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Multi-scale assessment of rotavirus vaccination:

determinants of immunological, clinical and population-level effects

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An abstract of

A dissertation submitted to the Faculty of the

James T. Laney School of Graduate Studies of Emory University

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in Epidemiology

2019

Abstract

Multi-scale assessment of rotavirus vaccination:
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By Julia Marie Baker

Starting in 2006, rotavirus vaccines have been integrated into 96 national immunization programs. Yet, rotavirus gastroenteritis continues to cause 128,500-215,000 deaths among children under 5 years of age annually. This research aimed to generate insights that will help mitigate two major challenges preventing global success of the rotavirus vaccine and to understand the population-level effects of vaccination.

Rotavirus vaccines are substantially less immunogenic in high child mortality settings when compared to low child mortality settings. In **Aim 1**, we assessed individual and country-level factors associated with rotavirus vaccine immunogenicity. We pooled individual-level data on vaccinated infants from 22 clinical trials conducted across child mortality settings. Using multilevel logistic regression, we found oral polio vaccination given concomitantly with the first two rotavirus vaccine doses reduced seroconversion by 37% (OR=0.63, 95% CI=0.47, 0.84).

Given the need for improved rotavirus vaccine performance, a simple and effective method for evaluating new vaccination strategies and potential vaccine candidates is essential. In **Aim 2**, we assessed serum IgA as a correlate of protection for reduced risk of rotavirus gastroenteritis in high and low child mortality settings. Survival analysis methods were applied to follow-up data for vaccinated infants from pooled, individual-level clinical trial data. While no clear threshold indicating perfect protection across settings was identified, seroconversion served as a strong indicator of reduced risk of severe rotavirus gastroenteritis (child mortality setting: low: HR=0.04, 95% CI=0.01, 0.32; high: HR=0.48, 95% CI=0.26, 0.90).

Aim 3 estimated the longer-term, population-wide impacts of rotavirus vaccination in the United States. Time series data on monthly rates of rotavirus gastroenteritis hospitalizations in the pre- and post-vaccine periods were analyzed using negative binomial logistic regression. Declines in rotavirus gastroenteritis and a shift from annual to biennial patterns were apparent across age groups. The results highlight the important role infants play in rotavirus transmission and the underappreciated burden in older populations. Overall, rotavirus gastroenteritis hospitalizations decreased by 69% (95% CI=62%, 76%).

Seroconversion is valuable for identifying drivers of differential vaccine immunogenicity and for predicting risk of rotavirus gastroenteritis. Introduction of the rotavirus vaccine can lead to altered longer-term patterns in rotavirus gastroenteritis across age groups.

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Acknowledgements

Each member of my dissertation committee has been an integral part of my education. I can hardly express the depth of my gratitude for Dr. Ben Lopman who has provided unwavering support and guidance over the last three years. His encouragement to be ambitious and bold throughout my research has given me the confidence and drive necessary for both successfully completing this dissertation and for becoming an accomplished public health researcher. Dr. Michael Haber has been a kind and generous mentor providing guidance on the statistical components of my research when I felt lost and overwhelmed. Dr. Juan Leon's thoughtful feedback and practical advice provided an important balance to my sometimes overly wild ideas. Dr. Jackie Tate has been a calm and steady support since the beginning of my research and has demonstrated how to be an accomplished and respected woman making a real-world impact in global public health.

This dissertation is dedicated to my family. I'm endlessly grateful for the values my parents instilled in me, especially an appreciation for curiosity, social awareness and education. Their support and encouragement brought me to the public health field and has allowed me to achieve my educational goals. I also couldn't have accomplished all that I have over the last decade without the support of my better half, Johnny. His love, humor, and encouragement have made life a wonderful adventure and he has boosted my confidence when I needed it most. I'm grateful to my daughter, Ginny, who has been a pure joy and who occasionally sacrificed her favorite bedtime story in exchange for an update on the latest rotavirus literature. Finally, thank you to my little bundle, due in September, who has been my constant companion through the long days and late nights as I finished up my research.

My peers, the tremendous faculty, and my support network in the Rollins School of Public Health have allowed my journey through the doctoral program to be a success. I would probably be working on my analysis for years to come if it weren't for Molly Steele's endless willingness to troubleshoot my R code and her comedic relief in the process. I'm grateful to my friends in the doctoral program who have provided a much-needed balance to the challenges of academic life.

