, 10, and 14 weeks of age. Infants recruited

after 6 weeks of age were given the treatment doses at the time of enrollment with approximately 4 weeks between all subsequent doses. The placebo contained all elements of the vaccine except the viral antigens. Infants were excluded if the infant had symptoms of active gastrointestinal disease, if the parents were unable to understand study procedures and give consent, or if they were currently or expected to participate in a study of investigational products during the 6 weeks after the final treatment dose. Baseline demographic and health information was collected for each participant. There was active surveillance at local clinics and hospitals for any occurrence of gastroenteritis occurring after study entry. Gastroenteritis was defined as three or more watery or looser than normal stools within a 24 hour period or forceful vomiting. Stool samples and patient histories were collected from infants presenting with symptoms of gastroenteritis. Infants were followed for approximately two years. Stool samples were analyzed for the presence of rotavirus antigens using an enzyme immunoassay. RT-PCR was used to confirm wild-type rotavirus and to determine rotavirus P and G genotypes. Severity of disease was determined using the 20-point modified Vesikari clinical score for infants with PCR confirmed RVGE [13, 112, 113].

Study Data

In this analysis, the objective was to describe the timing and predictors of severe RVGE among those not receiving the rotavirus vaccine; therefore, only the placebo groups of each trial were analyzed. There were 1,641 and 3,753 infants randomized to receive only the placebo treatment in cohort 1 and 2, respectively. In cohort 1, infants were excluded if they were not randomized, their randomization code was broken at the investigator site, the study vaccine dose was not administered according to the protocol, or they did not have at least one day of follow-

up. In cohort 2, infants were excluded if they received at least one dose of RV5. Each cohort was analyzed separately, but the results are presented in parallel.

Statistical Analysis

Prior to analysis, we categorized variables measured at enrollment including demographic information, breastfeeding and growth status, history of or current infection, history of or current antibiotic use, routine vaccinations, severe RVGE, and anti-rotavirus IgA seropositivity. Breastfeeding status was classified as exclusive versus non-exclusive. We classified relevant nutrition indicators by using underweight, stunted, and wasting cutoffs specified by the WHO [102]. Length of infants was not recorded in Bangladesh; therefore, stunted and wasting status were not determined for Bangladeshi infants. We also classified current or prior infections and antibiotic use using data collected in medical histories taken at baseline (e.g., enrollment). Topical antibiotics were not included in current or prior antibiotic use. Routine vaccines were also classified to determine the number of doses received prior to or at enrollment for all vaccines except BCG, which was classified based on receipt before enrollment. Severe RVGE was defined as a Vesikari or modified-Vesikari score of > 11. For cohort 1, we summarized anti-rotavirus IgA antibody measures and considered participants seropositive if anti-rotavirus IgA antibody concentration was ≥ 20 U/ml. Antibody data were not available for cohort 2. We also determined the age at first severe RVGE episode. For cohort 1, age of enrollment was provided as weeks completed; therefore, age of severe RVGE may not be exact but within 6 days of the actual age the event occurred.

To describe the timing of first severe RVGE episode among children in LMICs, we estimated the incidence rates, cumulative incidence, and age distribution of severe RVGE overall

and for each country. Specifically, rates and exact 95% CIs [103] were estimated as the number of first severe RVGE episodes from enrollment through 1 or 2 years of follow-up divided by the person-time accumulated. To estimate the cumulative incidence and 95% CI of first severe RVGE episode, we obtained the complement of the extended Kaplan-Meier survival curve overall and stratified by country. Use of the extended Kaplan-Meier survival curve allowed for late entry on an age-specific time scale [104]. In cohort 1 and 2, follow-up began at 6 weeks of age and continued until the event occurred or infants were censored. Any infant who was recruited into either study before 6 weeks of age began accumulating person-time at 6 weeks of age. Follow-up time in cohort 1 was within 6 days of exact number of days followed from 6 weeks of age, because age at enrollment was provided in weeks completed and follow-up was provided as days from enrollment. Finally, among those experiencing a severe RVGE episode, we described the age distribution of first episodes overall and by country.

We estimated the association between baseline factors and rates of first severe RVGE using a Cox proportional hazards model with the exact method to analyze tied events. Baseline factors considered in both cohorts were sex (female/male), underweight status (yes/no), current or prior infection (yes/no) at enrollment, current or prior antibiotic use (yes/no) at enrollment, and timely routine vaccination [BCG receipt prior to enrollment (0 vs. $\geq$ 1), DTP-HB/HIB or DTaP and HBV receipt prior or at enrollment (0 vs. $\geq$ 1), and OPV receipt prior or at enrollment ($\leq$ 1 dose vs. 2)]. Stunted (yes/no) and wasting (yes/no) were considered as potential predictors in cohort 1. Exclusive breastfeeding (yes/no) was considered as a potential predictor in cohort 2. Due to the low number of severe RVGE cases, we analyzed each cohort separately adjusted for country in the multivariable model rather fit individual models for each country in the cohorts. To be included as a potential predictor of first severe RVGE episode, there had to have been at

least 10 severe RVGE events within each strata of each factor. To examine the proportional hazards assumption, we inspected the plot of log(time) and log(-log(Survival)) for each variable. Similar to the methods described above, follow-up began at 6 weeks of age for both cohorts with late entry adjustment for those enrolled later. Crude and adjusted HRs and 95% CIs were estimated and were considered statistically significant at a cutoff of $\alpha = 0.05$. As a sensitivity analysis, we determined the crude and adjusted HRs for cohort 2 excluding Mali and Kenya, because there were problems with gastroenteritis surveillance in those countries.

If more than 10% of participants in either cohort discontinued follow-up, we examined the potential for differential dropout (right censoring) within each level of each predictor to determine if censoring could be informative.

This analysis was approved by the University of North Carolina at Chapel Hill Institutional Review Board. All analyses were performed using SAS Clinical Trial Data Transparency (Version 4.5.2; SAS Institute Inc., Cary, NC, USA).

Results

There were 1,614 and 3,752 children included in the analysis from cohort 1 and 2, respectively (Table 4.1). The median lengths of follow-up to censoring or first severe RVGE were 327 and 518 days for cohorts 1 and 2, respectively (Table 4.2). The majority of infants were African race and enrolled at 6 – 7 weeks of age. About 80% of infants in cohort 2 were exclusively breastfed at enrollment. In cohort 1, about 20% of children were stunted and about 5% were underweight and 5% had wasting, whereas in cohort 2, about 10% were stunted, 10% were underweight, and about 20% had wasting. About 5% of children had a history of or a current infection at enrollment in cohort 1, whereas about 20% had reported a history of or a

current infection in cohort 2. History of or current antibiotic use at enrollment was reported in about 10% and 5% of cohort 1 and 2, respectively. Percentage of those receiving routine vaccinations was relatively high in cohort 1 whereas routine vaccination receipt was lower in cohort 2. About 10% of infants in cohort 1 were anti-rotavirus IgA seropositive at enrollment.

There were 101 and 205 first episodes of severe RVGE in cohorts 1 and 2, respectively (Table 4.2). The overall incidence of severe RVGE was similar in both cohorts (~ 5 events per 100 child-years). However, there was some variability in rates of first severe RVGE by country. Rates were highest in Bangladesh and Malawi (6.7 and 8.8 events per 100 child-years, respectively), and lowest in Kenya and Vietnam (2.1 and 2.7 events per 100 child-years, respectively).

The cumulative incidence and 95% CI of severe RVGE from 6 week to 20 months of age was 79 (95% CI: 63, 95) severe RVGE events per 1,000 infants in cohort 1 and 63 (95% CI: 54, 72) in cohort 2. There was variability in the cumulative incidence by country (Figures 4.1 and 4.2).

Among those experiencing an episode of severe RVGE, the age distribution of onset is summarized by country in Figure 4.3. There was variability in the age of onset across different countries. The largest and smallest variability in episode timing was in Ghana [median: 35.1 weeks (interquartile range (IQR): 31.7, 69.1)] and South Africa [median: 28.6 weeks (IQR: 22.4, 35.4)], respectively.

Prior or current antibiotic use at enrollment was associated with about two times the rate of severe RVGE (adjusted HR: 2.03 (95% CI: 1.18, 3.48)) compared to those with no use in cohort 1 (Table 4.3). The direction of the association was consistent between the two cohorts with the rate of severe RVGE being about one and half times the rate in those with antibiotic use

(adjusted HR: 1.41 (95% CI: 0.80, 2.51)) compared to those with no use in cohort 2. No variables in cohort 2 were significant predictors of severe RVGE, but there was an relatively strong inverse association between no receipt of BCG before enrollment and receipt of ≥ 1 dose of BCG (adjusted HR: 0.65 (95% CI: 0.35, 1.21)). Results including and excluding Kenya and Mali in the analysis were similar (Appendix Table 3).

In cohort 1, 17% of the study population dropped out or were lost to follow-up prior to the end of the study. In cohort 2, only 3.9% of participants discontinued follow-up. Additional information on lost to follow-up in cohort 1 can be found in Appendix Table 4.

Discussion

We analyzed data from the placebo groups of two large trials conducted in seven LMICs to describe the timing and predictors of severe RVGE. The cumulative incidence of severe RVGE was 6 – 8 % at 20 months of age. The cumulative incidence increased steadily over the first two years of life and was low at 6 months of age. Antibiotic use was associated with about 1.4 to 2 times the rate of severe RVGE.

Although every child under 5 years of age was thought to experience symptomatic or asymptomatic rotavirus infection prior to vaccine introduction [28, 49, 50], the cumulative risk of severe RVGE among unvaccinated infants is likely much lower and was about 6 to 8% at 20 months of age among children in the LMICs included. In this study, we found the rates of severe RVGE in cohort 1 and 2 were similar or slightly higher than previously reported estimates. A study in India reported the incidence of severe RVGE in the first year of life to be 5 events per 100 child-years [98], while another study in Pakistan reported approximately 2 events per 100 child-years [114]. In cohort 1, almost all severe RVGE cases occurred in the first year of life.

This could be due to fewer cases occurring in the second year of life or because of decreased surveillance of gastroenteritis in that period. By contrast, several severe RVGE events occurred after 12 months of age in cohort 2.

There were differences in the rates and cumulative incidence of severe RVGE in different LMICs. Rates of severe RVGE were highest in Malawi and Bangladesh. Rates of first severe RVGE episode were similar in Ghana and Mali. However, early surveillance measures were unsuccessful in Mali, which resulted in very few cases being observed in the first 12 months of life [105]. Actual rates of severe RVGE in Mali were likely higher. Kenya and Vietnam all had similar rates of first RVGE episodes. However, this was likely an artifact of data collection, because few cases were observed in Kenya during the second year of the study due to civil unrest that disrupted the study and surveillance for gastroenteritis.

Severe RVGE occurred early among infants in LMICs with the cumulative incidence increasing steadily over the first one to two years of life and low incidence at 6 months of age. Although some studies have described age-specific rates of RVGE or the distribution of age of RVGE onset [50, 51, 93, 97], these studies have not differentiated episodes of severe and non-severe RVGE or first versus subsequent episodes of RVGE. Therefore, due to different methods of presenting data, it is difficult to compare these estimates to those from prior studies.

In both cohorts, we found that antibiotic use early in life was associated with severe RVGE. To our knowledge, antibiotic use has not been reported to be associated with severe RVGE, but has been linked to increased diarrheal incidence. Antibiotics reduce the diversity of the gut microbiota and can have a profound impact on the early development of the infant microbiota [115]. It is likely microbial colonization and diversification play a critical role in susceptibility to diarrheal diseases. Recent studies conducted in India found an increased risk of

diarrheal disease in children receiving antibiotics at < 6 months of age compared to those who did not [116] and a shorter time to subsequent diarrheal episode when the first episode was treated with antibiotics [117]. It is also possible that antibiotic use is an indication of children who are sicker and exposed to more pathogens, and are therefore, more likely to develop severe RVGE.

In cohort 2, there was an inverse association between no receipt of BCG prior to enrollment compared to receipt of one or more doses. BCG is a single dose vaccine given to prevent tuberculosis and is recommended to be given to infants as soon as possible after birth [118]. Although it is a single dose vaccine, there were some infants in both cohorts with receipt of more than one dose of BCG recorded. It is unexpected that there was an inverse association between infants who are not vaccinated with BCG by the time they were recruited into the study (about 6 weeks of age) and incidence of severe RVGE. We expected to observe children with better routine healthcare, as indicated by receipt of routine immunization, to be the same or less likely than those without it to experience severe RVGE. This association merits further investigation as it is possible there were important unmeasured covariates responsible for inducing this association.

There are some limitations to this research. First, these data were collected as part of clinical trials. Therefore, the participants may not be generalizable to the broader study population of infants in each country, because trials generally have strict inclusion and exclusion criteria. However, it should be noted that there were few exclusion criteria for the RV5 trial, and it seems plausible that the study population was relatively representative of all infants being vaccinated. Consequently, this limitation may not be concerning, because the rates and cumulative risk for first severe RVGE episode in the cohort 1 and 2 were similar, indicating the

RV1 trial cohort may, like the RV5 trial cohort, also be generalizable to the broader population of infants. However, these similarities could be due to differences in countries, region, or study timing and not due to the generalizability of the RV1 population. Second, the study inclusion and exclusion criteria for both cohort 1 and 2 did not specifically prohibit children who received the placebo or vaccine from being in the same household or neighborhood. If vaccinated and unvaccinated children were nearby, there may have been potential for herd protection, which would decrease the number of unvaccinated children with RVGE. As a result, the incidence of severe RVGE in this study would not represent a completely unvaccinated cohort. Third, we were unable to estimate severe RVGE risk from birth, because the trial recruited children around 6 weeks of age. This means we have likely underestimated the incidence of severe RVGE in early childhood. Similarly, due to staggered enrollment by age, the earliest time period we had sufficient sample size to analyze the trial populations was at 6 weeks of age. This resulted in the exclusion of one severe RVGE episode from the cumulative incidence estimates and predictor analysis, because the event occurred prior to 6 weeks of age in cohort 1. Early events would be essential to include when weighing the option of beginning vaccination prior to 6 weeks of age. However, if vaccination will begin at 6 weeks of age or later, events prior to 6 weeks of age could not be prevented and are not essential to include in comparisons. Similarly, there was only active surveillance at local clinics and hospitals, and not households, for gastroenteritis in the RV5 trial, which likely resulted in underascertainment of severe RVGE episodes. Also, early surveillance measures were unsuccessful in Mali, which resulted in very few cases being observed in the first 12 months of life [105] and there was civil unrest in in Kenya in the second year of the study, which resulted in underascertainment of cases. The cumulative incidence estimates for these countries likely underestimate the true cumulative incidence. Fourth, limited

covariate data were collected, and as a result, we were restricted in our ability to identify predictors of severe RVGE. There are likely other unmeasured factors that predict the occurrence of severe RVGE. Fourth, there appeared to be differential dropout by some covariates in cohort 1. This could have resulted in some factors not being identified as predictors, because these factors were associated with early dropout.

There are several strengths of this analysis. The large sample size of infants prospectively followed allowed us to analyze severe RVGE events, which is often unfeasible in small studies. With these data, we could analyze the timing and predictors of first severe RVGE episodes, which are the most clinically relevant outcomes of interest. Having information on when severe events occur is essential for understanding the potential impact of altering vaccine schedules. Here, we were able to report the cumulative incidence over the first two years of life, so this information could be available when weighing the potential advantages and disadvantages of shifting vaccine schedules. In addition, the study participants came from a broad geographic area representing several different LMICs including both urban and rural areas. Also, severe RVGE outcomes were validated as wild-type PCR-confirmed cases of RVGE. Finally, we analyzed data focusing on the age of severe RVGE and used methods that allowed for late-entry into the study such that the time scale could be age, which is the most relevant time scale for understanding the timing of severe RVGE cases and the potential impact of altering vaccine schedules.

In conclusion, the cumulative risk of severe RVGE from 6 weeks to 20 months of age in children in LMICs was about 6 to 8% and increased relatively steadily over the first one to two years of life with low incidence at 6 months of age. Early antibiotic use was associated with increased rates of first severe RVGE events. These data provide important insights on the

epidemiology of rotavirus in LMICs in the pre-vaccine era that can help inform the use of rotavirus vaccines in LMICs.

Table 4.1 Characteristics of the placebo groups of cohort 1 and 2 at enrollment.

Baseline Characteristic	Cohort 1 N = 1,614	Cohort 2 N = 3,752
Demographic		
Age (weeks completed), Median (IQR)	6 (6, 7)	7 (6, 9)
Female Sex, %	48.5	49.6
African Race, %	97.0	72.8
Asian Race, %	0.0	27.1
Exclusively Breastfed, %	--	81.3
Growth Status		
Stunted, %	22.1[*]	10.6[†]
Underweight, %	4.5	11.1
Wasting, %	4.2[*]	20.7[†*]
Prior/Current Infection, %	4.2	19.4
Prior/Current Antibiotic[‡] Use, %	9.2	5.6
Routine Vaccines		
≥ 1 BCG[§], %	95.2	74.0
≥ 1 DTP-HB/HIB or DTaP & HBV, %	99.8	68.6
OPV		
0 Dose, %	0.0	8.3
1 Dose, %	11.8	47.9
2 Doses, %	87.6	37.6
≥ 3 Doses, %	0.6	6.2
Rotavirus IgA Sub-Cohort, N = 156		
Seropositive, %	11.5	--
IgA, GMC	13.3	--

[*] Missing 1 – 15 observations
[†] Excluding Bangladesh
[‡] Excluding topical antibiotics
[§] Administered prior to enrollment

Table 4.2 Rate of severe RVGE by cohort and country.

Cohort/Country	N	Median days of follow-up*	Severe RVGE		
			Events	Child-Years	Rate† (95% CI)
Cohort 1	1,614	327	101	1,790	5.6 (4.6, 6.9)
Malawi	581	553	62	705	8.8 (6.7, 11.3)
South Africa	1,033	323	39	1,084	3.6 (2.6, 4.9)
Cohort 2	3,752	518	205	4,876	4.2 (3.6, 4.8)
Ghana	1,102	527	57	1,431	4.0 (3.0, 5.2)
Kenya	651	483	15	700	2.1 (1.2, 3.5)
Mali	981	539	61	1,331	4.6 (3.5, 5.9)
Bangladesh	568	540	56	831	6.7 (5.1, 8.8)
Vietnam	450	480	16	583	2.7 (1.6, 4.5)

RVGE, rotavirus gastroenteritis
* From enrollment
† Per 100 Child-Years

Table 4.3 Predictors of first severe RVGE episode in cohort 1 and 2.

Characteristic	Cohort 1* N = 1,613 Events = 100		Cohort 2* N = 3,746 Events = 205	
	Unadjusted† HR (95% CI)	Adjusted† HR (95% CI)	Unadjusted† HR (95% CI)	Adjusted† HR (95% CI)
Demographic				
Female Sex vs. Male (ref)	1.44 (0.97, 2.15)	1.43 (0.96, 2.12)	0.86 (0.65, 1.13)	0.86 (0.65, 1.13)
Exclusively Breastfed vs. Not (ref)	--	--	0.75 (0.48, 1.15)	0.75 (0.48, 1.16)
Growth Status				
Stunted vs. Not (ref)	0.78 (0.49, 1.26)	0.75 (0.46, 1.21)	--	--
Underweight vs. Not (ref)	‡	‡	0.82 (0.52, 1.30)	0.81 (0.51, 1.29)
Wasting vs. Not (ref)	‡	‡	--	--
Current/Prior Infection vs. None (ref)	‡	‡	0.99 (0.64, 1.52)	0.89 (0.56, 1.40)
Current/Prior Antibiotic‖ Use vs. None (ref)	1.97 (1.15, 3.36)	2.03 (1.18, 3.48)	1.40 (0.81, 2.41)	1.41 (0.80, 2.51)
Routine Vaccines				
BCG§; No Dose vs. ≥ 1 Dose (ref)	‡	‡	0.63 (0.34, 1.17)	0.65 (0.35, 1.21)
DTP-HB/HIB¶; No Dose vs. ≥ 1 Dose (ref)	‡	‡	1.00 (0.71, 1.42)	1.08 (0.71, 1.66)
OPV; ≤ 1 Dose vs. ≥ 2 Doses (ref)	0.79 (0.45, 1.42)	0.80 (0.45, 1.43)	0.94 (0.70, 1.25)	0.95 (0.66, 1.36)

* One infant in cohort 1 experienced an event prior to 6 weeks of age and six infants from cohort 2 entered and exited the study before 6 weeks of age.
† Adjusted for country
‡ < 10 events in each strata
§ Excluding topical antibiotics
‖ Administered prior to enrollment
¶ Or DTaP & HBV, which were the standard vaccines given in Asian countries

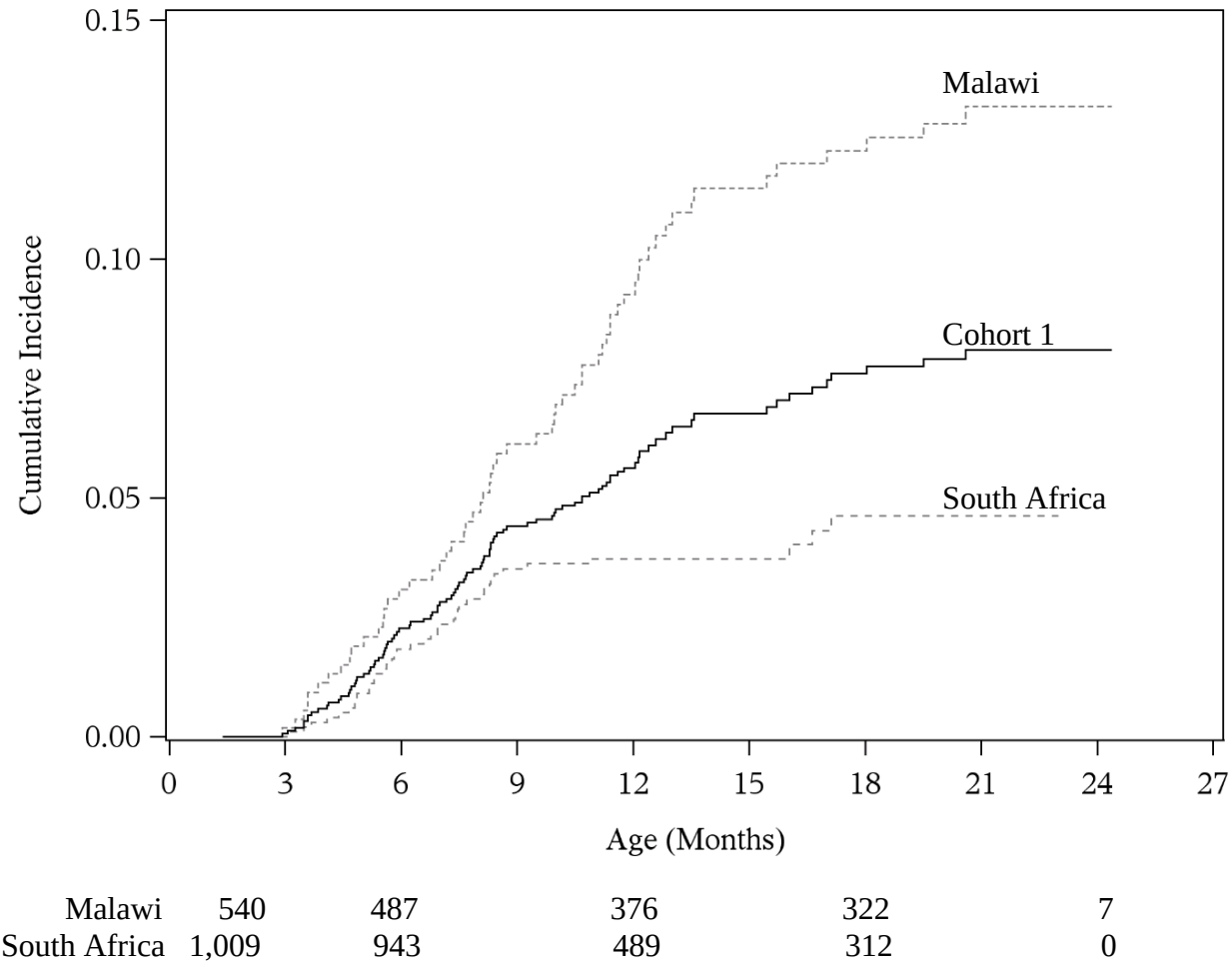
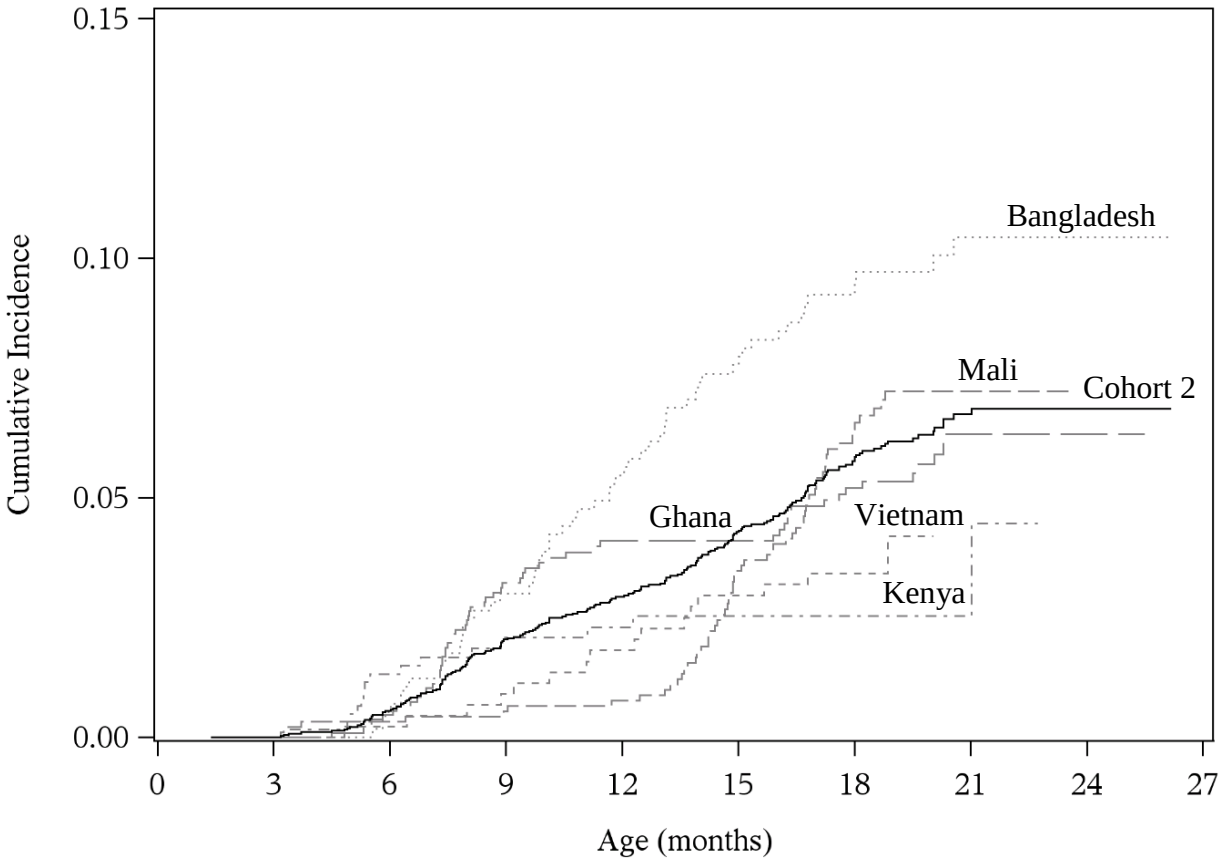


Figure 4.1 Cumulative incidence of severe RVGE from 6 weeks of age in cohort 1. Countries are labeled above gray lines; cohorts are labeled above black lines. Number at risk at start of follow-up and at 6 months intervals is labeled at corresponding time points for each country below the x-axises.



Ghana	568	564	535	380	71
Kenya	1,102	1,066	828	726	68
Mali	647	591	427	275	0
Bangladesh	979	913	887	658	0
Vietnam	450	441	430	302	0

Figure 4.2 Cumulative incidence of severe RVGE from 6 weeks of age in cohort 2. Countries are labeled above gray lines; cohorts are labeled above black lines. Number at risk at start of follow-up and at 6 months intervals is labeled at corresponding time points for each country below the x-axises.

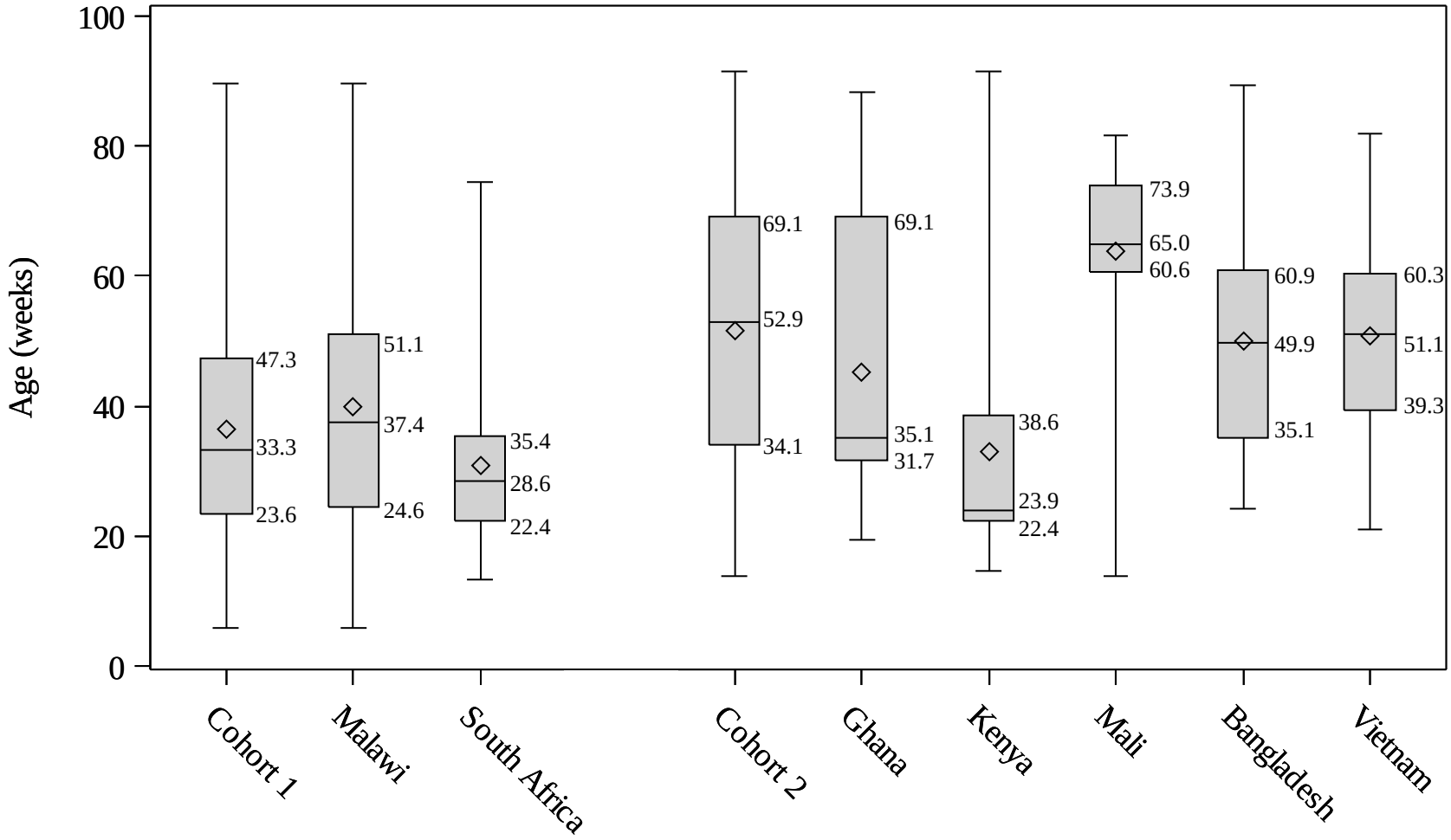


Figure 4.3 IQR of age of first severe RVGE onset. First quartile, median, and third quartile are labeled with their numeric values. Mean values are represented by diamonds.

CHAPTER 5: RESULTS AIM 2

Background

Rotavirus was the leading cause of severe gastroenteritis in children under five years of age prior to the availability and introduction of rotavirus vaccines into routine immunization programs beginning in 2006 [1, 2]. In 2003, it was estimated rotavirus caused 111 million episodes of gastroenteritis, two million hospitalizations, and up to almost 500,000 deaths each year [1]. While severe rotavirus infections have led to hospitalizations in HICs, the majority of rotavirus-associated deaths have occurred in the world's poorest countries [1].

Rotavirus vaccines, as of May 2016, are part of routine immunization programs in 81 countries [62]. These countries mostly use RV1 and RV5 rotavirus vaccines [55, 61]. Despite the success of these vaccines at reducing health care encounters and hospitalizations [65-67], RV1 and RV5 effectiveness is lower in LMICs compared to HICs [68, 69]. Reasons for lower effectiveness in LMICs compared to HICs have been investigated in recent years. While breastfeeding appears to not have a large effect on rotavirus vaccination seroconversion [17-19], concomitant vaccination with OPV [20, 21], malnutrition [22, 23], interference by transplacental maternal antibodies [24, 25], and environmental enteropathy and the infant microbiota [26, 27] all may contribute to the lower vaccine effectiveness observed in LMICs.

Some of the factors shown to decrease rotavirus vaccine performance may be mitigated by altering vaccine schedules. A few studies have investigated the influence of vaccine schedules on vaccine performance [24, 25, 119], but these studies have generally been restricted to immunologic endpoints. Since there is no known correlate of protection for anti-rotavirus IgA

levels [44, 45], there is still uncertainty about the effect alternative schedules have on clinical endpoints (i.e., severe RVGE). One trial in Malawi and South Africa compared a two versus three dose series of RV1 using clinical endpoints [11], but no data have been reported on the effect of timing of rotavirus vaccines as they are administered (i.e., two doses of RV1, three doses of RV5) using clinical endpoints.

In this study, we analyzed data from two, large rotavirus vaccine trials to understand the association between timing of rotavirus vaccine doses and incidence of severe RVGE among children in LMICs. This is the first study to present data on the association between timing of two doses of RV1 or three doses of RV5 and severe RVGE. These data provide important information on determining the optimal timing of rotavirus vaccine doses to improve effectiveness of these vaccines in LMICs.

Methods

Parent Study Data

This analysis used data from two randomized Phase III placebo-controlled multicenter clinical trials of RV1 and RV5 (Clinical Trial Number: NCT00241644 and NCT00362648, respectively) conducted in LMICs. The trials have been described in depth elsewhere [11, 12, 91], but a brief summary of each trial is below.

The RV1 clinical trial was conducted in South Africa and Malawi from 2005 – 2009. Healthy infants aged 5 – 10 weeks were enrolled and randomized to receive three doses of placebo, a dose of placebo followed by two RV1 doses, or three RV1 doses at approximately 6, 10, and 14 weeks of age. Infants enrolled after 6 weeks of age were treated at the time of enrollment with about 4 weeks between all subsequent doses administered. Demographic and

health characteristics of infants was collected at enrollment and, in some cases, at each dose. Enrolled infants were actively followed for occurrence of gastroenteritis from time of enrollment until study conclusion at one year of age with a subset followed for up to two years of age. Study staff visited parents or guardians weekly to collect diary cards and also visited health clinics serving the study population. Stool samples were collected and tested for rotavirus using an ELISA (Rotaclone, Meridian Bioscience) followed by RT-PCR confirmation. Gastroenteritis was defined as three or more, looser than normal stools within a 24 hour period. Severity of RVGE was determined using the 20-point Vesikari clinic score [112].

The RV5 study was conducted in Ghana, Kenya, Mali, Bangladesh, and Vietnam from 2007 – 2009. Infants 4 – 12 weeks were enrolled and randomly assigned to receive three doses of vaccine or placebo at approximately 6, 10, and 14 weeks of age. Infants enrolled after 6 weeks of age were treated at the time of enrollment with about 4 weeks between all subsequent doses administered. Infants were not included if they displayed symptoms of an active gastrointestinal infection, if parents were unable to follow the study protocol or provide consent, or if infants were participating in another study investigating a product within six weeks of the final dose of treatment. Demographic and health information was collected for participants at enrollment and, in some cases, at each dose. During the study, there was active surveillance for gastroenteritis at local clinics and hospitals. Any participant presenting with gastroenteritis provided a stool sample for testing of rotavirus using an enzyme immunoassay followed by RT-PCR confirmation. Gastroenteritis was defined as three or more watery or looser than normal stools within a 24 hour period or forceful vomiting. Severity of RVGE was classified using the 20-point modified Vesikari score [13, 112, 113].

Study Data

We analyzed data from the placebo and vaccinated arms of the RV1 and RV5 trials. In the RV1 trial, we include infants randomized to receive three doses of placebo or one dose of placebo and two doses of RV1, respectively. Infants randomized to the arm to receive three doses of RV1 were excluded, because RV1 is administered as a two dose series, and we were interested in analyzing the association between timing as the vaccine is administered and severe RVGE. Timing of doses in the RV1 trial referred to the timing of RV1 doses, meaning the first dose of the vaccine administered at 10 weeks, and not the placebo dose administered at approximately 6 weeks, was considered the first dose. Infants in the RV1 trial were excluded if the infants were not randomized, the randomization code was broken at the investigator site, the study vaccine dose was not administered according to the protocol, or infants had less than one day of follow-up after 12 weeks of age. In the RV5 trial, we included infants randomized to receive three doses of placebo or three doses of RV5. We excluded infants who received a mixed series of placebo and RV5 doses or infants with less than one day of follow-up after 12 weeks of age. Each trial was analyzed separately, but the results are presented in parallel.

Overview of Study Design

We used a novel study design to understand if timing of rotavirus vaccine doses affected risk of severe RVGE. We began by defining five different aspects of vaccine timing, specified below. For a specific aspect of timing (e.g., timing of first dose), we compared two or more predefined schedules related to that aspect of timing (e.g., first dose given at < 10 versus ≥ 10 weeks). We compared the schedules by assessing the cumulative risk of severe RVGE on an age specific time scale beginning at the latest time any infant enrolled in either trial (i.e., 12 weeks of

age). Using an age-specific time scale was essential, because we compared different timing of doses and needed to account for events that occurred early in life. Specifically, we did this by partitioning the follow-up time of infants such that infants could contribute person-time and severe RVGE events to more than one of the predefined schedules developed to assess an aspect of dose timing. This meant that schedules with delayed vaccine doses had early events included in the estimates. Person-time for each infant was included in each schedule until that infant deviated from the predefined timing requirements allowed for each dose of that particular schedule. At that point, the infant was censored from that schedule. Figure 5.1 shows an example of two hypothetical infants and how the events and person-time for that infant were assigned for one aspect of timing. Once we had appropriately partitioned follow-up time and events from 12 weeks of age, we estimated the association between each schedule and incidence of severe RVGE.

To adjust the estimates, since timing was not randomized, we used the estimated associations observed in the in the placebo group, which should only differ from the null due to confounding, to calibrate the estimates within the rotavirus vaccinated group to obtain adjusted estimates. To verify that imbalances in covariates were similar between the rotavirus vaccinated and placebo groups, we calculated standardized mean differences to verify the assumption among measured covariates before calibrating estimates. Additional details of the approach are stated below.

Defining Schedules for Aspects of Timing

We classified the timing of doses *a priori* using five main aspects of dose timing: 1) whole schedules focused on timing of first dose holding interval(s) between doses constant; 2)

timing of first dose; 3) timing of last dose; 4) length of interval(s) between doses; and 5) number of doses received at ≥ 10 weeks of age. For each aspect of timing, we developed two or more schedules to compare based that aspect of dose timing. Each of these schedule comparisons required infants to receive a complete series of RV1 or RV5 except for the last comparison, which was comparing the number of doses received at ≥ 10 weeks of age. We conducted a sensitivity analysis by excluding those without a full RV5 series from the analysis to ensure estimates were not due to receiving fewer vaccine doses. All predefined schedules were developed based on biologic plausibility, the potential for realistic interventions (e.g., alterations in rotavirus schedules that would fit at times routine vaccines are given as part of the EPI), and the nature of the data. The schedules for each aspect of timing are specified in Table 5.1. Due to the number of associations, we *a priori* chose our primary aspect of timing to be the whole schedule focused on timing of the first dose holding interval(s) between doses constant at 4 – 6 weeks. Weeks of age completed were used for all schedule definitions (e.g., 6 weeks and 5 days of age was categorized as 6 weeks of age).

Outcome

We classified the outcome, first episode of severe RVGE, as infants experiencing RVGE with a Vesikari or modified-Vesikari score of > 11. For all analyses, we analyzed data on an age-specific time scale with follow-up beginning at the latest time any infant enrolled in either trial (i.e., 12 weeks of age). As mentioned above, we partitioned the follow-up time of infants such that infants could contribute person-time and severe RVGE events to more than one schedule for each aspect of timing. This approach allowed us to account for any early severe RVGE events that occurred. Specifically, infants contributed person-time to each schedule for each aspect of

timing from 12 weeks of age until the earliest of any of the following instances occurred: 1) the infant was lost to follow-up, 2) the infant experienced a severe RVGE event, or 3) the timing of the infant's actual doses deviated from the predefined timing requirements allowed for that specific schedule. Appendix Table 1 includes detailed information on inclusion and censoring from each schedule, and Appendix Table 2 illustrates detailed examples of three hypothetical infants in each trial and the person-time contributed for each schedule for each aspect of timing.

Covariates

We categorized covariate data on demographic information; breastfeeding and growth status; and concomitant infection, antibiotic use, and vaccination. Demographic data were measured at enrollment. Breastfeeding status was measured at enrollment and the two subsequent study visits in the RV5 trial. Infants were classified as exclusively or non-exclusively breastfed. Growth status was classified as underweight, stunted, and wasting status at enrollment using the WHO cutoffs [102]. Stunted and wasting status could not be determined for Bangladeshi infants in the RV5 trial, because length of infants was not recorded. Current or prior infections and antibiotic use were classified using medical histories taken at enrollment and the two subsequent study visits in both the RV1 and RV5 trials. Antibiotic use did not include topical antibiotics. Concomitant vaccination was determined using the data collected at enrollment and the two subsequent study visits in both trials.

Statistical Analysis

To estimate the association between timing of rotavirus vaccine doses and incidence of severe RVGE, we estimated RDs and RRs between different schedules for each aspect of timing

at 6, 12, and 18 months of age using the difference or ratio of cumulative risk estimates obtained from the complement of the Kaplan-Meier function curve at those time points [106]. We *a priori* chose to focus on RDs and RRs at 12 months of age as our primary time point of interest. We did not estimate RDs and RRs at specific time points if any schedule had less than five severe RVGE events at that time point. We also estimated HRs using a Cox proportional hazard model with the exact method to account for tied events.

There was potential for bias in estimating the association between dose timing and severe RVGE, because of informative censoring from specific schedules and confounding. We used data from the placebo groups to account for both potential sources of bias under the assumption any factors influencing the timing of receipt of doses in the placebo groups were the same factors as those in the rotavirus vaccinated groups. Specifically, we accounted for bias by calibrating the estimates obtained among vaccinated infants with the estimates obtained among unvaccinated infants. Since the placebo doses should not influence the incidence of severe RVGE, any association between timing of placebo doses and incidence of severe RVGE was due to bias. Consequently, the association observed in the placebo group provided a quantitative estimate of amount of bias for each comparison made within the vaccinated groups. These data were used to calibrate (i.e., adjust) the estimates among those in the vaccinated groups.

Before calibrating estimates, we empirically verified that any imbalances in measured covariates between schedules were similar in the placebo and vaccinated groups. We did this by calculating the SMDs between covariates in each schedule for each aspect of timing for the placebo and vaccinated groups. The SMD was calculated as $(p1 - p2)/\text{sqrt}((p1(1 - p1) + p2(1 - p2))/2)$, where p1 was the proportion (or mean) of the binary covariate for a specific schedule (e.g., first dose at < 6 weeks) and p2 was the proportion in a different schedule (e.g., first dose at

≥ 6 weeks). We obtained a SMD for each covariate in the placebo and vaccinated groups and compared the SMDs between the estimates for the placebo and vaccinated groups. If the imbalance in covariates was similar between the placebo and vaccinated groups, we assumed calibration of the estimates in the vaccinated group would yield a marginal, adjusted estimate.

To estimate the adjusted association between rotavirus vaccine dose timing and severe RVGE, we obtained difference and ratio measures comparing schedules for each aspect of timing, as described above, for both the placebo and vaccinated groups of each trial. We then calibrated the estimates obtained among those vaccinated with the estimates obtained among those in the placebo groups by subtracting the difference measures and dividing the ratio measures (i.e., $RD_{RV} - RD_{Placebo}$, $\frac{RR_{RV}}{RR_{Placebo}}$, and $\frac{HR_{RV}}{HR_{Placebo}}$). A nonparametric bootstrap with 2,000 sample draws with replacement was used to obtain the point estimates and 95% empirical CIs [107]. The median of the distribution of calibrated estimates was reported for the difference and ratio estimates and the 2.5^{th} and 97.5^{th} percentiles of the distribution were reported for the lower and upper bounds of the 95% empirical CIs.

This analysis was approved by the University of North Carolina at Chapel Hill Institutional Review Board. All analyses were performed using SAS Clinical Trial Data Transparency (Version 4.5.2; SAS Institute Inc., Cary, NC, USA).

Results

There were 3,114 and 7,341 children included in this analysis from the RV1 and RV5 trials, respectively (Table 5.2). Among included infants, there was more variability in the timing of second and third (RV5 trial only) doses than the first dose. There was an equal distribution of males and females and most infants came from African LMICs. A high percentage of infants in

the RV1 trial were stunted, whereas a high percentage were wasting in the RV5 trial. Many infants in the RV5 trial were exclusively breastfed through dose three. Concomitant infections were only present in less than approximately one percent of infants at any given dose in both trials. Concomitant antibiotic use at doses was about 15% at dose one and two in the RV1 trial, but was lower in the RV5 trial (approximately 6% at dose two and three). Concomitant vaccination was high for all doses in the RV1 trial and about 50% for doses in the RV5 trial.

Infants were followed for a median time of 286 and 301 days after 12 weeks of age in both the placebo and RV1 vaccinated groups, respectively. Median follow-up was 483 days after 12 weeks of age in both the placebo and RV5 vaccinated groups. A total of 154 (100 and 54 in placebo and RV1 groups, respectively) and 324 (205 and 119 in placebo and RV5 groups, respectively) first severe RVGE events occurred in the RV1 and RV5 trials, respectively.

The cumulative risk of severe RVGE stratified by schedule and treatment status (i.e., placebo or vaccinated) for the primary aspect of dose timing, timing of first dose with 4 – 6 week interval(s) between all subsequent doses, is shown in Figures 5.2 and 5.3. All other schedules for all other aspects of timing are presented in Appendix Figures 1 – 7.

The distribution of covariates between schedules for each aspect of timing were very similar for the placebo and vaccinated groups. Generally, there was < 10% difference between the SMDs of covariates between schedules for each aspect of timing in the placebo and vaccinated groups (Appendix Figures 8 – 16). All except 13 of the 331 (4%) covariate comparisons had differences of < 10% between the placebo and vaccinated groups. This meant, in most instances, the distribution of covariates by schedules for each aspect of timing was very similar in the placebo and rotavirus vaccinated groups. Most (N = 6) of the differences that occurred between the placebo and vaccinated groups happened for schedule comparisons for

those beginning RV1 vaccination at 10 – 12 versus 13 – 16 weeks (with an interval of 4 – 6 weeks between the next dose). The majority of remaining differences between the placebo and vaccinated groups (N = 6) occurred for a single covariate and were 10 to < 15%. One difference that occurred was 15 to < 20%.