Lastly, thank you to everyone else who made this research possible, including my network of collaborators at Emory University, collaborators at the Centers for Disease Control and Prevention Division of Viral Diseases, the Data Sharing Team at GlaxoSmithKline, and the study participants.

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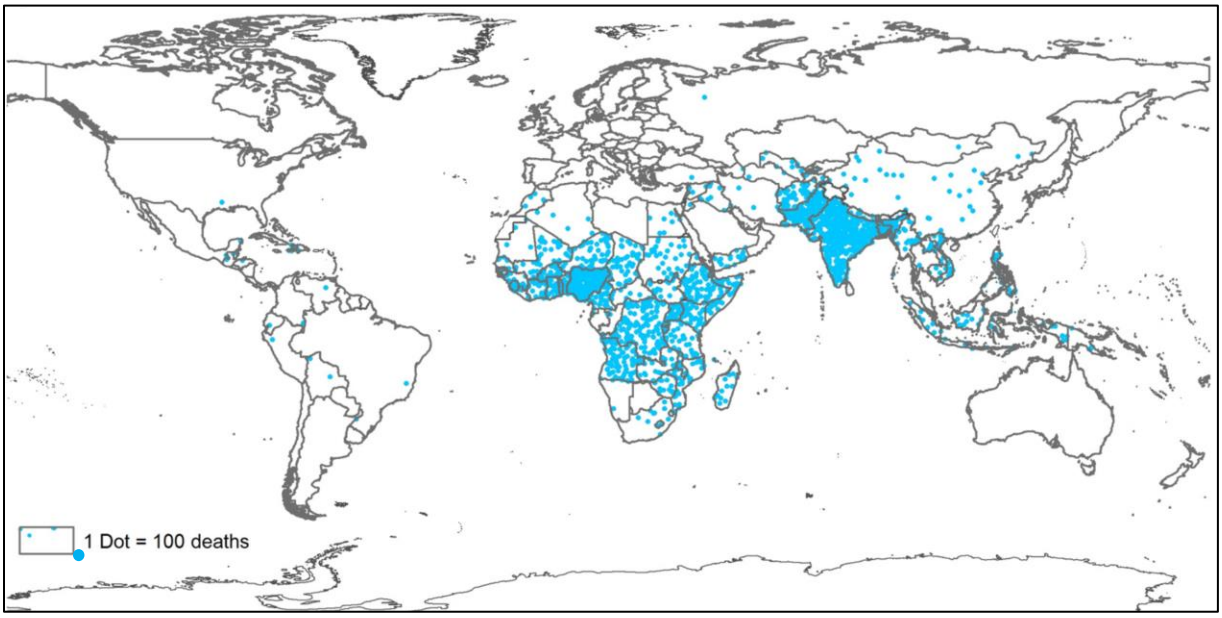
1 Background

1.1 Child mortality and rotavirus burden of disease among young children

It is estimated that 5.5 million children died worldwide in 2017, approximately 39 per 1,000 live births.[1] While this represents a tremendous decline of 58% since 1990, child mortality remains an imperative global challenge.[1] Diarrheal disease contributes to child morbidity and mortality both directly and indirectly. Worldwide, children under 5 years of age experience 1.1 billion episodes of diarrheal disease resulting in approximately 446,000 deaths[2] (the second leading cause of death outside of neonatal deaths)[3] each year. Further compounding the burden, diarrheal disease is considered a primary cause of malnutrition,[4] which is estimated to contribute to 45% of deaths among young children.[5]

Rotavirus gastroenteritis is estimated to cause between 128,500 and 215,000 deaths among infants and children under 5 years of age annually[6,7]—more than one quarter of all diarrhea-related deaths in this age group.[6,8,9] An estimated 85% of rotavirus-related deaths occur in Africa and Asia and nearly half of all rotavirus deaths occur in the countries of India, Nigeria, Pakistan and Democratic Republic of Congo.[7] Almost every child will experience rotavirus infection by their fifth birthday[10,11] and, in some high incidence settings, almost all children will have been infected by the time they turn 2-3 years of age.[10,12] Severe disease and life-threatening complications from rotavirus most frequently occur with the first infection in early childhood.[13]

Figure 1. Number of rotavirus deaths among children under 5 years of age by country, 2013