Calibrated RDs and RRs between schedules for each aspect of timing at 12 months of age are presented in Figures 5.4 and 5.5, respectively. There was about a 4% increase in 12-month risk for those beginning their first dose of RV5 at < 6 versus ≥ 6 weeks [12-month RD: 4.02% (95% CI: 1.13, 7.14)]. This was similar, but attenuated when we compared those beginning RV5 vaccination at 3 – 6 weeks of age to those beginning at 10 – 12 weeks of age (holding intervals between all subsequent doses at 4 – 6 weeks) [12-month RD: 2.07% (95% CI: 0.09, 4.02)]. Similarly, receiving three doses at ≤ 15 versus > 15 weeks of age (meaning the first dose and second dose must be received at ≤ 7 and ≤ 11 weeks of age, respectively) was slightly less than a 2% increase in 12-month risk [12-month RD RV5: 1.89% (95% CI: 0.59, 3.17); 12-month RD RV1: 1.41% (95% CI: -1.14, 4.09)]. In addition, those receiving only one dose of RV5 at ≥ 10 weeks of age had a higher risk of severe RVGE compared to those receiving three doses at ≥ 10 weeks [12-month RD: 2.59% (95% CI: 0.69, 4.71)]. When the analysis comparing one versus three doses of RV5 at ≥ 10 weeks of age excluded infants missing the second or third dose of vaccine, the association seen was similar [12-month RD: 2.55% (95% CI: 0.50, 4.72)]. In addition, there was about a 4% increase in 12-month risk for those with a 4 week interval between RV1 doses compared to a 6 week interval [12-month RD: 4.04% (95% CI: -0.0004, 8.16)]. This was diminished to about 2% when comparing a 5 week interval to a 6 week interval, but this estimate was relatively imprecise [12-month RD: 1.90% (95% CI: -2.17, 5.90)].

There were fewer than five events for at least one schedule for each aspect of timing at 6 months of age, and for the first dose at < 6 versus ≥ 6 weeks of age schedule at 12 months of age. Therefore, we did not estimate RDs and RRs for these time points and schedules. Since we could not estimate RDs and RRs for the first dose at < 6 versus ≥ 6 weeks of age schedule at 12 months of age, we presented the 18-month RDs and RRs for this comparison. HRs were similar to RRs and a presented in Appendix Figure 17. Uncalibrated, calibrated and placebo RDs and RRs at 12 and 18 months are presented in Appendix Figure 18 and 19, respectively.

Discussion

We analyzed data from two clinical trial conducted in LMICs to understand the association between timing of rotavirus vaccine doses and severe RVGE. There was a dose-response relationship between age at first RV5 dose and severe RVGE. Earlier administration of first RV5 dose was associated with an increased severe RVGE risk and that risk declined with increased age of first dose until approximately 8 – 9 weeks of age. An interval of 4 versus 6 weeks between RV1 doses was associated with increased risk of severe RVGE when RV1 was administered on an approximately 10/14 week schedule. These data were the first to compare timing of doses among vaccinated infants using clinical endpoints and provide insights to inform administration of rotavirus vaccines in LMICs.

Collectively, data from the RV5 trial indicated earlier vaccination of the first RV5 dose resulted in a higher risk of severe RVGE with this risk declining over time until approximately 8 – 9 completed weeks of age. Importantly, the study design allowed early events to be counted in more than one schedule if the doses infants had received were consistent with more than one predefined schedule for an aspect of timing. This means these associations were present even when accounting for the occurrence of early severe RVGE events. The results reported here are

consistent with trials investigating alternative rotavirus schedules reporting immunologic endpoints. Vaccination with RV1 at 10/14 versus 6/10 weeks of age resulted in higher one-month seroconversion proportions (i.e., seroconversion defined from pre-vaccination to one-month after completing vaccination) in Ghana and Pakistan, which had seroconversion proportion differences comparing 10/14 versus 6/10 week schedules of 17.0% (95% CI: 6.4, 27.1) and 10.1% (-0.4, 20.3), respectively [24, 25]. In addition, in a previous analysis conducted by the first author, there was significant heterogeneity of efficacy for those with a first dose of RV5 received at < 8 weeks compared to ≥ 8 weeks of age [120]. The current analysis built upon the prior analysis in several ways. This analysis compared the association of timing of rotavirus doses and severe RVGE among vaccinated infants. We also used an age-specific time scale that allowed us to include early events (events prior to vaccination completion) and infants with a partial series until they deviated from the predefined schedules. In addition, this analysis focused on several aspects of rotavirus vaccine timing, included the use of RV1 trial data, and provided estimates of associations on an absolute scale, which is important for considering public health impacts.

Lower incidence of severe RVGE with delays in first rotavirus vaccine dose could be due to a number of biologic factors including a decline in transplacentally-acquired anti-rotavirus IgG antibodies, development in the immune system, or changes in the microbiota that allows for a stronger immune response with slightly older ages at first dose of rotavirus vaccine. There is some evidence that anti-rotavirus IgG antibodies inhibit response to rotavirus vaccines. In Ghana, a trial investigating the impact of alternative RV1 vaccines schedules on seroconversion found seroconversion was higher among infants with the lowest compared to highest quartile of pre-vaccination IgG levels [25]. A similar result was also found in Pakistan [24]. In addition to

maternal antibodies, early development of the infant immune system in conjunction with the evolution of the gut microbiota could play a role in the association observed between dose timing and severe RVGE. Early in life, the immune system is developing and can be impacted by the development of the microbiota [121]. The development of the immune system may be delayed leading to a reduced immune response when rotavirus vaccine doses are given earlier in life. Similarly, the composition of the microbiota itself is constantly evolving early in life and may influence the vaccine response. A case control study of 6 week old Ghanaian infants found RV1 vaccine responders (post-vaccination IgA antibody levels ≥ 20 IU/mL) had microbiotas more closely resembling Dutch infants than Ghanaian non-responders (post-vaccination IgA antibody levels ≥ 20 IU/mL) [27]. It may be possible such differences in the microbiota between responders and non-responders can be mitigated with increasing age leading to improved vaccine response when vaccinated at later ages.

We also found an increase in risk of severe RVGE for those infants with a 4 week interval between RV1 doses compared to a 6 week interval between doses. This association was attenuated and much less precise comparing the 5 week to 6 week interval between RV1 doses. For RV5, there was no association between length of interval between doses and severe RVGE. However, it is difficult to investigate these associations, because RV5 is a three rather than two dose vaccine, which resulted in a number of possible combinations of intervals between doses. It is possible we did not observe an association, because we had to collapse across groups of different interval lengths to obtain sufficient sample sizes for comparisons. A trial conducted in Vietnam comparing a vaccine schedule with an interval of 4 – 5 weeks between doses (schedule of 8.8/13.2 weeks) to another schedule with an interval of 8 – 9 weeks between doses (schedule of 8.6/17.4 weeks) did report a higher seroconversion proportion for the schedule with the longer

interval between doses [88] (one-month seroconversion proportion difference (95% CI): -0.25 (-0.36, -0.14)). However, when a similar interval comparison of 4 – 5 weeks to 8 – 9 weeks for infants in the Philippines was made for a schedule with an earlier start (schedule of 6.5/15.1 weeks) versus a later start (schedule of 10.6/15.2 weeks), the schedule with a longer interval between doses but with an earlier start had a lower seroconversion proportion [88] (one-month seroconversion proportion difference (95% CI): -0.11 (-0.23, 0.01)). A longer delay between doses may provide a greater booster effect for RV1, but possibly only when the first dose is administered around 10 weeks of age like it was in the RV1 trial.

There are a number of limitations of this analysis. Since this was an analysis of previously collected data, we were restricted in our ability to analyze timing of different dosing schedules. Ideally, it would have been informative to have a larger number of infants vaccinated at different times, so we could have more precisely estimated the association between different schedules. This was particularly problematic for the RV1 trial, because there were fewer vaccinated infants and these infants had less variability in the timing of their doses. We were also unable to assess events that occurred before 12 weeks of age, because there was staggered enrollment on an age scale, and we chose to begin follow-up at the age every infant had begun follow-up. In the RV5 cohort, no events occurred prior to 12 weeks of age, but three events did occur prior to 12 weeks of age (one and two in the placebo and RV1 groups, respectively) in the RV1 trial. Similarly, there was only active surveillance at local clinics and hospitals, and not households, for gastroenteritis in the RV5 trial, which likely resulted in underascertainment of severe RVGE episodes. This means there may have been earlier events that were unaccounted for in the analysis, because they were not captured in the study. In addition, there may be residual confounding of some estimates that we were unable to account for in this analysis.

Although the vast majority of schedules for each aspect of timing had similar covariate imbalances in the placebo and vaccinated groups, there were a few variables that did not deviate in a similar manner. This means that after calibration of estimates, these estimates may have some residual confounding due to the differences in the imbalance of covariates. Therefore, we particularly urge readers to interpret estimates of the comparison of the whole RV1schedule of 13 – 16 versus 10 – 12 weeks with a 4 – 6 week interval between doses with caution, because there were a large number of imbalances between the placebo and vaccinated groups. For this comparison, it is difficult to predict the direction and magnitude of bias that may be due to the differences. For all other comparisons, we expect there is little bias due to the difference in imbalance of covariates between the placebo and vaccinated groups, because the differences between SMDs were relatively small, restricted to one to two covariates, and direct adjustment for these factors with each schedule comparison did not substantially impact the estimates in either the placebo or vaccinated groups.

Despite these limitations, there are a number of strengths of this analysis. The data from these trials allowed us to assess if timing of rotavirus vaccine doses is associated with incidence of severe RVGE, which has not been previously reported. We were also able to use data from multiple data sources of two rotavirus vaccines to understand the association between rotavirus vaccine timing and severe RVGE more broadly. In addition, we were able to use a novel study design to account for early rotavirus events to ensure later schedules would be penalized for any early events that occurred prior to receipt of the vaccine. Similarly, we leveraged data in the placebo groups to account for bias and adjust estimates of the association between timing of rotavirus vaccine doses and incidence of severe RVGE.

In this analysis of two clinical trials of rotavirus vaccines in LMICs, we found that there was an association of rotavirus vaccine dose timing on incidence of severe RVGE. The increase in 12- or 18-month risk associated with earlier administration of first RV5 and RV1 doses ranged from 1.5 – 4% and declined with increasing age at first dose. In addition, a 4 week versus 6 week interval between RV1 doses was associated with about a 4% increase in 12-month risk when RV1 was administered at about 10/14 weeks of age. Although these percentages are small, these associations are quite large when considering millions of children are vaccinated with rotavirus vaccines each year. Countries should carefully consider these data when determining vaccination strategies to prevent the most cases of severe RVGE.

Table 5.1 Description of predefined rotavirus vaccine schedules developed for each aspect of dose timing. Timing is in weeks of age unless otherwise indicated. The primary comparison of interest is bolded.

Aspect of Timing	N*	N†	Schedule Number	RV Type	Dose 1	Dose 2	Dose 3
Whole Schedules	**1,167**	**1,073**	**1**	**RV5**	**3 – 6**	**4 – 6 wks after 1st dose**	**4 – 6 wks after 2nd dose**
	1,669	**1,516**	**2**	**RV5**	**7 – 9**	**4 – 6 wks after 1st dose**	**4 – 6 wks after 2nd dose**
	784	**720**	**3**	**RV5**	**10 – 12**	**4 – 6 wks after 1st dose**	**4 – 6 wks after 2nd dose**
	1,299	**1,212**	**3**	**RV1**	**10 – 12**	**4 – 6 wks after 1st dose**	**NA**
	207	**166**	**4**	**RV1**	**13 – 16**	**4 – 6 wks after 1st dose**	**NA**
First Dose	519	506	1	RV5	< 6	≤ 10 wks after 1st dose	≤ 10 wks after 2nd dose
	3,147	3,063	2	RV5	≥ 6	≤ 10 wks after 1st dose	≤ 10 wks after 2nd dose
	2,882	2,805	3	RV5	< 10	≤ 10 wks after 1st dose	≤ 10 wks after 2nd dose
	784	764	4	RV5	≥ 10	≤ 10 wks after 1st dose	≤ 10 wks after 2nd dose
Last Dose	1,527	1,216	1	RV5	≤ 7	≤ 11	≤ 15
	3,520	2,353	2	RV5	≤ 12	≤ 10 wks after 1st dose	> 15 & ≤ 10 wks after 2nd dose
	1,048	420	1	RV1	≤ 11	≤ 15	NA
	1,559	1,056	2	RV1	≤ 16	> 15 & ≤ 10 wks after 2nd dose	NA
Interval between Doses	2,589	1,310	1	RV5	≤ 12	4 wks after 1st dose	4 wks after 2nd dose
	3,345	1,453	2‡	RV5	≤ 12	4 or 5 wks after 1st dose	4 or 5 wks after 2nd dose
	3,474	546	3§	RV5	≤ 12	4, 5 or 6 wks after 1st dose	4, 5, or 6 wks after 2nd dose
	1,559	337	1	RV1	≤ 16	4 wks after 1st dose	NA
	1,559	926	2	RV1	≤ 16	5 wks after 1st dose	NA
	1,559	167	3	RV1	≤ 16	6 wks after 1st dose	NA
Number of Doses ≥ 10 Weeks of Age	1,030	2	0‖	RV5	< 10	< 10	< 10
	3,666	397	1‖	RV5	< 10	< 10	≥ 10 & ≤ 32
	3,271	2,432	2‖	RV5	< 10	≥ 10 & ≤ 32	≥ 10 & ≤ 32
	784	765	3‖	RV5	≥ 10	≥ 10 & ≤ 32	≥ 10 & ≤ 32

NA, not applicable; wks, weeks

* Number of infants beginning follow-up at 12 weeks of age in this schedule. Total sample size across schedules for an aspect of timing can sum to more than the total number of vaccinated infants in each trial because infants can begin in > 1 schedule.

† Number of infants being followed for event for a particular schedule at 6 months of age.

‡ At least one interval between doses must be 5 weeks;

§ At least one interval between doses must be 6 weeks.

[l] Other timing of doses resulting in the same number of doses received ≥ 10 are possible and are included in Appendix Table 1.

Table 5.2 Characteristics of the trial populations.

Infant Characteristics	Trial 1 (N = 3,114)		Trial 2 (N = 7,341)	
	RV1 (N = 1,560)	Placebo (N = 1,554)	RV5 (N = 3,666)	Placebo (N = 3,675)
Age at Vaccine or Placebo Receipt				
Dose 1[*], mean (SE)	11.2 (0.03)	11.3 (0.03)	7.6 (0.03)	7.5 (0.03)
Dose 2[†], mean (SE)	16.2 (0.04)	16.3 (0.04)	12.2 (0.04)	12.1 (0.04)
Dose 3[‡], mean (SE)	--	--	16.7 (0.04)	16.7 (0.04)
Demographic				
Female Sex, %	48.8	48.6	48.6	49.7
African Race, %	97.2	96.8	72.3	72.4
Asian Race, %	--	--	27.6	27.6
Growth Status at Enrollment				
Stunted, %	22.6	21.7[§]	10.1	10.4[‖]
Underweight, %	3.9	4.4	11.5	11.2
Wasting, %	3.7[§]	4.3[§]	23.1[§]	20.9[§‖]
Exclusively Breastfed				
At Dose 1, %	--	--	80.0	81.6
At Dose 2, %	--	--	75.2[§]	74.7
At Dose 3, %	--	--	69.9	70.1[§]
≥ 1 Concomitant Infections				
At Dose 1, %	0.5	0.9	5.5	5.6
At Dose 2, %	0.3	0.4	0.5	0.6
At Dose 3, %	--	--	1.1	1.3
≥ 1 Concomitant Antibiotic[¶]				
At Dose 1, %	12.9	14.1	2.2	2.8
At Dose 2, %	15.5	14.6	5.6	5.2
At Dose 3, %	--	--	6.2	6.1
Routine Vaccines				
≥ 1 Concomitant BCG				
At Dose 1, %	--	--	21.9	22.0
≥ 1 Concomitant DTP-HB/HIB[**]				
At Dose 1, %	99.3	99.3	45.7	47.0
At Dose 2, %	99.1	99.5	43.6	43.8
At Dose 3, %	--	--	42.0	42.2
≥ 1 Concomitant OPV				
At Dose 1, %	99.3	99.3	54.1	55.6
At Dose 2, %	99.2	99.5	51.1	51.0
At Dose 3, %	--	--	48.2	48.1

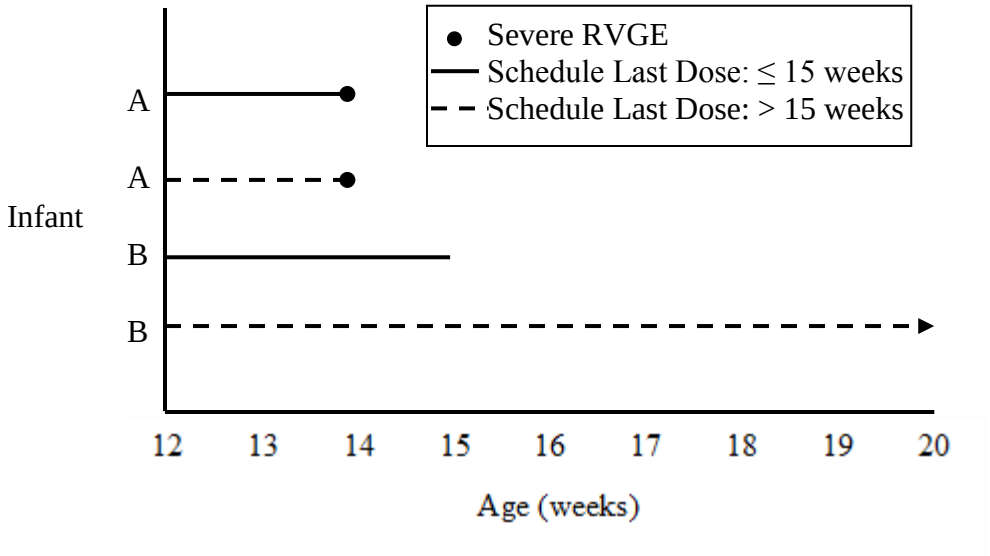
SE, standard error

[*] There were 25 and 21 in the placebo and RV1 groups, respectively, missing dose one.

[†] There were 63 and 45 in the placebo and RV1 groups, respectively, missing dose two. There were 17 and 10 in the placebo and RV5 groups, respectively, missing dose two.

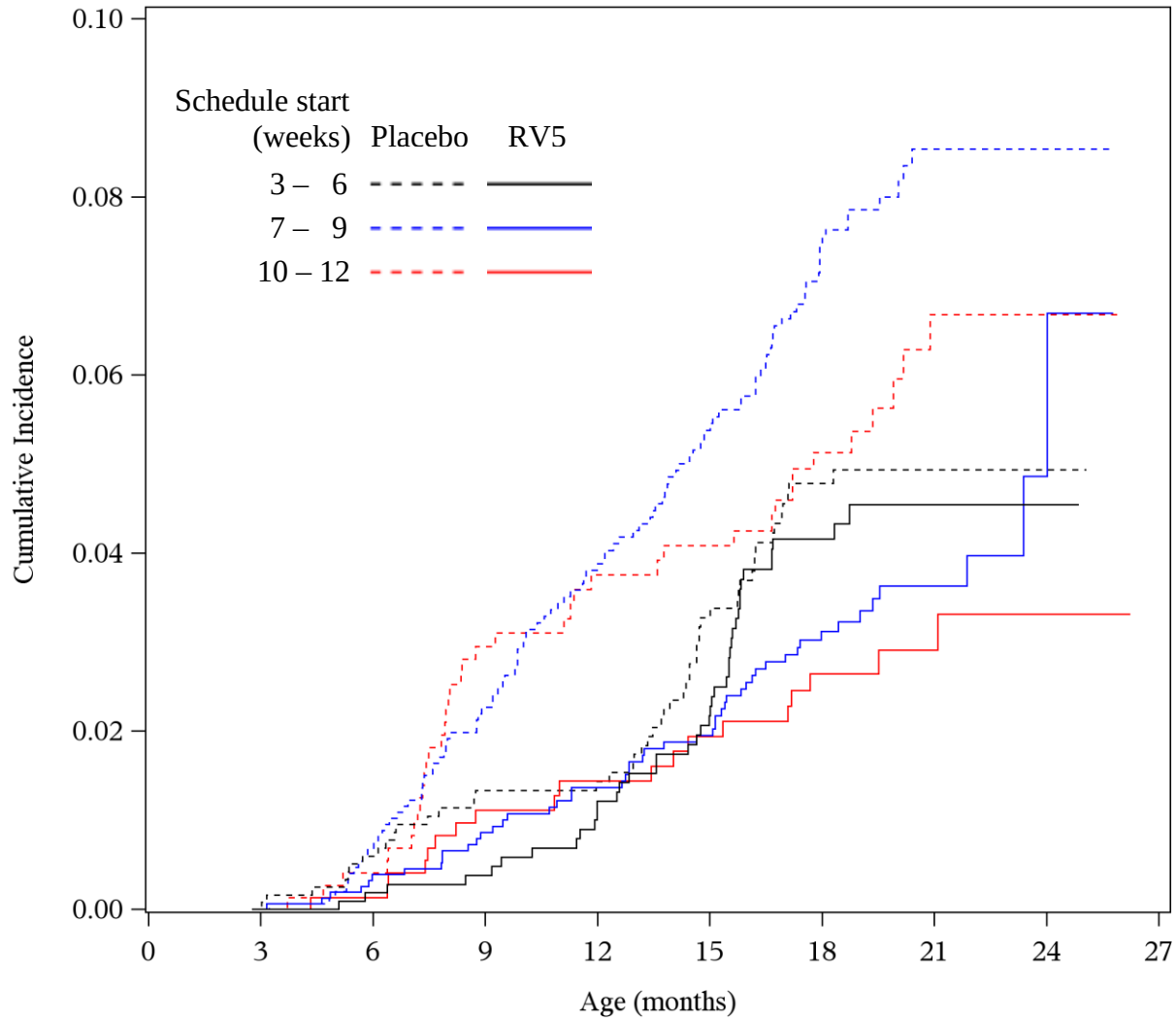
[‡] There were 57 and 47 in the placebo and RV5 groups, respectively, missing dose three.

[§] Missing 1 – 15 observations, excluding those missing doses of vaccine or placebo.
[‖] Excluding Bangladesh
[¶] Excluding topical antibiotics
[**] Or DTaP & HBV, which were the standard vaccines given in Asian countries



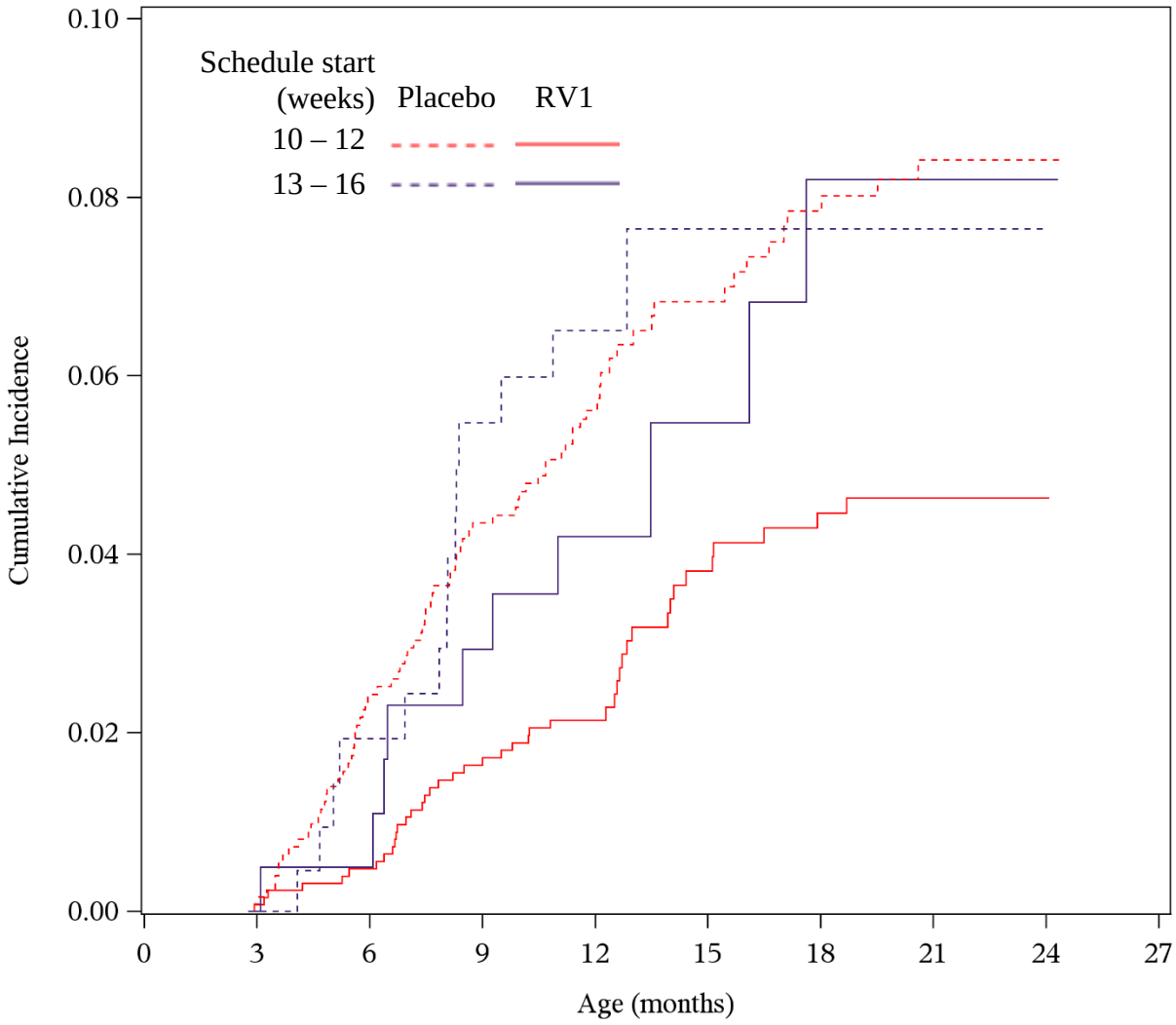
A: event at 14 weeks (counted in both groups).
B: no event before 20 weeks; infant vaccinated with final dose > 15 weeks.

Figure 5.1 Example of two infants and how events and person-time are assigned for the schedules for timing of the last dose.



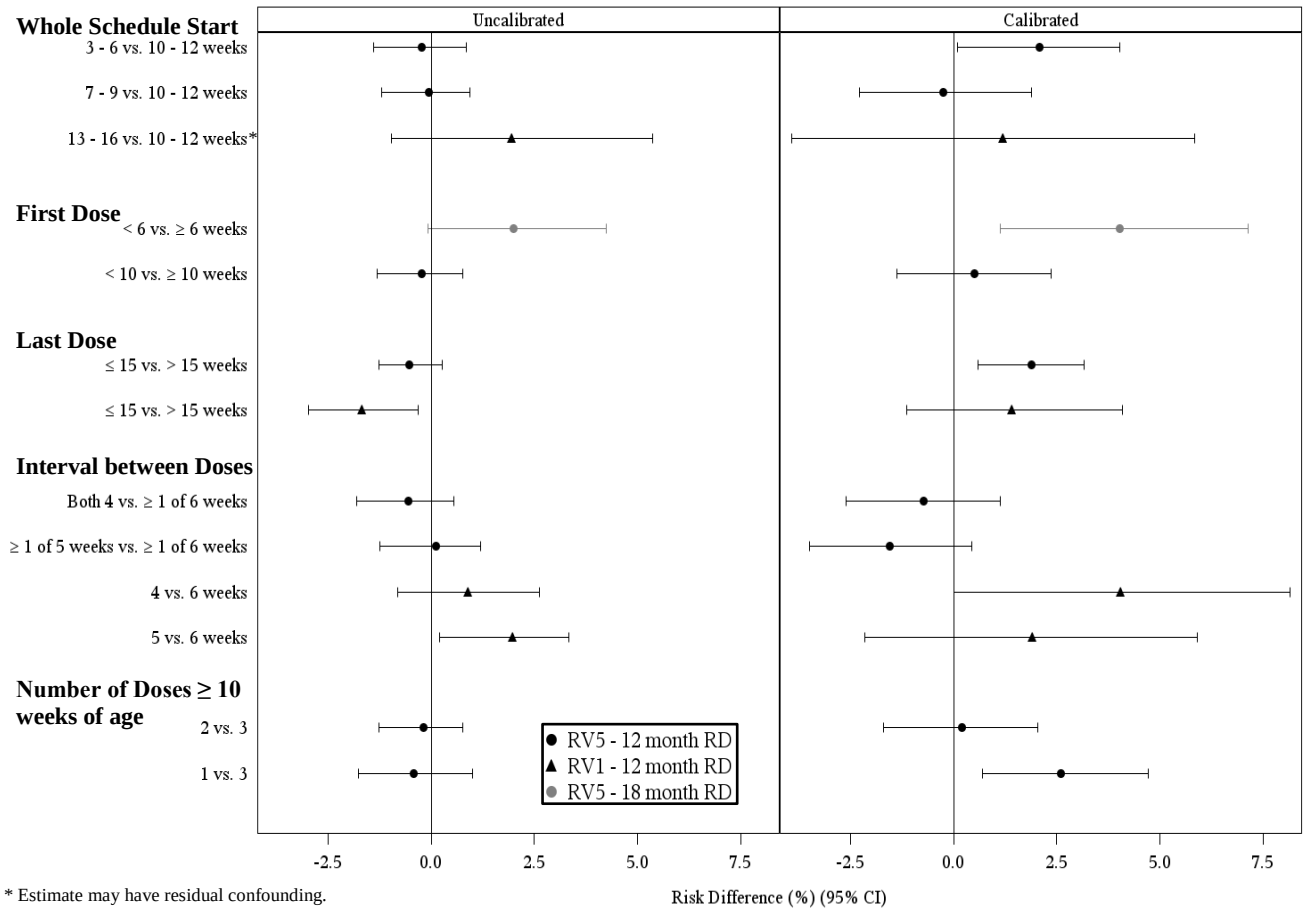
RV5	3 – 6	1,167	1,073	943	640	7
	7 – 9	1,669	1,516	1,351	1,003	52
	10 – 12	784	720	600	527	45
Placebo	3 – 6	1,250	1,134	980	687	9
	7 – 9	1,589	1,451	1,293	948	64
	10 – 12	780	713	590	514	39

Figure 5.2 Cumulative incidence of severe RVGE by timing of first dose with 4 – 6 week intervals between subsequent doses in the placebo and RV5 vaccinated groups. Number at risk at 12 weeks, 6 months, 12 months, 18 months, and 24 months of age for schedule is below the x-axis.



RV1	10 – 12	1,299	1,212	767	573	7
	13 – 16	207	166	100	67	1
Placebo	10 – 12	1,255	1,128	708	530	6
	13 – 16	253	196	107	73	1

Figure 5.3 Cumulative incidence of severe RVGE by timing of first dose with a 4 – 6 week interval between doses in the placebo and RV1 vaccinated groups. Number at risk at 12 weeks, 6 months, 12 months, 18 months, and 24 months of age for schedule is below the x-axis.



* Estimate may have residual confounding.

Figure 5.4 Uncalibrated and calibrated RDs and 95% CIs.

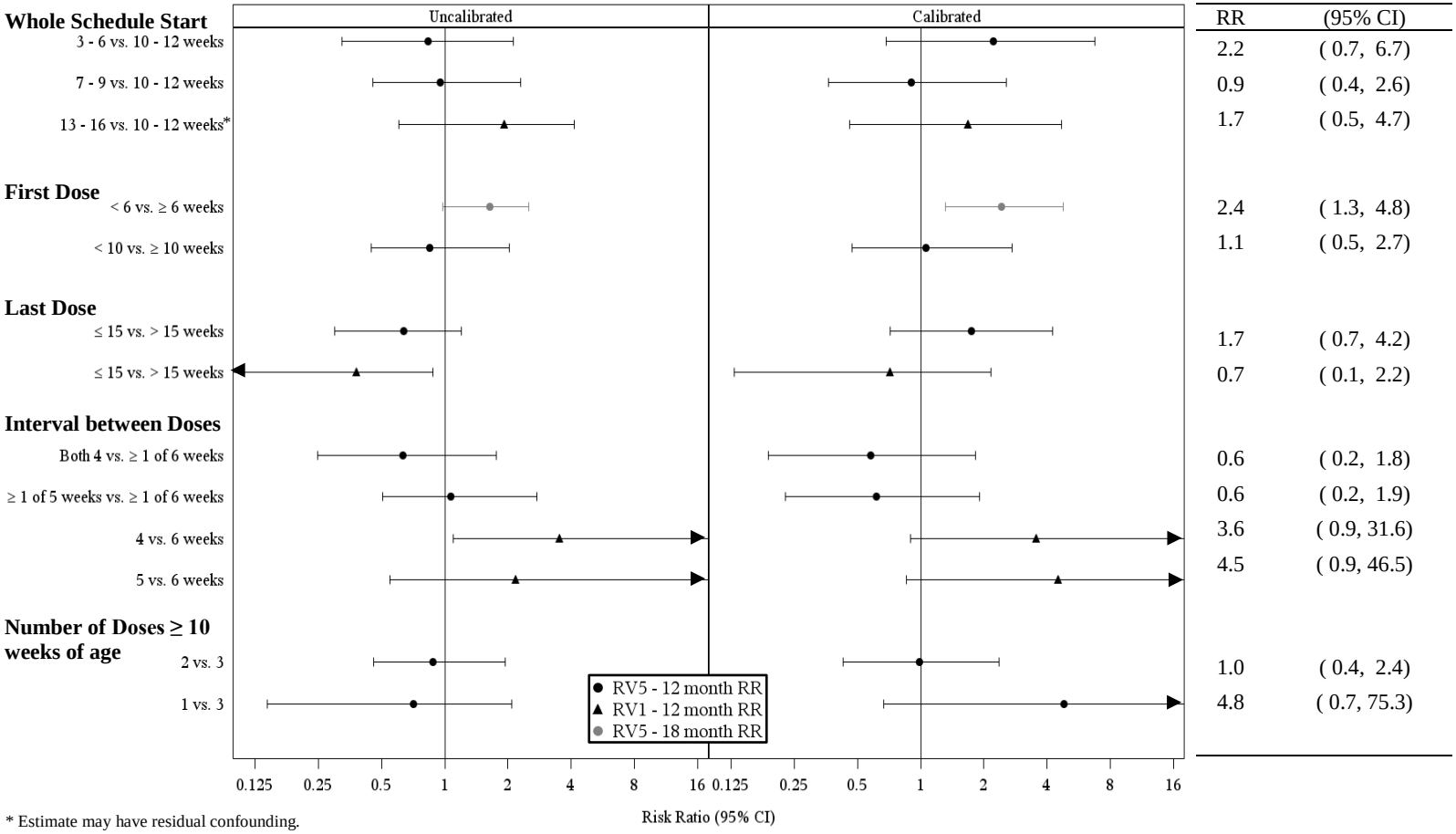


Figure 5.5 Uncalibrated and calibrated RRs and 95% CIs.

CHAPTER 6: DISCUSSION

Summary of Objective and Results

The purpose of this dissertation was to investigate when children in LMICs experienced severe RVGE episodes and if the timing of rotavirus vaccine doses was associated with risk of severe RVGE in these areas. We did this by analyzing the natural history of rotavirus among unvaccinated infants in two trial populations and by estimating the association between timing of rotavirus vaccine doses and risk of severe RVGE among vaccinated infants in each trial. A summary of the results from each research aim is below.

Aim 1: To describe the natural history of severe RVGE among infants in the placebo groups of the rotavirus vaccine trials in LMICs.

Aim 1a: To describe the timing of first episode of severe RVGE.

Aim 1b: To estimate the association between incidence of first severe RVGE and baseline factors, including demographic information; breastfeeding and growth status; and concomitant infection, antibiotic use, and vaccination.

The cumulative risk of severe RVGE was 6 – 8% at 20 months of age. Risk increased steadily over the first one to two years of life and was low at 6 months of age. Antibiotic use was associated with about 1.4 to 2 times the rate of severe RVGE in cohort 2 and 1, respectively.

Aim 2: To estimate the association between timing of rotavirus vaccine doses and incidence of severe RVGE among vaccinated infants in the rotavirus vaccine trials in LMICs.

The timing of rotavirus vaccine doses was associated with the incidence of severe RVGE. There was a dose-response relationship between age at first RV5 dose and incidence of severe RVGE. Earlier administration of first RV5 dose was associated with an increased risk of severe RVGE and that risk declined with increased age of first dose until approximately 8 – 9 weeks of age. An interval of 4 versus 6 weeks between RV1 doses was also associated with an increased risk of severe RVGE. However, this occurred when RV1 was administered on an approximately 10/14 week schedule.

Timing of Severe RVGE in LMICs

In Aim 1, we found that 6 – 8% of children experienced severe RVGE by 20 months of age, and the cumulative risk of severe RVGE increased relatively steadily over the first 12 – 18 months of age. Cumulative risk at 6 months of age was low. This is important to note, because if countries were to shift the timing of rotavirus vaccine doses, it would be important to avoid a period with a large increase in risk such that infants who would have been vaccinated are no longer vaccinated for this period of increased risk. Instead, we expect if there are shifts in the rotavirus vaccine schedules such that complete protection occurred at a slightly later age, there would be relatively small increases in the number of severe RVGE events. Table 6.1 summarizes the maximum number of early cases we would expect to occur if we delayed RV1 and RV5 schedules. These estimates assume the severe RVGE episodes would be fully prevented with the commonly used schedule of 6/10 (RV1) or 6/10/14 weeks (RV5) and that there is no partial

protection from an incomplete later series of 10/14 (RV1) or 8/12/16 weeks (RV5). In short, these estimates provided a "worst case scenario" for the number of severe RVGE episodes we could expect to occur with a shift in schedule if there was no benefit in terms of RVGE to changing these schedules.

Rotavirus Vaccines and Schedules Currently in Use in LMICs

In LMICs, the overwhelming majority of countries (37 of 44, 84%) use RV1. Currently, the WHO recommends rotavirus vaccines be given as soon as possible after 6 weeks of age [6], and 23 (62%) of the 37 LMICs administering RV1 in their routine immunization program use the 6/10 week schedule [62, 122]. The remaining 14 (38%) countries administer RV1 on a 4/16 week (N = 7), 4/12 week (N = 3), 6/10/14 week (N = 2), 6/12 week (N = 1), or 8/12 week (N = 1) schedule [122]. The following vaccination schedules are used for the 7 countries using RV5: 8/12/16 week (N = 3), 6/10/14 week (N = 2), 4/16 week (N = 1), or 4/16/24 week (N = 1).

Practical Challenges of Altering Rotavirus Vaccine Schedules in LMICs

There are three important practical challenges to think through when considering changing the timing of rotavirus vaccine doses. First, the changes made to the timing of rotavirus vaccine doses must conform to the schedule used for other routine vaccines. Unfortunately, this is a relatively large constraint, because vaccinations before a year of age often occurs around birth, 6 weeks, 10 weeks, 14 weeks, and 9 months of age for children in many LMICs. The most practical options for changing a two dose RV1 series would be to administer it at 10/14 or 6/14 weeks of age. The countries currently using RV5 use one of the following schedules for their routine vaccination: 6/10/14, 8/12/16 or 8/16/24 week. A shift in the RV5 series would likely be

infeasible unless countries shifted their schedule for all routine vaccines, which is likely unrealistic. Fortunately, the majority of LMICs use RV1, and it is the vaccine with the largest potential to feasibly alter the vaccine schedule. Second, any changes made to timing of rotavirus vaccine doses must be made considering the probability that children in the country will return, and return approximately on time for subsequent vaccinations. Delay or discontinuation of routine vaccinations is a major concern for public health officials in many LMICs. However, the limited data available describing this indicates there is a large amount of heterogeneity in both the completeness and timeliness of vaccine doses [123, 124]. If the country specific data indicates it is probable that children will not return, or return significantly delayed for subsequent rotavirus vaccine doses, it is likely not be beneficial to delay vaccination. Third, it is important to consider the tradeoff between possible increases in vaccine performance and increases in cases that can occur when considering changes in vaccine schedule. This dissertation provides some data using clinical endpoints to make this judgment.

Limitations

There were a few limitations of this dissertation. Unfortunately, we were unable to make comparisons between the most frequently used schedule (6/10 weeks) and other feasible schedules (e.g., 6/14 or 10/14 weeks) for RV1, which is the most widely used rotavirus vaccine in LMICs. This was due to the schedules that were used in the trials of RV1. However, we were able to use data from both the RV1 and RV5 trials to understand how timing of rotavirus vaccine doses affects the risk of severe RVGE. In addition, it was a limitation that infants were not followed from birth. Ideally, we would have followed infants from birth, so we did not have left truncation of data (i.e., late-entry) on an age-specific time scale and could observe very early

severe RVGE episodes. For the purpose of comparing schedules beginning at 6 weeks of age or later, this limitation was less important, because any events occurring before protection would be conferred at 12 or 16 weeks of age (i.e., two weeks after the 6/10 or 6/10/14 week schedule is complete), could not be prevented. However, if there was important partial protection from incomplete vaccine series before 12 weeks of age, it would be beneficial to begin follow-up earlier.

Strengths

This dissertation has several strengths. We were able to determine if timing of rotavirus vaccine doses was associated with the incidence of severe RVGE. Given the very limited data on rotavirus vaccine timing using clinical endpoints, the results of this dissertation were able to provide critical, missing information. Importantly, the results of this research using clinical endpoints were consistent with the results of vaccine trials assessing timing using immunogenic endpoints. The totality of this evidence can be used to inform vaccine administration strategies that will prevent the most cases of severe RVGE in LMICs. In addition, the results provide crucial information on the timing of first episode of severe RVGE, which is the most clinically relevant outcome prevented by rotavirus vaccination. Prior to this dissertation, no study had reported information the cumulative incidence of severe RVGE in LMICs, in large part, because sample sizes of previous studies were too small to do so. This dissertation also used a novel study design to account for early severe RVGE episodes and confounding while leveraging data from two sources to compare patterns seen across different locations with different rotavirus vaccines.

Future Directions

The scientific literature and results of this dissertation provide evidence that altering vaccine schedules may result in fewer episodes of severe RVGE. Since we were unable to directly compare RV1 given at 6/10 weeks to other schedules, it would be highly informative to have a multisite randomized control trial of children followed from birth for severe RVGE until one to two years of age and assigned to infants to different dosing schedules to determine the efficacy of different rotavirus vaccine schedules. The most compelling comparisons arms would be 6/10, 10/14, and 6/14 weeks of RV1. However, given the cost of such a trial, it is unlikely to occur. Countries should carefully consider the evidence that is available, because these data may be the only data available from large study populations reporting incidence of severe RVGE.

Conclusions

This dissertation research and the previous scientific literature indicate that severe RVGE episodes may be prevented in children by altering the vaccine schedules used in LMICs. Results of different timing classifications from the RV5 trial indicate infants with earlier first dose had a higher risk of severe RVGE compared to those with later first dose. This increased risk attenuated over the age of first dose with no increase seen when first dose was given at 8 – 9 weeks compared to later ages. There was also an increased risk of severe RVGE associated with a 4 versus 6 week interval between RV1 doses that were administered at approximately a 10/14 week schedule. Changes to rotavirus vaccine schedules in LMICs must be considered in light of the standard immunization schedule, retention and timeliness of vaccinations, and potential for severe RVGE early in life.

Table 6.1 Number of expected severe RVGE cases that would occur if altering vaccination schedules had no benefit.

Cohort/Country	Expected Increase in RVGE Cases per 1,000 Infants (95% CI)*	
	RV1 6/10 vs. 10/14	RV5 6/10/14 vs. 8/12/16
Cohort 1	5 (1, 8)	2 (0, 4)
Malawi	9 (1, 17)	2 (0, 6)
South Africa	2 (0, 5)	2 (0, 5)
Cohort 2	1 (0, 2)	0 (0, 1)
Ghana	0	0
Kenya	2 (0, 5)	0
Mali	2 (0, 5)	1 (0, 3)
Bangladesh	0	0
Vietnam	0	0

* Estimates assume infants are fully protected at two weeks following the final dose of vaccine. Therefore, the expected increase if for the period from 12 to 16 weeks (RV1) and 16 to 18 weeks (RV5). These estimates also assume the severe RVGE episodes would be prevented had infants been vaccinated at 6/10 (RV1) or 6/10/14 (RV5) weeks and that there is no partial protection for an incomplete series for later vaccine schedules.

APPENDIX: SUPPLEMENTAL INFORMATION

Appendix Table 1. Detailed description of entry, follow-up, and censoring for schedule comparisons for aspects of timing. Timing is in weeks of age unless otherwise indicated.

<table>
<tr><th>Aspect of Timing</th><th>Schedule</th><th>RV Type</th><th>Dose 1</th><th>Dose 2</th><th>Dose 3</th><th>Description of Entry, Follow-up, and Censoring for Schedules*</th></tr>
<tr><td>Whole Schedules</td><td>1</td><td>RV5</td><td>3 – 6</td><td>4 – 6 wks after 1st</td><td>4 – 6 wks after 2nd</td><td>Infants with 1st dose at 3, 4, 5, or 6 wks of age begin in this schedule and continue follow-up until severe RVGE, end of study, or censoring. Infants are censored at the earliest occurrence of any of the following:<table><tr><th>Reason for Censoring</th><th>Time of Censoring</th></tr><tr><td>Lost to follow-up/dropout</td><td>Time of dropout</td></tr><tr><td>Receipt of 2nd dose < 4 wks after 1st dose</td><td>Time of 2nd dose</td></tr><tr><td>No 2nd dose by 6 wks after 1st dose</td><td>6 wks after 1st dose</td></tr><tr><td>Receipt of 3rd dose < 4 wks after 2nd dose</td><td>Time of 3rd dose</td></tr><tr><td>No 3rd dose by 6 wks after 1st dose</td><td>6 wks after 2nd dose</td></tr></table></td></tr>
<tr><td>Whole Schedules</td><td>2</td><td>RV5</td><td>7 – 9</td><td>4 – 6 wks after 1st</td><td>4 – 6 wks after 2nd</td><td>Infants with 1st dose at 7, 8, or 9 wks of age begin in this schedule and continue follow-up until severe RVGE, end of study, or censoring. Infants are censored at the earliest occurrence of any of the following:<table><tr><th>Reason for Censoring</th><th>Time of Censoring</th></tr><tr><td>Lost to follow-up/dropout</td><td>Time of dropout</td></tr><tr><td>Receipt of 2nd dose < 4 wks after 1st dose</td><td>Time of 2nd dose</td></tr><tr><td>No 2nd dose by 6 wks after 1st dose</td><td>6 wks after 1st dose</td></tr><tr><td>Receipt of 3rd dose < 4 wks after 2nd dose</td><td>Time of 3rd dose</td></tr><tr><td>No 3rd dose by 6 wks after 1st dose</td><td>6 wks after 2nd dose</td></tr></table></td></tr>
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<tr><td>Whole Schedules</td><td>3</td><td>RV1</td><td>10 – 12</td><td>4 – 6 wks after 1st</td><td>NA</td><td>Infants with 1st dose at 10, 11, or 12 wks of age begin in this schedule and continue follow-up until severe RVGE, end of study, or censoring. Infants are censored at the earliest occurrence of any of the following:<table><tr><th>Reason for Censoring</th><th>Time of Censoring</th></tr><tr><td>Lost to follow-up/dropout</td><td>Time of dropout</td></tr><tr><td>Receipt of 2nd dose < 4 wks after 1st dose</td><td>Time of 2nd dose</td></tr><tr><td>No 2nd dose by 6 wks after 1st dose</td><td>6 wks after 1st dose</td></tr></table></td></tr>
<tr><td>Whole Schedules</td><td>4</td><td>RV1</td><td>13 – 16</td><td>4 – 6 wks after 1st</td><td>NA</td><td>Infants missing first RV1 or with 1st dose > 12 wks of age begin in this schedule and continue follow-up until severe RVGE, end of study, or censoring. Infants are censored at the earliest occurrence of any of the following:<table><tr><th>Reason for Censoring</th><th>Time of Censoring</th></tr><tr><td>Lost to follow-up/dropout</td><td>Time of dropout</td></tr><tr><td>No 1st dose by 16 wks of age</td><td>16 wks of age</td></tr><tr><td>Receipt of 2nd dose < 4 wks after 1st dose</td><td>Time of 2nd dose</td></tr><tr><td>No 2nd dose by 6 wks after 1st dose</td><td>6 wks after 1st dose</td></tr></table></td></tr>
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<tr><td>Last Dose</td><td>1</td><td>RV5</td><td>≤ 7</td><td>≤ 11</td><td>≤ 15</td><td>Infants with 1st dose at ≤ 7 wks of age begin in this schedule and continue follow-up until severe RVGE, end of study, or censoring. Infants are censored at the earliest occurrence of any of the following:<table><tr><th>Reason for Censoring</th><th>Time of Censoring</th></tr><tr><td>Lost to follow-up/dropout</td><td>Time of dropout</td></tr><tr><td>No 2nd dose by 11 wks of age</td><td>11 wks of age</td></tr><tr><td>No 3rd dose by 15 wks of age</td><td>15 wks of age</td></tr></table></td></tr>
<tr><td>Last Dose</td><td>2</td><td>RV5</td><td>≤ 12</td><td>≤ 10 wks after 1st</td><td>> 15 & ≤ 10 wks after 2nd</td><td>All infants begin in this schedule and continue follow-up until severe RVGE, end of study, or censoring. Infants are censored at the earliest occurrence of any of the following:<table><tr><th>Reason for Censoring</th><th>Time of Censoring</th></tr><tr><td>Lost to follow-up/dropout</td><td>Time of dropout</td></tr><tr><td>No 2nd dose by 10 wks after 1st dose</td><td>10 wks after 1st dose</td></tr><tr><td>Receipt of 3rd dose ≤ 15 wks of age</td><td>Time of 3rd dose</td></tr><tr><td>No 3rd dose by 10 wks after 2nd dose</td><td>10 wks after 2nd dose</td></tr></table></td></tr>
<tr><td>Last Dose</td><td>1</td><td>RV1</td><td>≤ 11</td><td>≤ 15</td><td>NA</td><td>Infants with 1st dose at ≤ 11 wks of age begin in this schedule and continue follow-up until severe RVGE, end of study, or censoring. Infants are censored at the earliest occurrence of any of the following:<table><tr><th>Reason for Censoring</th><th>Time of Censoring</th></tr><tr><td>Lost to follow-up/dropout</td><td>Time of dropout</td></tr><tr><td>No 2nd dose by 15 wks of age</td><td>15 wks of age</td></tr></table></td></tr>
<tr><td>Last Dose</td><td>2</td><td>RV1</td><td>≤ 16</td><td>> 15 & ≤ 10 wks after 2nd</td><td>NA</td><td>All infants begin in this schedule and continue follow-up until severe RVGE, end of study, or censoring. Infants are censored at the earliest occurrence of any of the following:<table><tr><th>Reason for Censoring</th><th>Time of Censoring</th></tr><tr><td>Lost to follow-up/dropout</td><td>Time of dropout</td></tr><tr><td>No 1st dose by 16 wks of age</td><td>16 wks of age</td></tr><tr><td>Receipt of 2nd dose ≤ 15 wks of age</td><td>Time of 2nd dose</td></tr><tr><td>No 2nd dose by 10 wks after 1st dose</td><td>10 wks after 1st dose</td></tr></table></td></tr>
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Aspect of Timing	Schedule	RV Type	Dose 1	Dose 2	Dose 3	Description of Entry, Follow-up, and Censoring for Schedules*
Interval between Doses	1	RV5	≤ 12	4 wks after 1st	4 wks after 2nd	All infants begin in this schedule and continue follow-up until severe RVGE, end of study, or censoring. Infants are censored at the earliest occurrence of any of the following: Reason for Censoring: Lost to follow-up/dropout — Time of Censoring: Time of dropout Reason for Censoring: Receipt of 2nd dose < 4 wks after 1st dose — Time of Censoring: Time of 2nd dose Reason for Censoring: No 2nd dose by 4 wks after 1st dose — Time of Censoring: 4 wks after 1st dose Reason for Censoring: Receipt of 3rd dose < 4 wks after 2nd dose — Time of Censoring: Time of 3rd dose Reason for Censoring: No 3rd dose by 4 wks after 2nd dose — Time of Censoring: 4 wks after 2nd dose
Interval between Doses	2	RV5	≤ 12	4 wks after 1st	5 wks after 2nd	All infants begin in this schedule and continue follow-up until severe RVGE, end of study, or censoring. Infants are censored at the earliest occurrence of any of the following: Reason for Censoring: Lost to follow-up/dropout — Time of Censoring: Time of dropout Reason for Censoring: Receipt of 2nd dose < 4 wks after 1st dose — Time of Censoring: Time of 2nd dose Reason for Censoring: No 2nd dose by 5 wks after 1st dose — Time of Censoring: 5 wks after 1st dose Reason for Censoring: Receipt of 3rd dose < 4 wks after 2nd dose — Time of Censoring: Time of 3rd dose Reason for Censoring: Receipt of 3rd dose at 4 wks after 2nd dose when 2nd dose received 4 wks after 1st dose — Time of Censoring: Time of 3rd dose Reason for Censoring: No 3rd dose at by 5 wks after 2nd dose — Time of Censoring: 5 wks after 2nd dose
Interval between Doses	2	RV5	≤ 12	5 wks after 1st	4 wks after 2nd	
Interval between Doses	2	RV5	≤ 12	5 wks after 1st	5 wks after 2nd	
Interval between Doses	3	RV5	≤ 12	4 wks after 1st	6 wks after 2nd	All infants begin in this schedule and continue follow-up until severe RVGE, end of study, or censoring. Infants are censored at the earliest occurrence of any of the following: Reason for Censoring: Lost to follow-up/dropout — Time of Censoring: Time of dropout Reason for Censoring: Receipt of 2nd dose < 4 wks after 1st dose — Time of Censoring: Time of 2nd dose Reason for Censoring: No 2nd dose by 6 wks after 1st dose — Time of Censoring: 6 wks after 1st dose Reason for Censoring: Receipt of 3rd dose < 4 wks after 2nd dose — Time of Censoring: Time of 3rd dose Reason for Censoring: Receipt of 3rd dose at 4 wks after 2nd dose when 2nd dose received 4 or 5 wks after 1st dose — Time of Censoring: Time of 3rd dose Reason for Censoring: Receipt of 3rd dose at 5 wks after 2nd dose when 2nd dose received 4 or 5 wks after 1st dose — Time of Censoring: Time of 3rd dose Reason for Censoring: No 3rd dose by 6 wks after 2nd dose — Time of Censoring: 6 wks after 2nd dose
Interval between Doses	3	RV5	≤ 12	5 wks after 1st	6 wks after 2nd	
Interval between Doses	3	RV5	≤ 12	6 wks after 1st	4 wks after 2nd	
Interval between Doses	3	RV5	≤ 12	6 wks after 1st	5 wks after 2nd	
Interval between Doses	3	RV5	≤ 12	6 wks after 1st	6 wks after 2nd	
Interval between Doses	1	RV1	≤ 16	4 wks after 1st	NA	All infants begin in this schedule and continue follow-up until severe RVGE, end of study, or censoring. Infants are censored at the earliest occurrence of any of the following: Reason for Censoring: Lost to follow-up/dropout — Time of Censoring: Time of dropout Reason for Censoring: No 1st dose by 16 wks of age — Time of Censoring: 16 wks of age Reason for Censoring: Receipt of 2nd dose < 4 wks after 1st dose — Time of Censoring: Time of 2nd dose Reason for Censoring: No 2nd dose by 4 wks after 1st dose — Time of Censoring: 4 wks after 1st dose
Interval between Doses	2	RV1	≤ 16	5 wks after 1st	NA	All infants begin in this schedule and continue follow-up until severe RVGE, end of study, or censoring. Infants are censored at the earliest occurrence of any of the following: Reason for Censoring: Lost to follow-up/dropout — Time of Censoring: Time of dropout Reason for Censoring: No 1st dose by 16 wks of age — Time of Censoring: 16 wks of age Reason for Censoring: Receipt of 2nd dose < 5 wks after 1st dose — Time of Censoring: Time of 2nd dose Reason for Censoring: No 2nd dose by 5 wks after 1st dose — Time of Censoring: 5 wks after 1st dose
Interval between Doses	3	RV1	≤ 16	6 wks after 1st	NA	All infants begin in this schedule and continue follow-up until severe RVGE, end of study, or censoring. Infants are censored at the earliest occurrence of any of the following: Reason for Censoring: Lost to follow-up/dropout — Time of Censoring: Time of dropout Reason for Censoring: No 1st dose by 16 wks of age — Time of Censoring: 16 wks of age Reason for Censoring: Receipt of 2nd dose < 6 wks after 1st dose — Time of Censoring: Time of 2nd dose Reason for Censoring: No 2nd dose by 6 wks after 1st dose — Time of Censoring: 6 wks after 1st dose

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<tr><td>Number of Doses ≥ 10 Weeks of Age</td><td>0</td><td>RV5</td><td>< 10</td><td>< 10</td><td>< 10</td><td rowspan="3">All Infants with 1st dose at < 10 wks of age begin in this schedule and continue follow-up until severe RVGE, end of study, or censoring. Infants are censored at the earliest occurrence of any of the following:<table><tr><th>Reason for Censoring</th><th>Time of Censoring</th></tr><tr><td>Lost to follow-up/dropout</td><td>Time of dropout</td></tr><tr><td>Receipt of 2nd dose ≥ 10 wks of age</td><td>Time of 2nd dose</td></tr><tr><td>Receipt of 3rd dose ≥ 10 wks of age</td><td>Time of 3rd dose</td></tr></table></td></tr>
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</table>

NA, not applicable, wks, weeks

*Censoring for those not receiving a first dose of vaccine is not described for the RV5 trial; all infants in the RV5 trial received at least 1 dose of vaccine, but some infants in the RV1 trial did not receive a dose of vaccine.

Appendix Table 2. Example of entry, follow-up, and censoring for each schedule for each aspect of timing for three hypothetical infants in the RV1 and RV5.

Aspect of Timing	Infant	Type	Dose Number			Schedule	Weeks of age
			1	2	3		12 13 14 15 16 17 18 19 20
Whole Schedules	A	RV5	5	10	18	3 – 6 weeks with 4 – 6 week intervals 7 – 9 weeks with 4 – 6 week intervals 10 – 12 weeks with 4 – 6 week intervals	
	B	RV5	10	16	20	3 – 6 weeks with 4 – 6 week intervals 7 – 9 weeks with 4 – 6 week intervals 10 – 12 weeks with 4 – 6 week intervals	->
	C	RV5	4	8		3 – 6 weeks with 4 – 6 week intervals 7 – 9 weeks with 4 – 6 week intervals 10 – 12 weeks with 4 – 6 week intervals	
	D	RV1	10	15		10 – 12 weeks with 4 – 6 week intervals 13 – 16 weeks with 4 – 6 week intervals	->
	E	RV1	10			10 – 12 weeks with 4 – 6 week intervals 13 – 16 weeks with 4 – 6 week intervals	
	F	RV1	14	18		10 – 12 weeks with 4 – 6 week intervals 13 – 16 weeks with 4 – 6 week intervals	->
First Dose	A	RV5	5	10	18	1st dose < 6 weeks 1st dose ≥ 6 weeks	->
	B	RV5	10	16	20	1st dose < 6 weeks 1st dose ≥ 6 weeks	->
	C	RV5	4	8		1st dose < 6 weeks 1st dose ≥ 6 weeks	
Last Dose	A	RV5	5	10	18	3rd dose ≤ 15 weeks 3rd dose > 15 weeks	->
	B	RV5	10	16	20	3rd dose ≤ 15 weeks 3rd dose > 15 weeks	->
	C	RV5	4	8		3rd dose ≤ 15 weeks 3rd dose > 15 weeks	
	D	RV1	10	15		2nd dose ≤ 15 weeks 2nd dose > 15 weeks	->
	E	RV1	10			2nd dose ≤ 15 weeks 2nd dose > 15 weeks	
	F	RV1	14	18		2nd dose ≤ 15 weeks 2nd dose > 15 weeks	->
Interval between Doses	A	RV5	5	10	18	Both 4 weeks ≥ 1 of 5 weeks ≥ 1 of 6 weeks	
	B	RV5	10	16	20	Both 4 weeks ≥ 1 of 5 weeks ≥ 1 of 6 weeks	->
	C	RV5	4	8		Both 4 weeks ≥ 1 of 5 weeks ≥ 1 of 6 weeks	
	D	RV1	10	15		4 weeks 5 weeks 6 weeks	->
	E	RV1	10			4 weeks 5 weeks 6 weeks	
	F	RV1	14	18		4 weeks 5 weeks 6 weeks	->
Number of Doses ≥ 10 Weeks of Age	A	RV5	5	10	18	1 dose at ≥ 10 weeks 2 doses at ≥ 10 weeks 3 doses at ≥ 10 weeks	->
	B	RV5	10	16	20	1 dose at ≥ 10 weeks 2 doses at ≥ 10 weeks 3 doses at ≥ 10 weeks	->
	C	RV5	4	8		1 dose at ≥ 10 weeks 2 doses at ≥ 10 weeks 3 doses at ≥ 10 weeks	Until 32

Appendix Table 3. Predictors of first severe RVGE episode in cohort 2 with and without Kenya and Mali included.

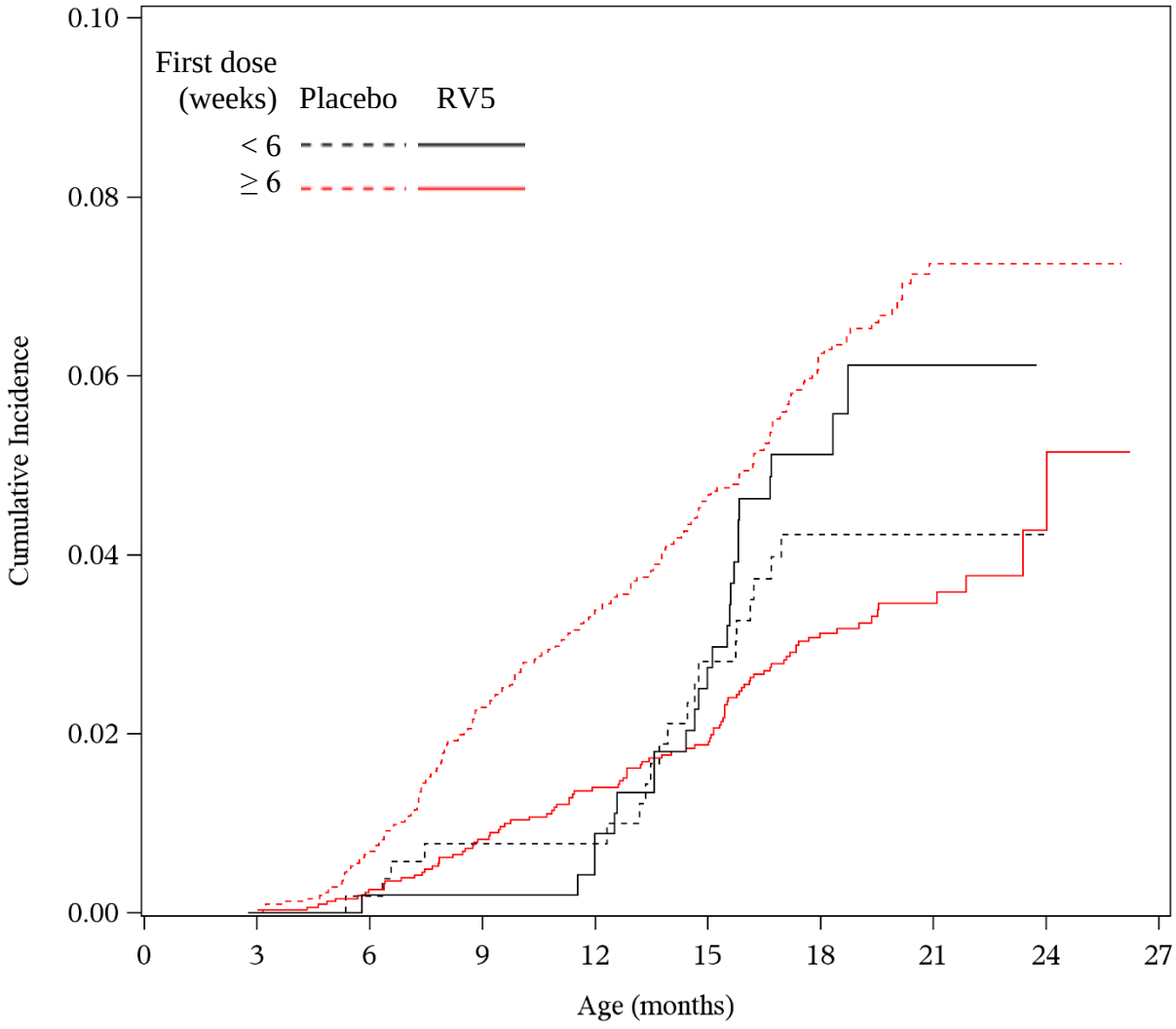
Characteristic	Cohort 2[*] N = 3,746 Events = 205		Cohort 2 without Kenya & Mali N = 2,120 Events = 129	
	Unadjusted[†] HR (95% CI)	Adjusted[†] HR (95% CI)	Unadjusted[†] HR (95% CI)	Adjusted[†] HR (95% CI)
Demographic				
Female Sex vs. Male (ref)	0.86 (0.65, 1.13)	0.86 (0.65, 1.13)	0.68 (0.48, 0.97)	0.69 (0.49, 0.98)
Exclusively Breastfed vs. Not (ref)	0.75 (0.48, 1.15)	0.75 (0.48, 1.16)	0.95 (0.50, 1.79)	0.99 (0.52, 1.87)
Growth Status				
Stunted vs. Not (ref)	--	--	--	--
Underweight vs. Not (ref)	0.82 (0.52, 1.30)	0.81 (0.51, 1.29)	0.98 (0.59, 1.63)	0.95 (0.57, 1.58)
Wasting vs. Not (ref)	--	--	--	--
Current/Prior Infection vs. None (ref)	0.99 (0.64, 1.52)	0.89 (0.56, 1.40)	1.11 (0.70, 1.77)	0.98 (0.60, 1.60)
Current/Prior Antibiotic[‖] Use vs. None (ref)	1.40 (0.81, 2.41)	1.41 (0.80, 2.51)	1.71 (0.97, 3.03)	1.66 (0.92, 3.01)
Routine Vaccines				
BCG[§]; No Dose vs. ≥ 1 Dose (ref)	0.63 (0.34, 1.17)	0.65 (0.35, 1.21)	0.67 (0.31, 1.42)	0.64 (0.29, 1.38)
DTP-HB/HIB[¶]; No Dose vs. ≥ 1 Dose (ref)	1.00 (0.71, 1.42)	1.08 (0.71, 1.66)	1.18 (0.72, 1.93)	1.11 (0.65, 1.90)
OPV; ≤ 1 Dose vs. ≥ 2 Doses (ref)	0.94 (0.70, 1.25)	0.95 (0.66, 1.36)	1.15 (0.80, 1.67)	1.17 (0.78, 1.76)

[*] Six infants from cohort 2 entered and exited the study before 6 weeks of age
[†] Adjusted for country
[‡] < 10 events in each strata
[§] Excluding topical antibiotics
[‖] Administered prior to enrollment
[¶] Or DTaP & HBV, which were the standard vaccines given in Asian countries

There were differential patterns in lost to follow-up within some strata of factors in cohort 1 (Appendix Table 4). There were more infants lost to follow-up who were stunted versus not and those with ≤ 1 dose of OPV versus ≥ 2 doses at enrollment.

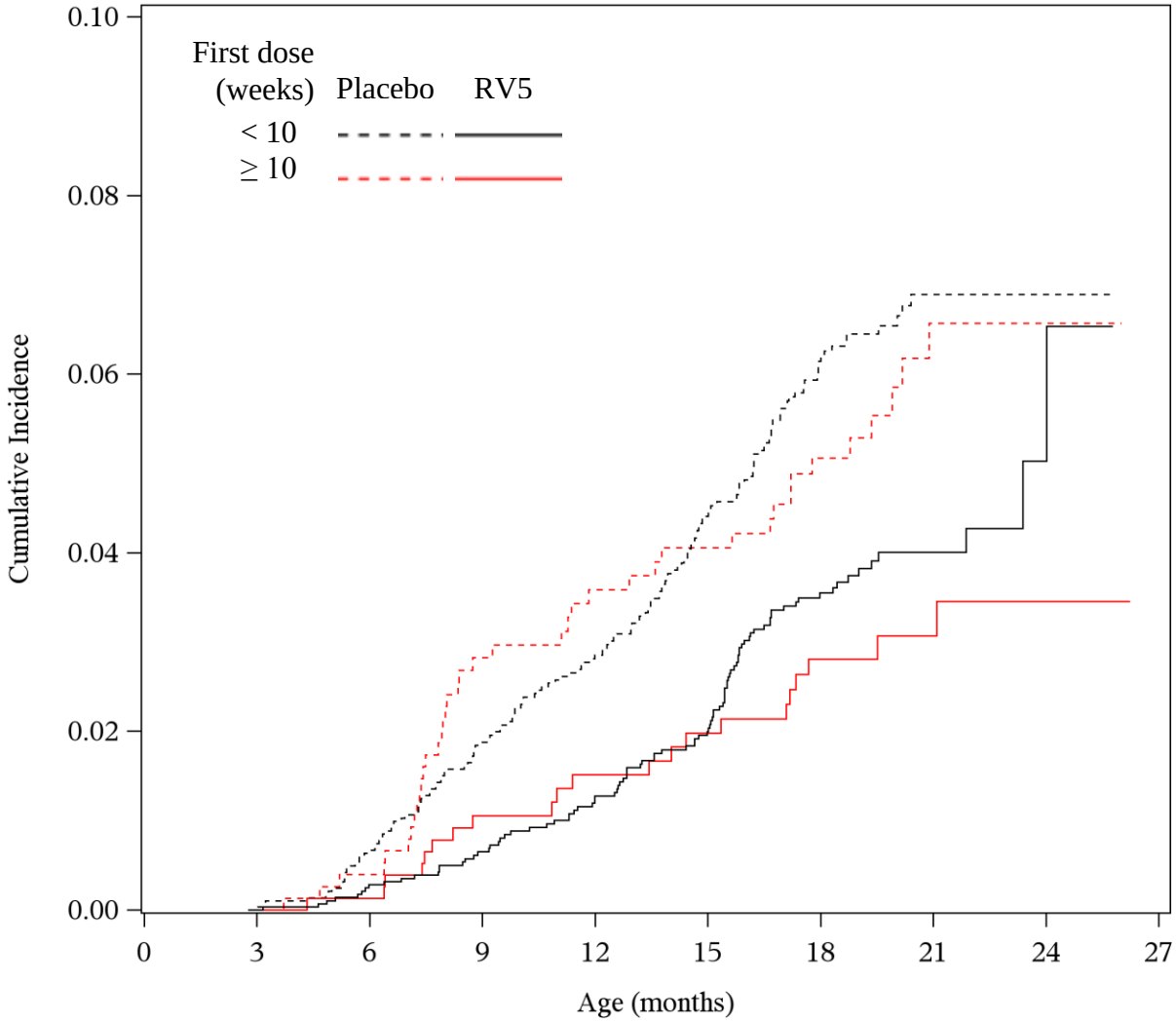
Appendix Table 4. Distribution of characteristics by follow-up status.

Characteristic	Infants with Complete Follow-Up, N (%)	Infants Lost to Follow-up, N (%)
Female Sex (vs. Male)	655 (49)	128 (46)
Growth Status		
Stunted (vs. Not)	279 (21)	77 (27)
Underweight (vs. Not)	57 (4)	16 (6)
Wasting (vs. Not)	54 (4)	13 (5)
Current/Prior Infection (vs. None)	48 (4)	19 (7)
Current/Prior Antibiotic Use (vs. None)	117 (9)	31 (11)
Routine Vaccination		
No BCG (vs. ≥ 1 Dose)	57 (4)	21 (7)
No DTP-HB/HIB (vs. ≥ 1 Dose)	1,330 (100)	280 (100)
≤ 1 Dose OPV (vs. ≥ 2 Doses)	138 (10)	52 (19)



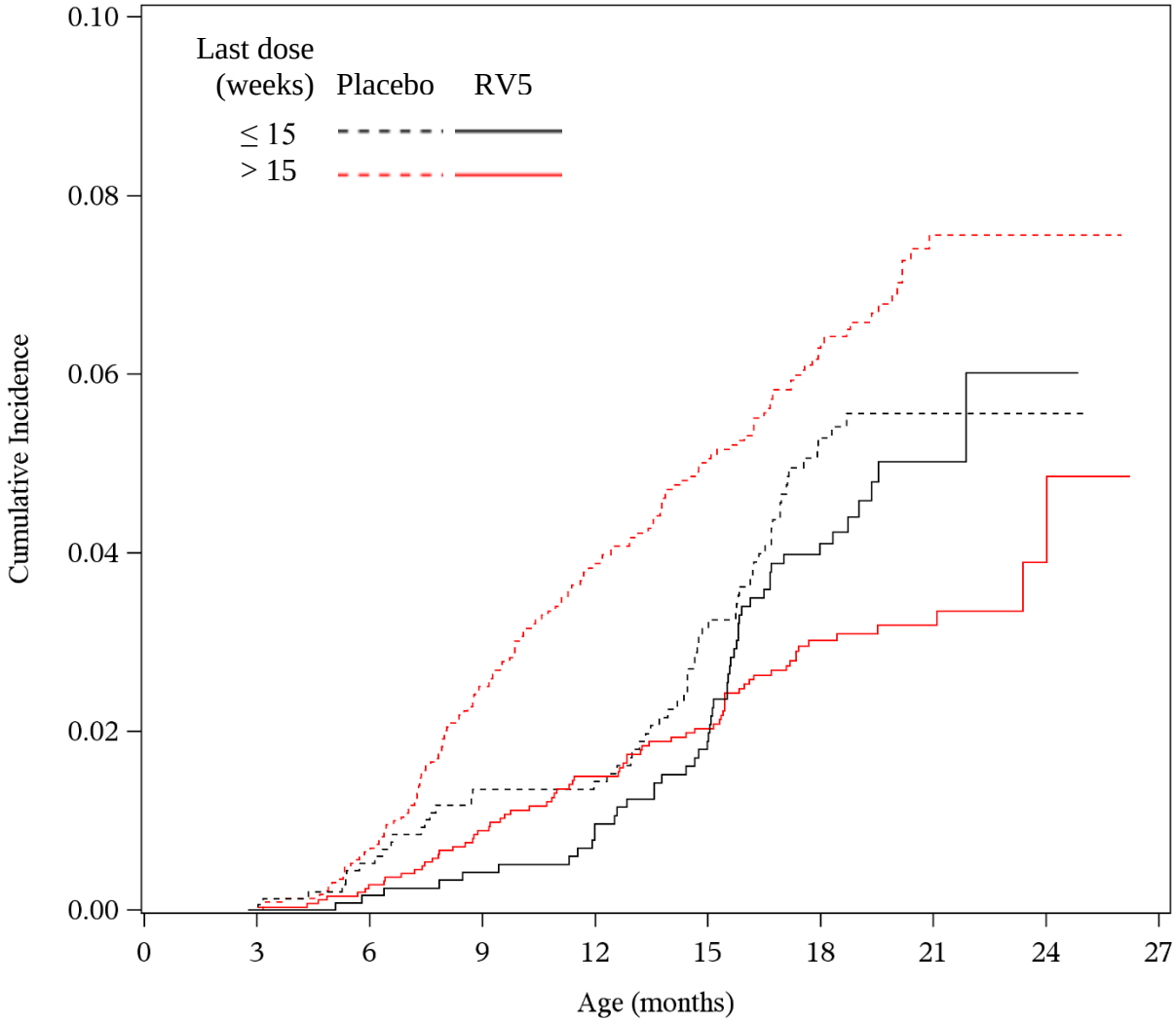
RV5	< 6	519	506	432	255	0
	≥ 6	3,147	3,063	2,700	2,055	109
Placebo	< 6	548	534	445	274	0
	≥ 6	3,127	3,011	2,640	1,993	114

Appendix Figure 1. Cumulative incidence of severe RVGE by timing of first dose (< 6 weeks vs. ≥ 6 weeks) in the placebo and RV5 vaccinated groups. Number at risk at 12 weeks, 6 months, 12 months, 18 months, and 24 months of age for each group is below the x-axis.



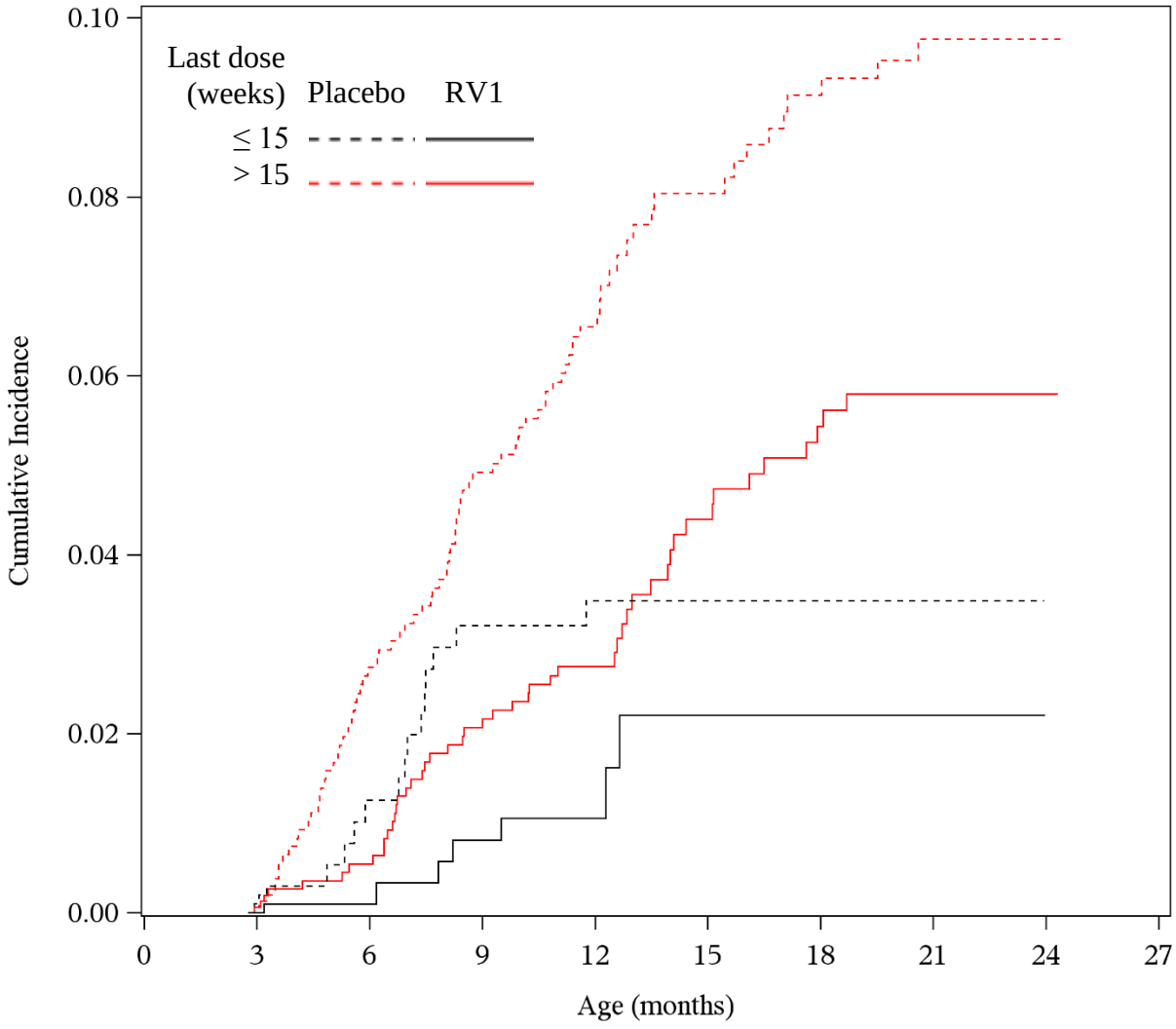
RV5	< 10	2,882	2,805	2,492	1,754	63
	≥ 10	784	764	640	556	46
Placebo	<10	2,895	2,798	2,465	1,733	74
	≥ 10	780	747	620	534	40

Appendix Figure 2. Cumulative incidence of severe RVGE by timing of first dose (< 10 weeks vs. ≥ 10 weeks) in the placebo and RV5 vaccinated groups. Number at risk at 12 weeks, 6 months, 12 months, 18 months, and 24 months of age for each group is below the x-axis.



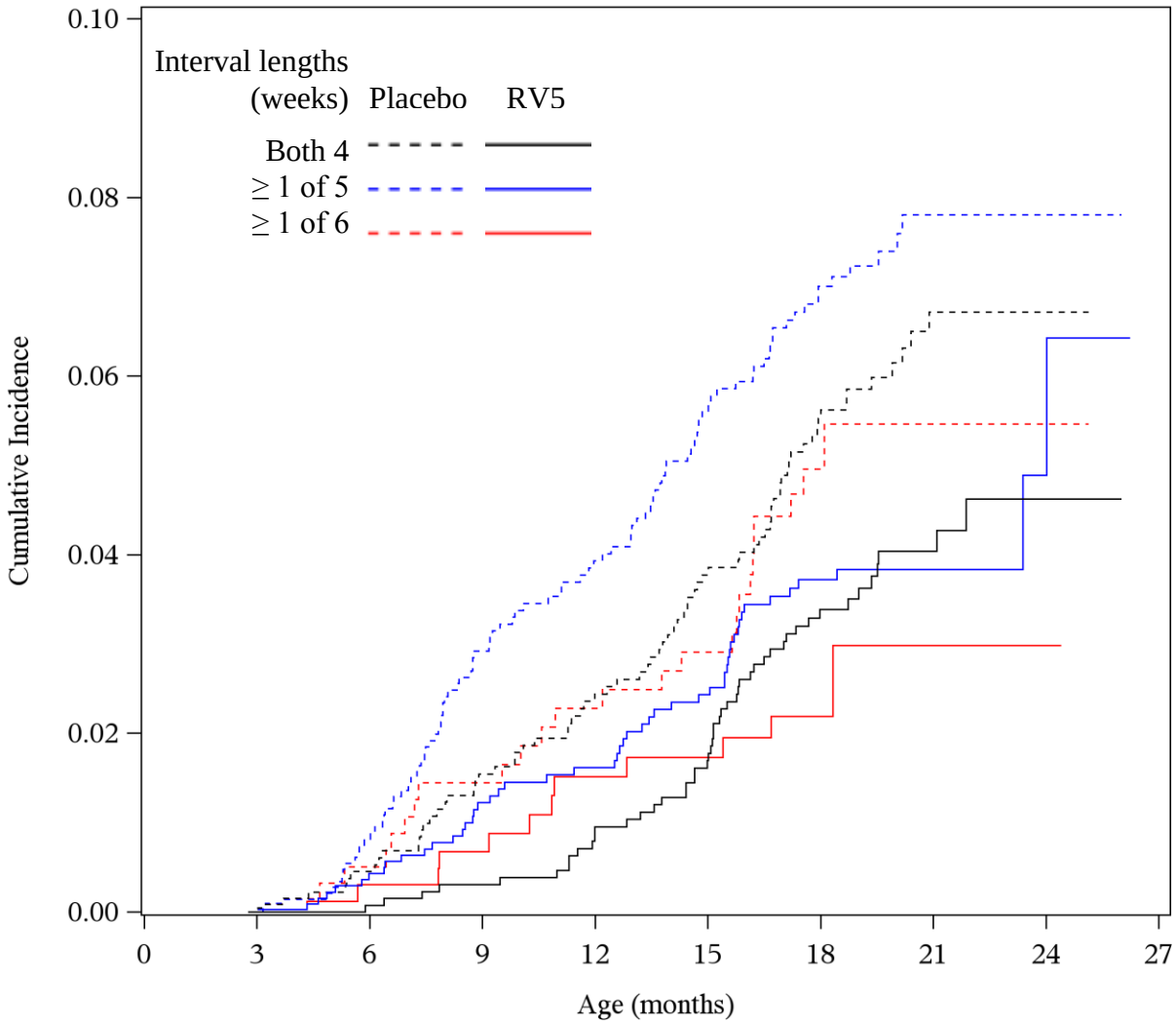
RV5	≤ 15	1,527	1,216	1,087	778	9
	> 15	3,520	2,353	2,045	1,532	100
Placebo	≤ 15	1,596	1,253	1,104	803	12
	> 15	3,534	2,292	1,981	1,464	102

Appendix Figure 3. Cumulative incidence of severe RVGE by timing of last dose (≤ 15 weeks vs. > 15 weeks) in the placebo and RV5 vaccinated groups. Number at risk at 12 weeks, 6 months, 12 months, 18 months, and 24 months of age for each group is below the x-axis.



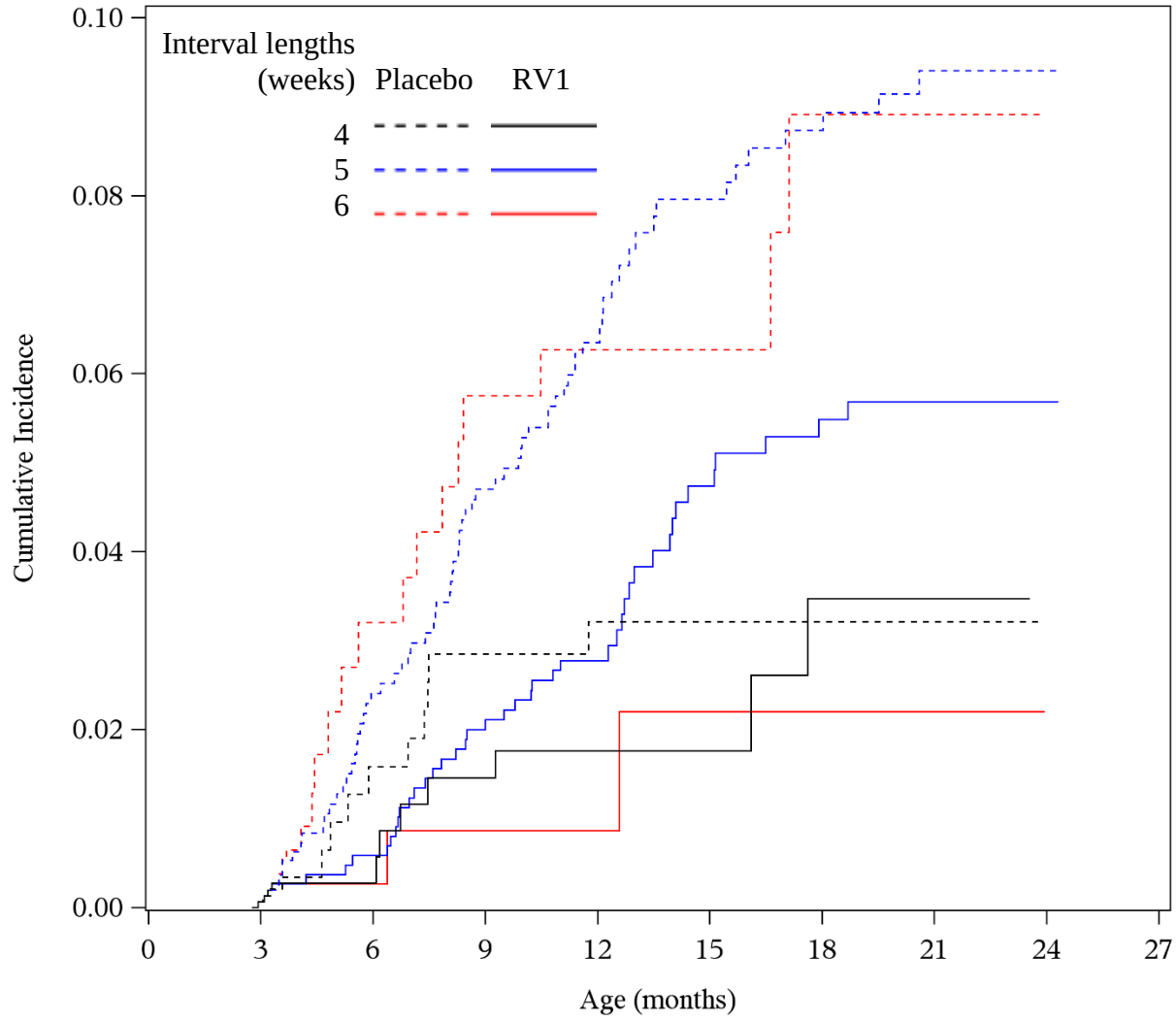
RV5	≤ 15	1,048	420	218	149	0
	> 15	1,559	1,056	702	531	8
Placebo	≤ 15	998	408	220	152	0
	> 15	1,553	996	636	481	7

Appendix Figure 4. Cumulative incidence of severe RVGE by timing of last dose (≤ 15 weeks vs. > 15 weeks) in the placebo and RV1 vaccinated groups. Number at risk at 12 weeks, 6 months, 12 months, 18 months, and 24 months of age for each group is below the x-axis.



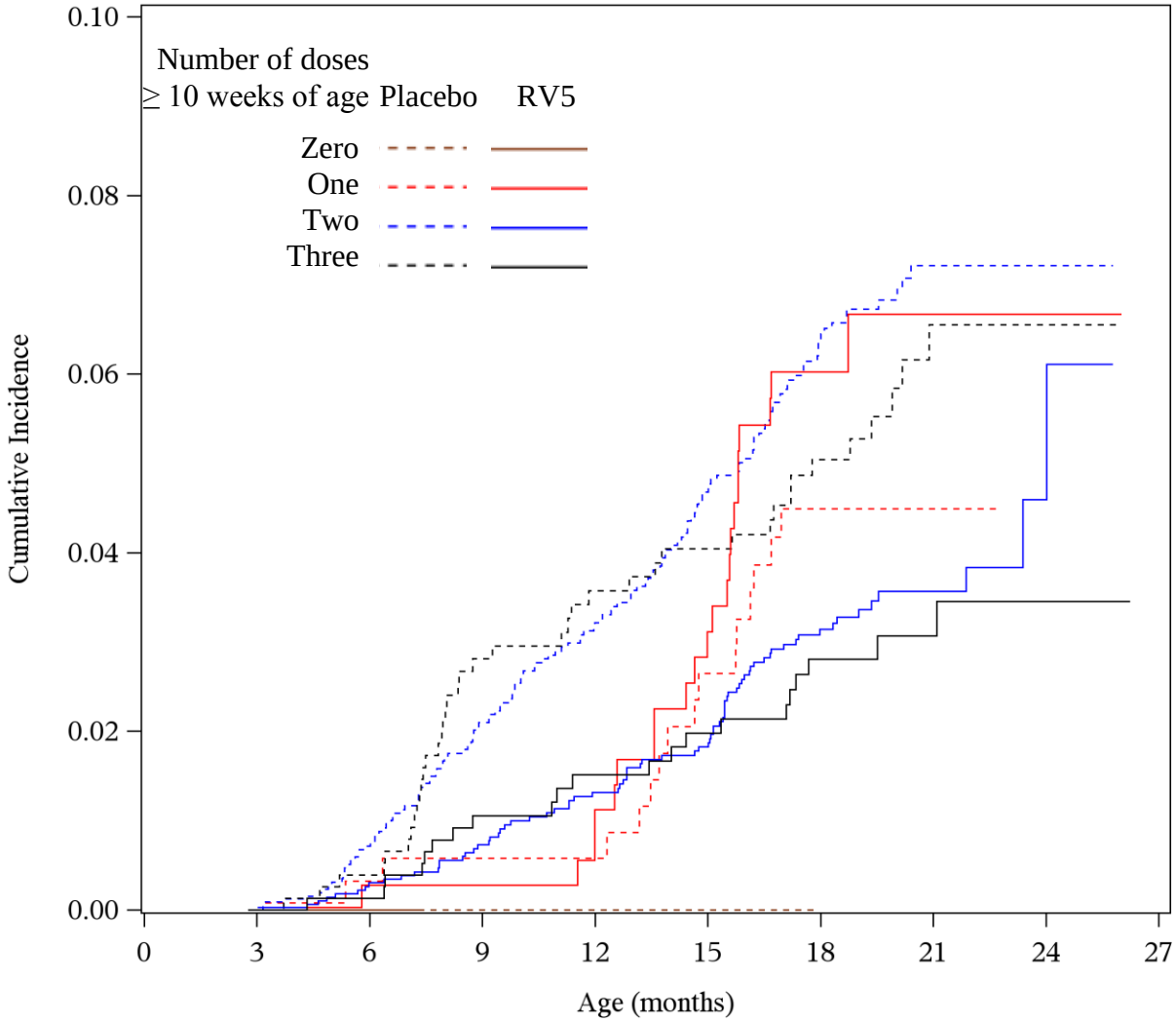
RV5	Both 4	2,589	1,310	1,219	1,003	39
	≥ 1 of 5	3,345	1,453	1,215	939	62
	≥ 1 of 6	3,474	546	460	228	3
Placebo	Both 4	2,531	1,298	1,187	968	41
	≥ 1 of 5	3,343	1,464	1,211	939	64
	≥ 1 of 6	3,478	536	465	242	7

Appendix Figure 5. Cumulative incidence of severe RVGE by length of interval between doses (both intervals 4 weeks vs. ≥ 1 interval of 5 weeks vs. ≥ 1 interval of 6 weeks) in the placebo and RV5 vaccinated groups. Number at risk at 12 weeks, 6 months, 12 months, 18 months, and 24 months of age for each group is below the x-axis.



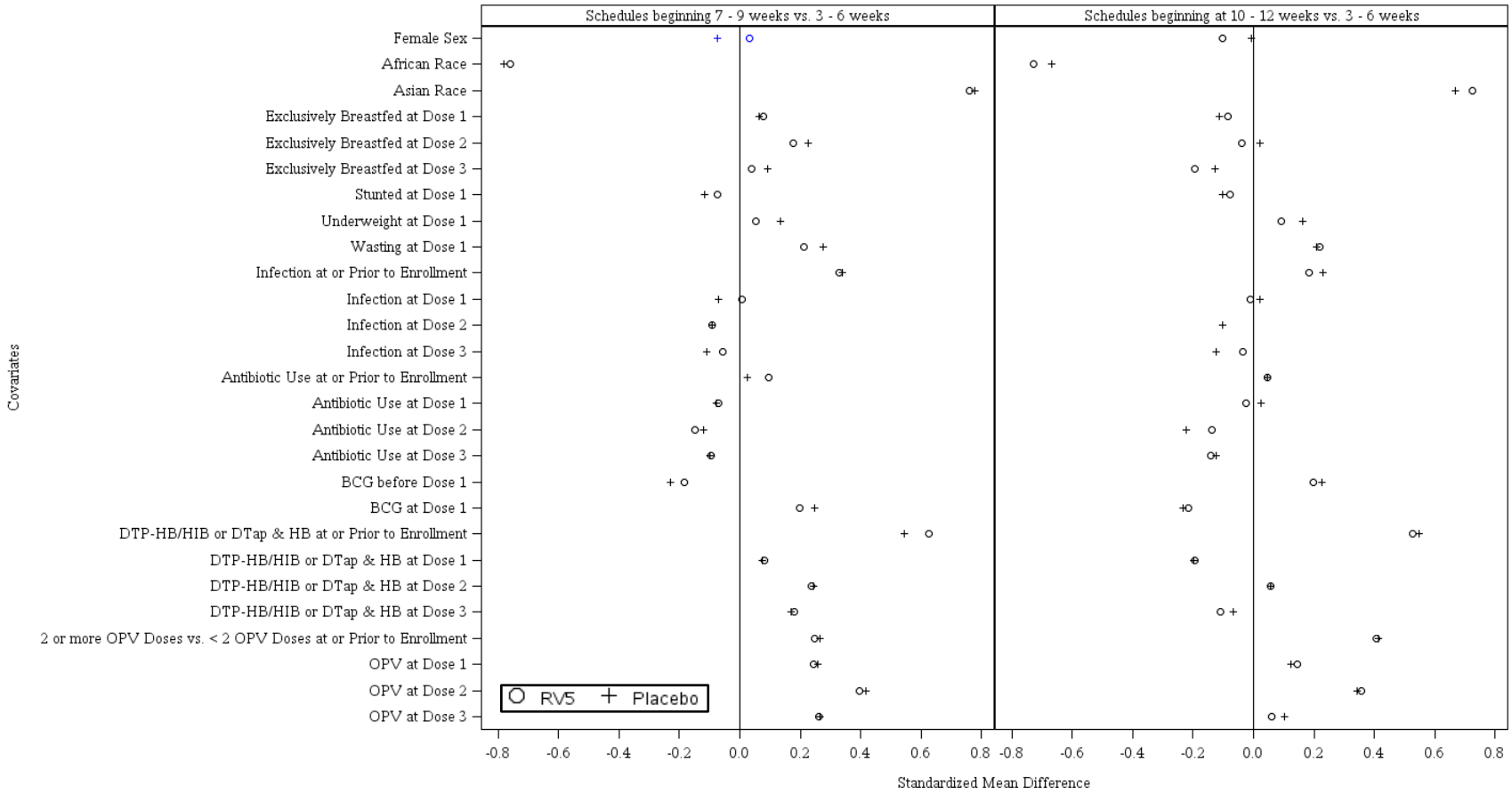
RV5	4	1,559	337	169	112	0
	5	1,559	926	631	484	8
	6	1,559	167	93	64	0
Placebo	4	1,553	313	154	96	0
	5	1,553	862	578	455	7
	6	1,553	190	102	67	0

Appendix Figure 6. Cumulative incidence of severe RVGE by length of interval between doses (4 weeks vs. 5 weeks vs. 6 weeks) in the placebo and RV1 vaccinated groups. Number at risk at 12 weeks, 6 months, 12 months, 18 months, and 24 months of age for each group is below the x-axis.

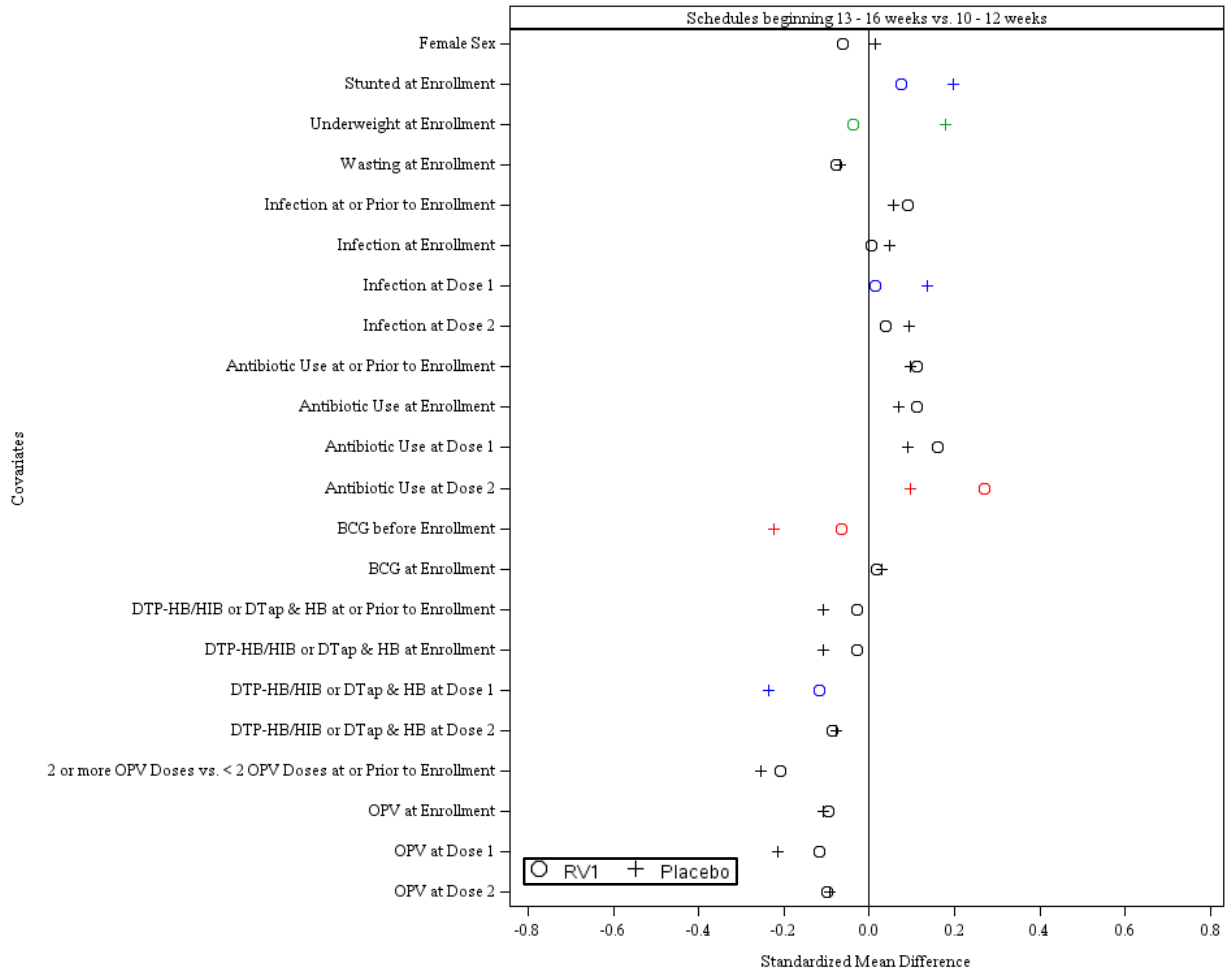


RV5	Zero	1,030	2	0	0	0
	One	3,666	397	351	200	2
	Two	3,271	2,432	2,152	1,559	63
	Three	784	765	640	556	46
Placebo	Zero	1,029	6	2	0	0
	One	3,675	405	340	208	0
	Two	3,279	2,434	2,140	1,532	74
	Three	780	751	621	535	40

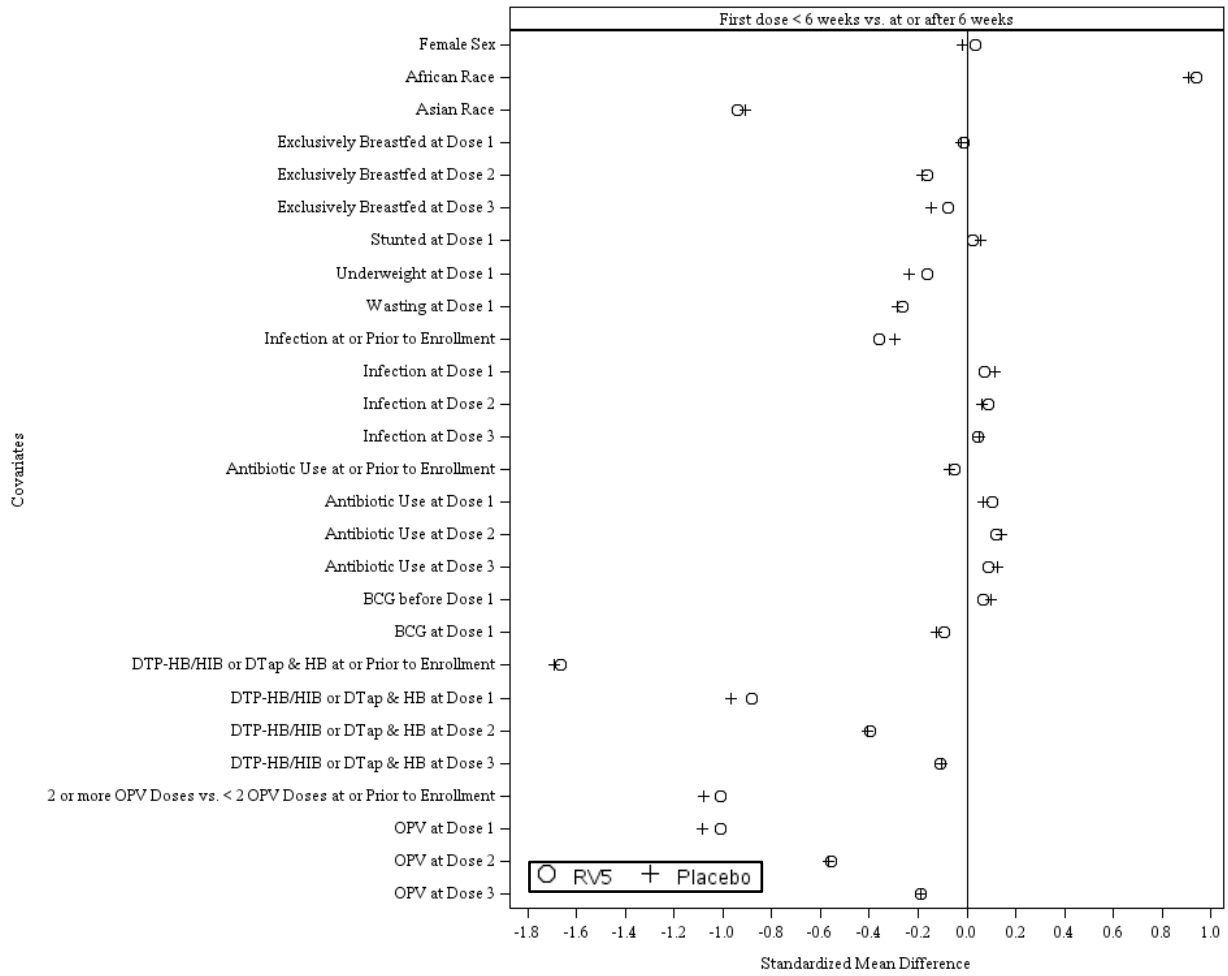
Appendix Figure 7. Cumulative incidence of severe RVGE by number of doses received ≥ 10 weeks age (zero vs. one vs. two vs. three) in the placebo and RV5 vaccinated groups. Number at risk at 12 weeks, 6 months, 12 months, 18 months, and 24 months of age for each group is below the x-axis.



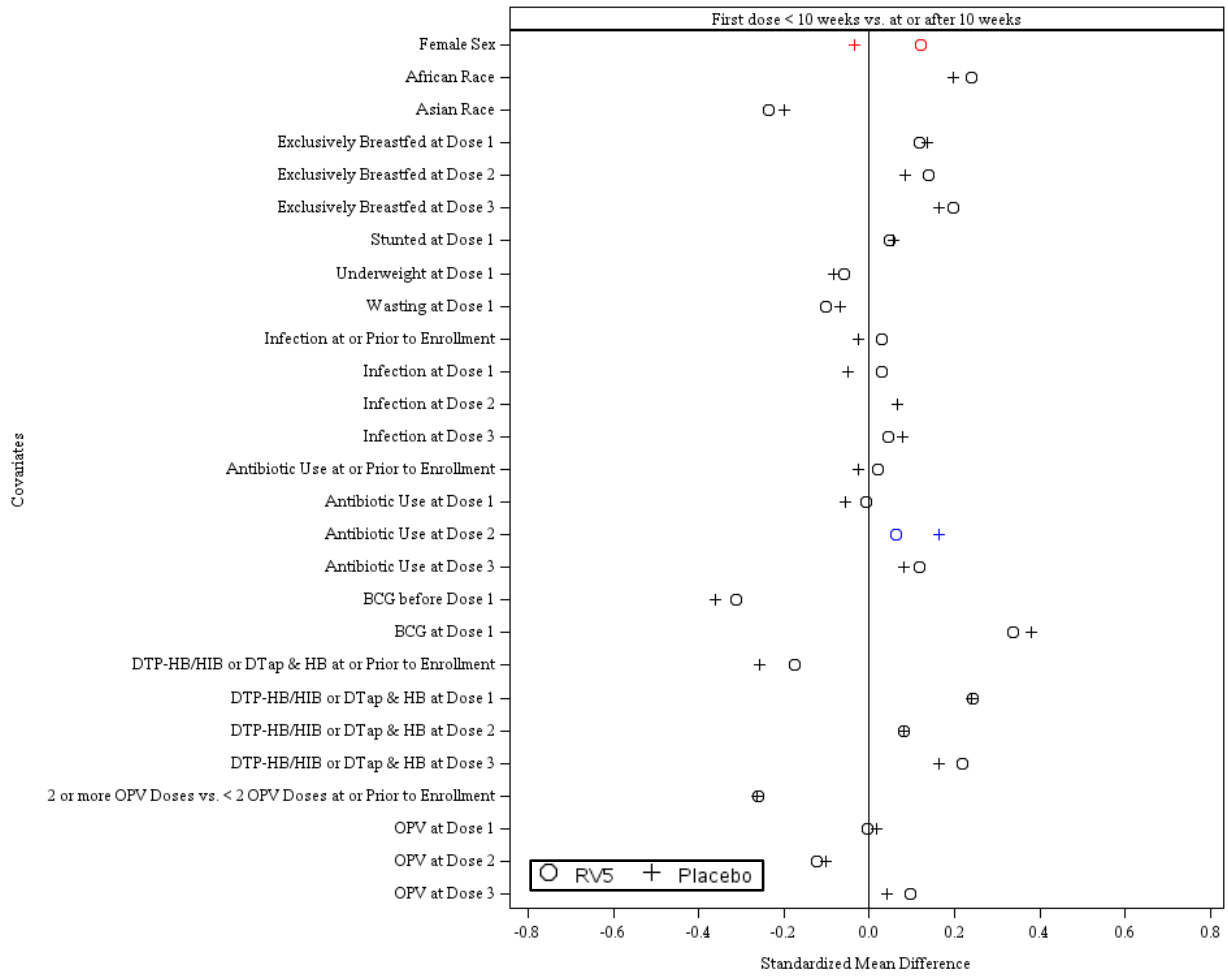
Appendix Figure 8. SMDs in the distribution of covariates between timing of first dose with 4 – 6 week intervals between subsequent doses. Black symbols represent a difference of < 10% between the SMDs of the RV5 and placebo groups. Blue symbols represent a difference of 10% to < 15% between the SMDs of the RV5 and placebo groups.



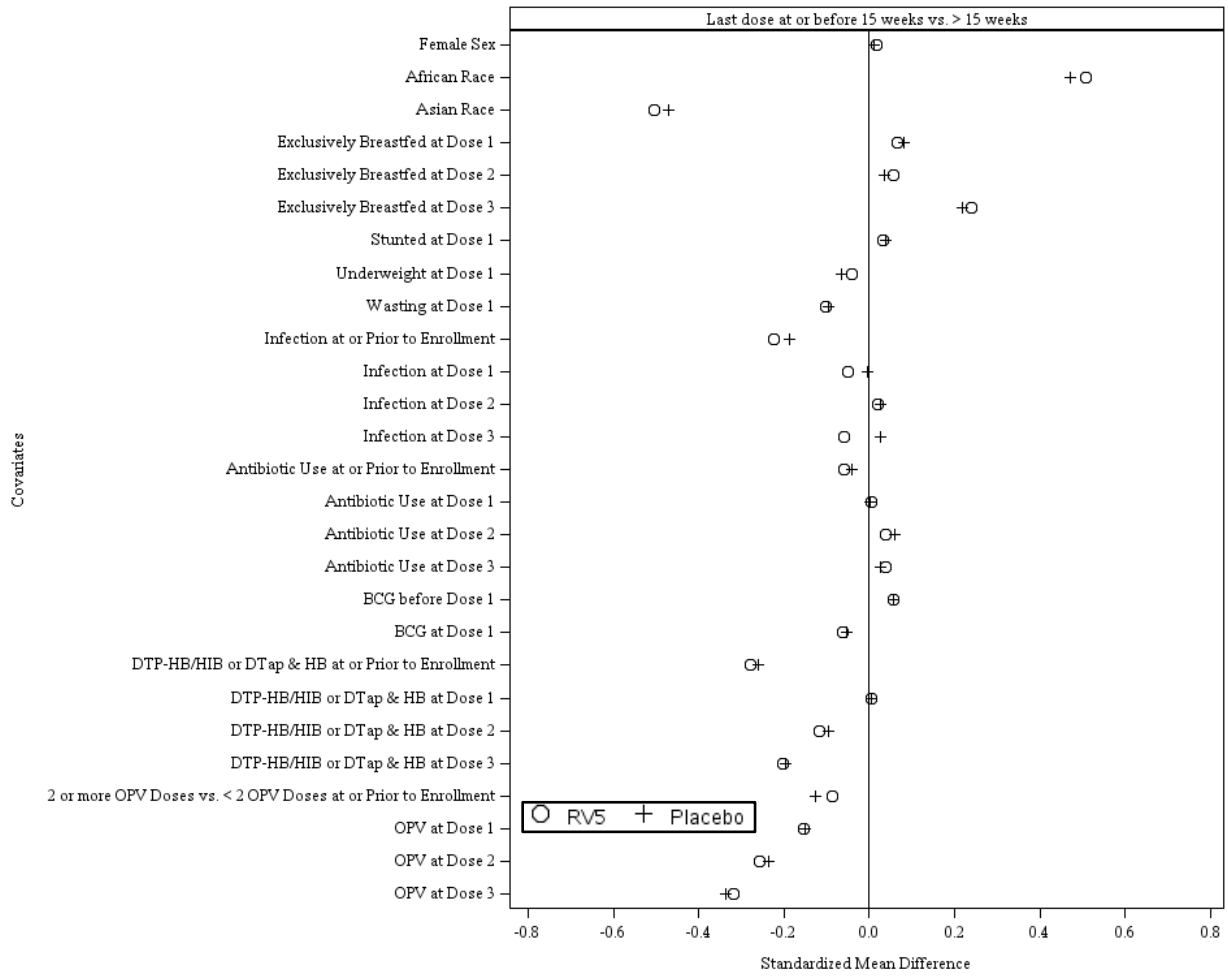
Appendix Figure 9. SMDs in the distribution of covariates between timing of first dose with a 4 – 6 week interval between doses. Black symbols represent a difference of < 10% between the SMDs of the RV1 and placebo groups. Blue symbols represent a difference of 10% to < 15% between the SMDs of the RV1 and placebo groups. Red symbols represent a difference of 15% to < 20% between the SMDs of the RV1 and placebo groups. Green symbols represent a difference of 20% to < 25% between the SMDs of the RV1 and placebo groups.



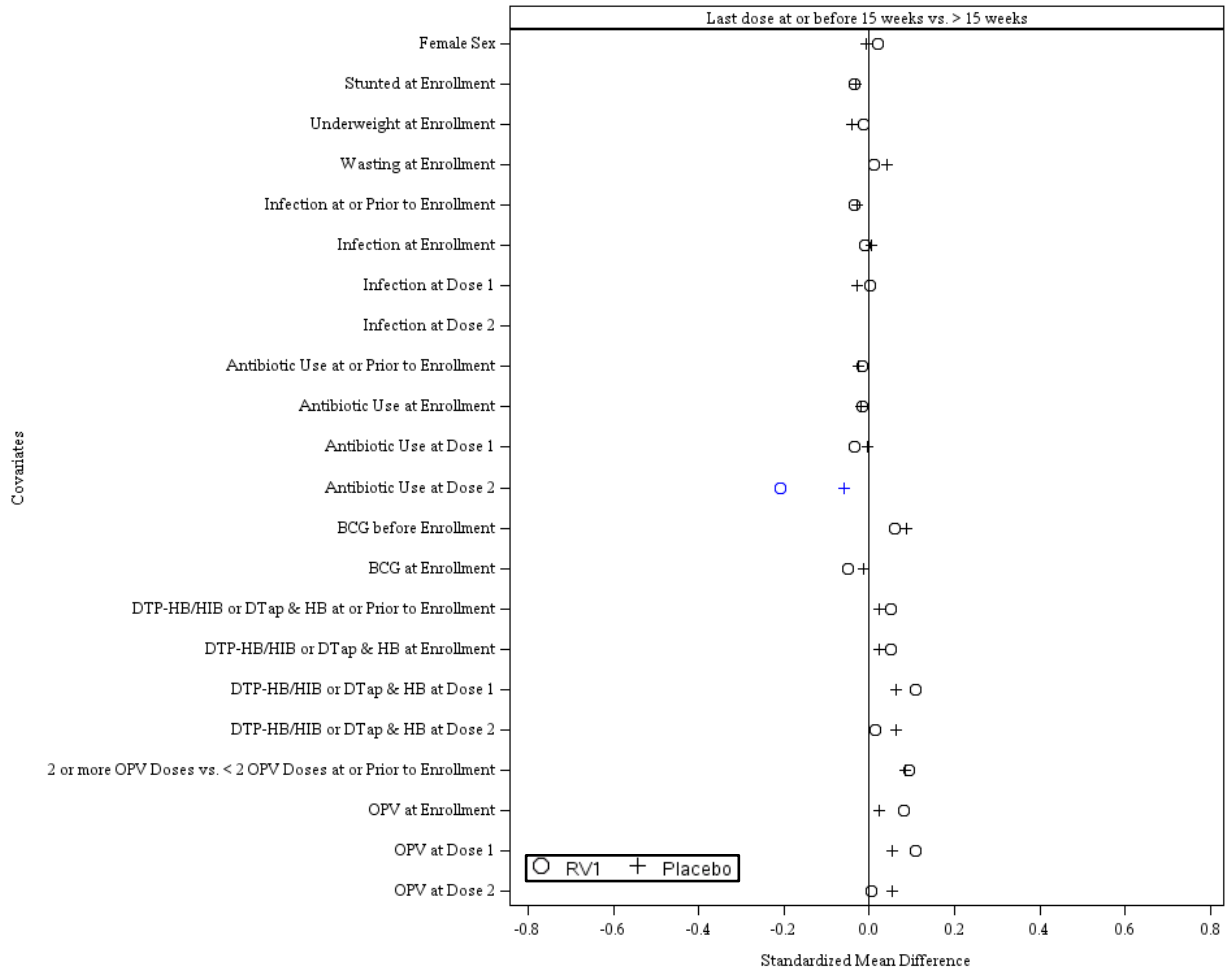
Appendix Figure 10. SMDs in the distribution of covariates between those receiving their first dose at < 6 weeks compared to ≥ 6 weeks. Black symbols represent a difference of < 10% between the SMDs of the RV5 and placebo groups.



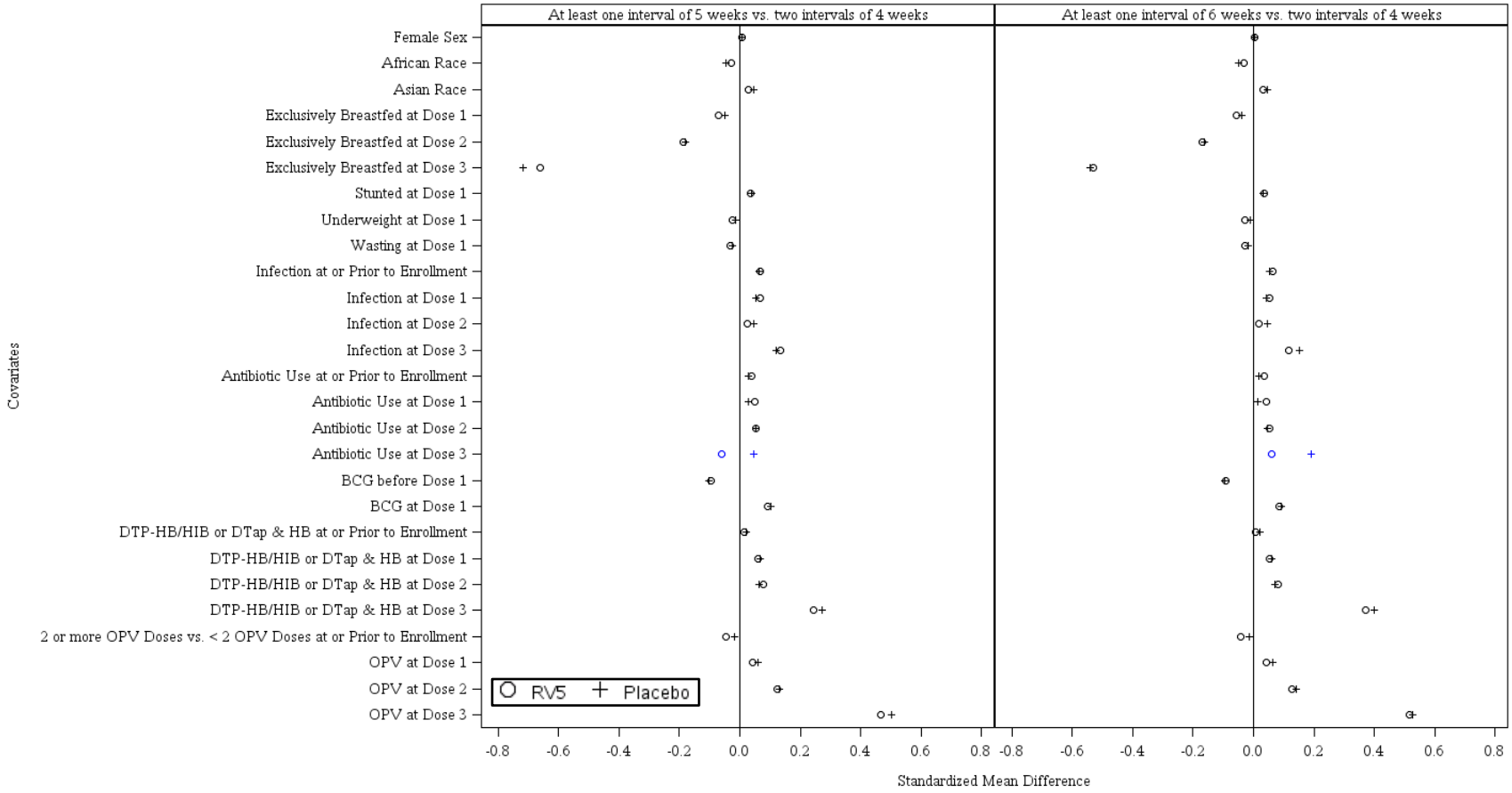
Appendix Figure 11. SMDs in the distribution of covariates between those receiving their first dose at < 10 weeks compared to ≥ 10 weeks. Black symbols represent a difference of < 10% between the SMDs of the RV5 and placebo groups. Blue symbols represent a difference of 10% to < 15% between the SMDs of the RV5 and placebo groups. Red symbols represent a difference of 15% to < 20% between the SMDs of the RV5 and placebo groups.



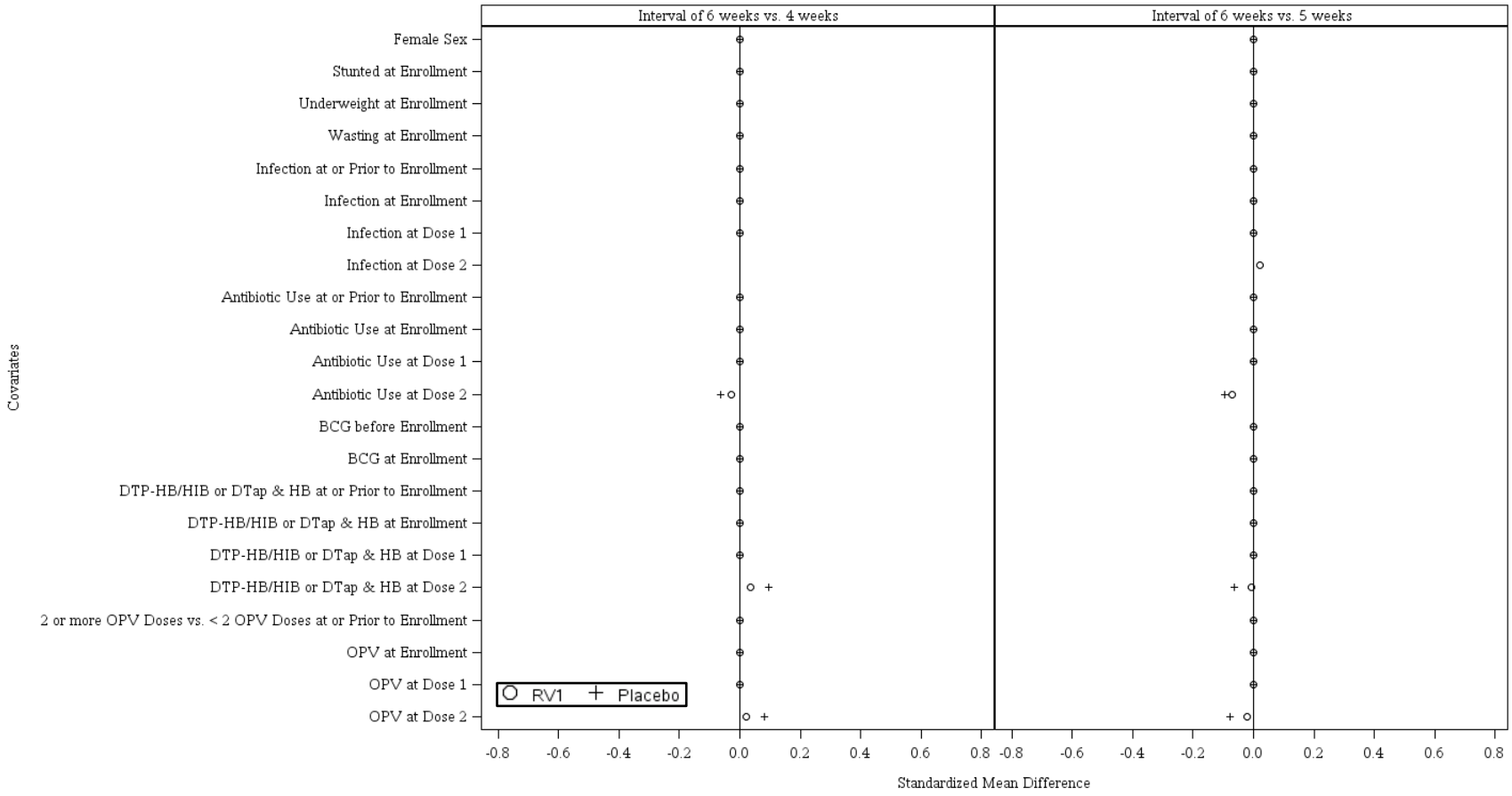
Appendix Figure 12. SMDs in the distribution of covariates between those receiving their last dose at ≤ 15 weeks compared to > 15 weeks. Black symbols represent a difference of < 10% between the SMDs of the RV5 and placebo groups.



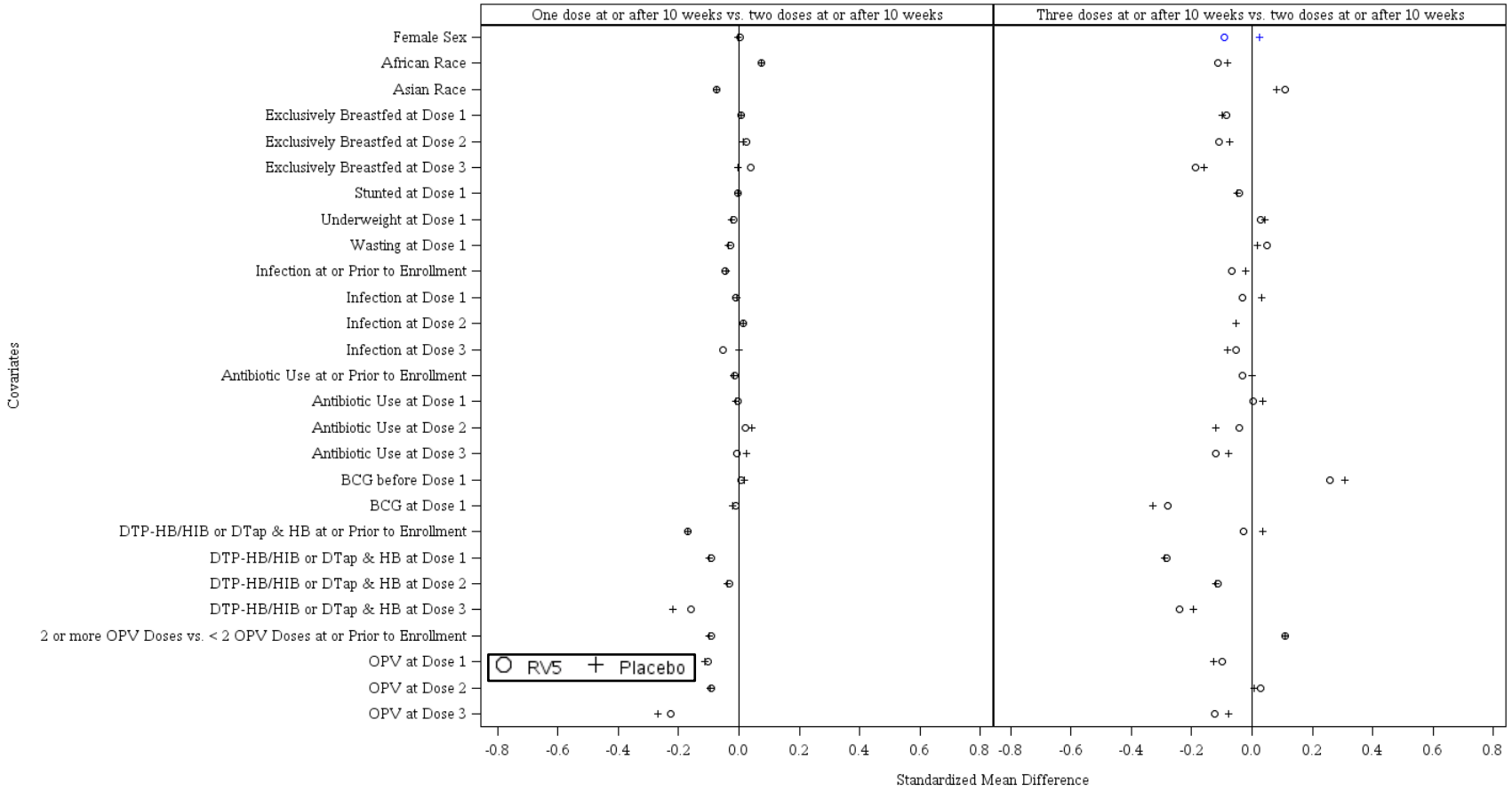
Appendix Figure 13. SMDs in the distribution of covariates between those receiving their last dose at ≤ 15 weeks compared to > 15 weeks. Black symbols represent a difference of < 10% between the SMDs of the RV1 and placebo groups. Blue symbols represent a difference of 10% to < 15% between the SMDs of the RV1 and placebo groups.



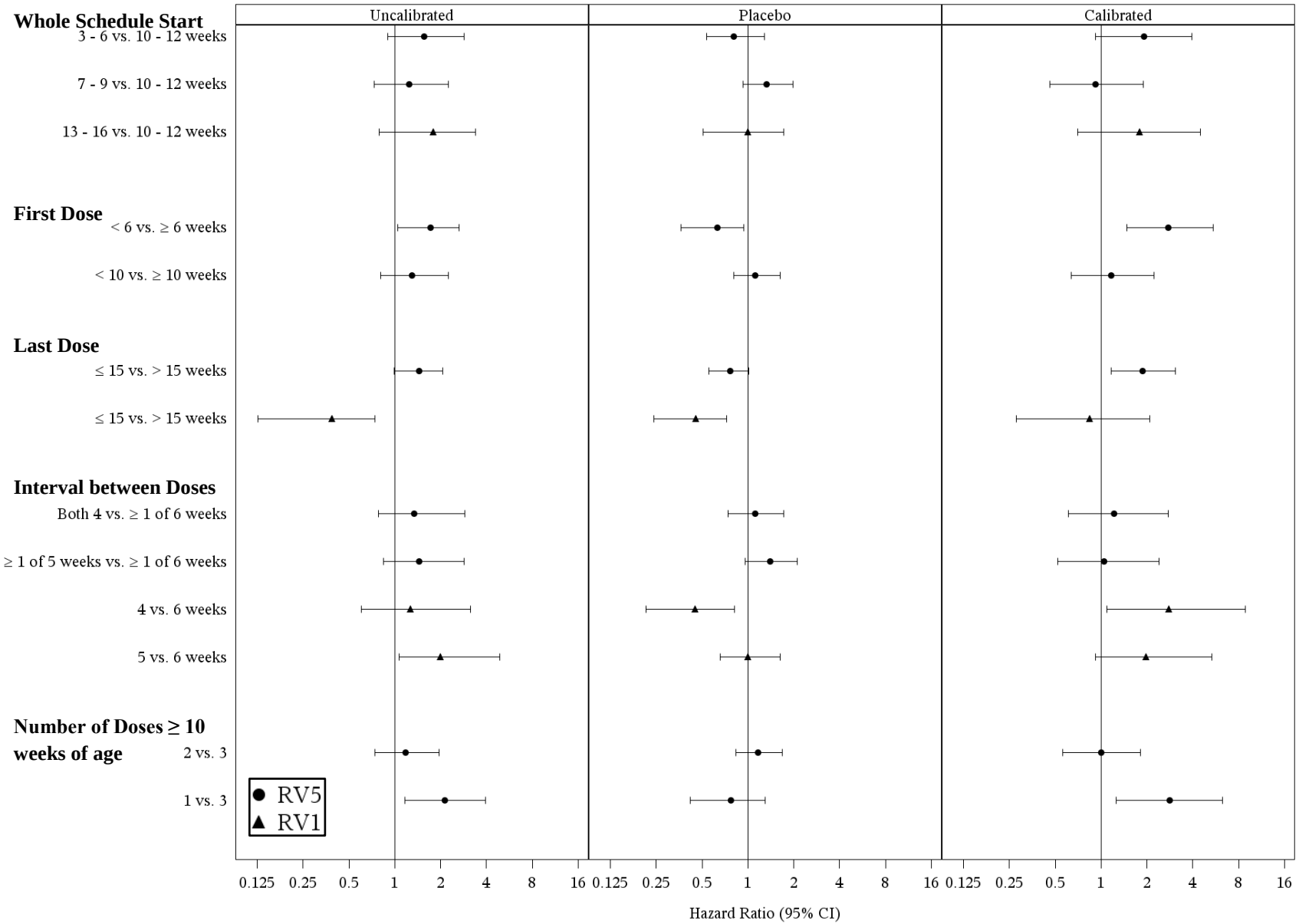
Appendix Figure 14. SMDs in the distribution of covariates between intervals of different lengths between doses. Black symbols represent a difference of < 10% between the SMDs of the RV5 and placebo groups. Blue symbols represent a difference of 10% to < 15% between the SMDs of the RV5 and placebo groups.



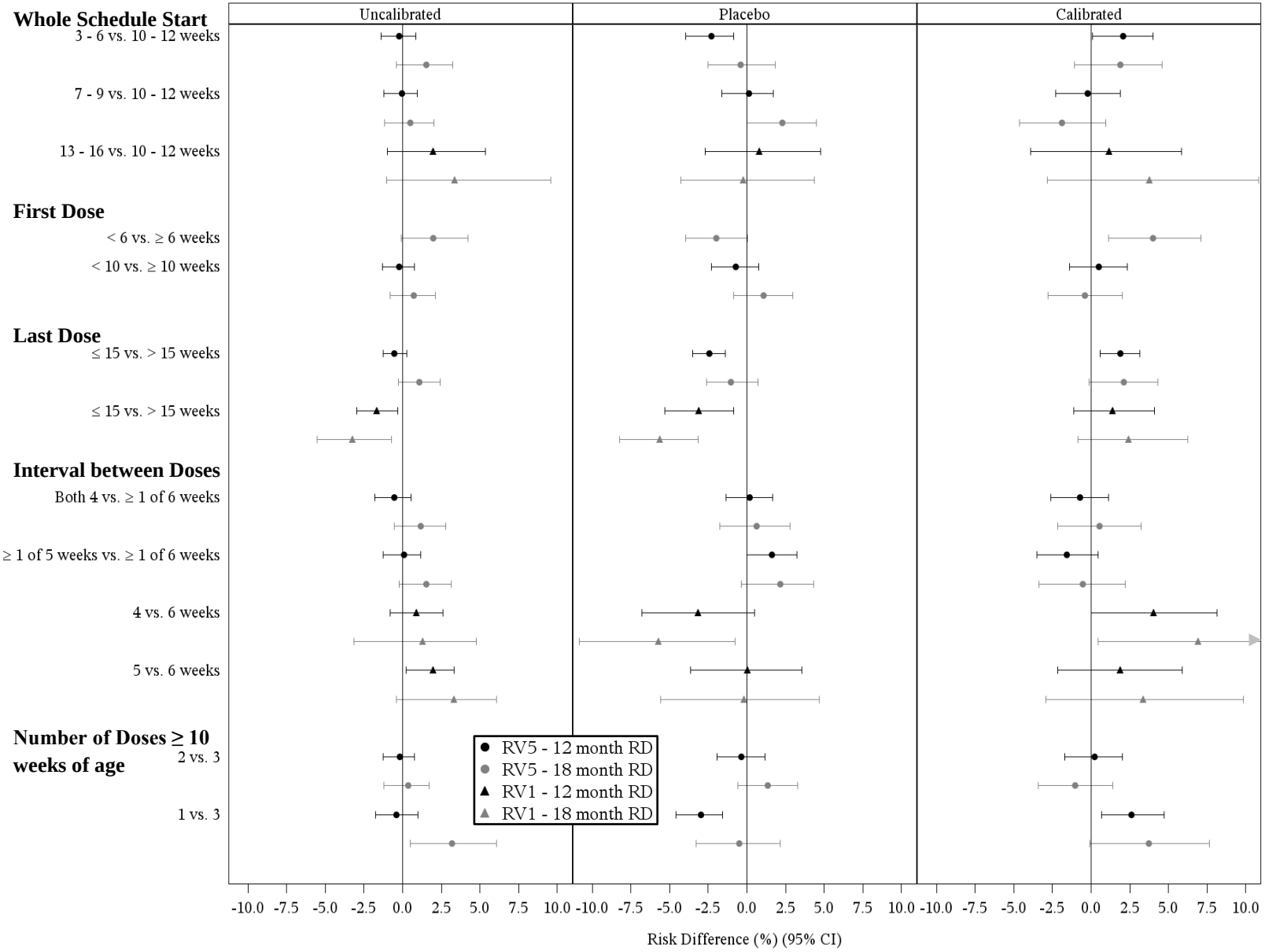
Appendix Figure 15. SMDs in the distribution of covariates between intervals of different lengths between doses. Black symbols represent a difference of < 10% between the SMDs of the RV1 and placebo groups.



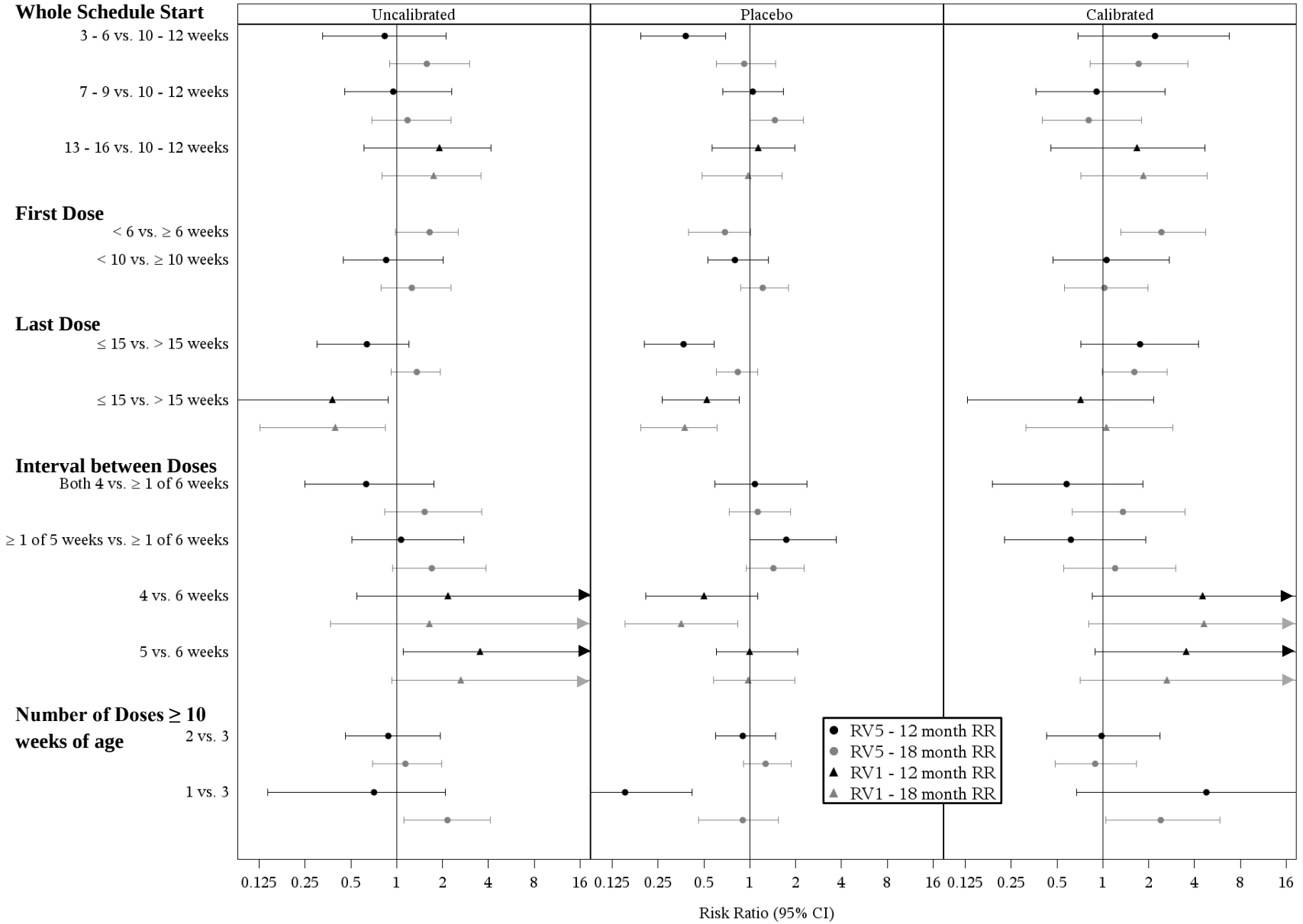
Appendix Figure 16. SMDs in the distribution of covariates between the number of doses received ≥ 10 weeks of age. Black symbols represent a difference of < 10% between the SMDs of the RV5 and placebo groups. Blue symbols represent a difference of 10% to < 15% between the SMDs of the RV5 and placebo groups.



Appendix Figure 17. Uncalibrated, placebo group, and calibrated HRs.



Appendix Figure 18. Uncalibrated, placebo group, and calibrated RDs.



Appendix Figure 19. Uncalibrated, placebo group, and calibrated RRs.

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