Tate, JE et al. Global, regional and national estimates of rotavirus mortality in children <5 years of age, 2000-2013. *Clinical Infectious Diseases.* 2016 May 1;62 Suppl 2:S96-S105. By permission of Oxford University Press and the Infectious Diseases Society of America

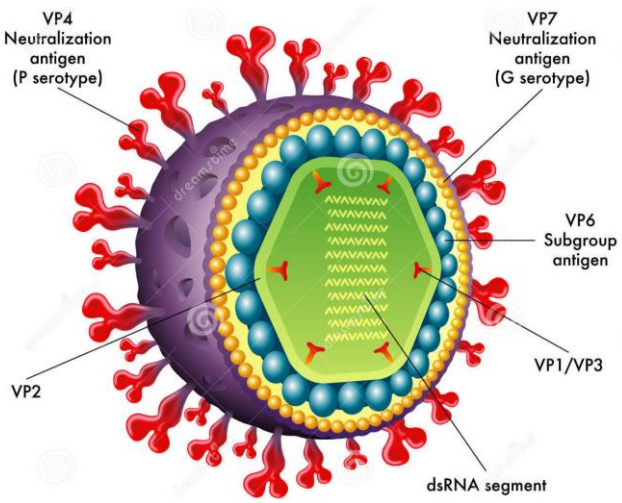
1.2 Rotavirus burden among older children and adults

While the primary burden of diarrheal disease and rotavirus gastroenteritis is felt among the youngest children, there is a potentially under-recognized rotavirus burden outside the pediatric age range.[14] Importantly, there is some uncertainty about the burden in this age group.[15] Some estimates suggest that almost one-quarter (23%) of rotavirus deaths occur among older children, adolescents and adults.[8] Across the age range, rotavirus is potentially the leading cause of diarrhea-related deaths in countries of all but the highest level of development[8] and is estimated to cause over 15% of the 1.3 million diarrhea-related deaths worldwide.[9]

1.3 Viral characteristics, transmission and infection

Rotavirus, first discovered in humans in 1973,[16] is a double-stranded RNA virus with a characteristic wheel-like appearance when viewed under an electron microscope.[17] The eight species of the virus (groups A through H) are known to infect humans and other animals, with Rotavirus A being the most frequent culprit in human disease causing up to 90% of cases.[18] The virus' 11 segment genome sequence codes for the production of structural and non-structural viral proteins shown in Figure 2[19] VP6, the proteins that make up the inner capsid (the middle layer of the virus' three-layer protein shell), determines the viral group. VP4 and VP7 are proteins on the outer surface of the virus and define the P and G rotavirus serotypes, respectively, with at least 42 different P-G combinations recognized.[20] VP8* is a surface protein located on VP4 and plays a critical role in infection, interacting with receptor proteins (histo-blood group antigens, HBGAs) on host mucosal cells.[18,21] Various strains, representing different combinations of genotypes, circulate in the environment as a result of genetic re-assortment.[18] Some regions, such as Africa, have particularly high diversity of strains in circulation[18,22,23] whereas in regions such as North America and Europe, one serotype (P1A[8]G1) is thought to cause a majority (70%) of infections.[22]

Figure 2. Rotavirus structure and viral proteins



Dreamstime.com stock photo

The virus is transmitted via the fecal-oral route, most commonly through person-to-person contact or contact with fomites.[13] Its stability allows it to survive in a variety of environments and spread through contamination of food, water, surfaces and objects. Once ingested, rotavirus damages mucosal cells lining the small intestine causing symptoms including vomiting and watery diarrhea that can last up to eight days, often accompanied by abdominal pain, fever, loss of appetite and/or dehydration. Disease is typically self-limiting and oral rehydration treatment may be given to prevent or reduce dehydration. Hospitalization and rehydration with intravenous fluids are used to treat the most severe episodes.[17]

The non-specific symptoms of rotavirus-related gastroenteritis are often clinically indistinguishable from gastroenteritis caused by other pathogens.[13] Identification of rotavirus as the cause of illness can be achieved through standard diagnosis techniques utilizing a fecal sample assay. Because the results of a rotavirus test do not alter the clinical management of gastroenteritis (i.e. rehydration), there is little incentive to conduct the test in the medical setting.[24] This rationale is apparent in reviews of medical records from children hospitalized in the United States for acute gastroenteritis in which only half were tested for rotavirus.[25,26] As a result of limited testing, estimates of the rotavirus disease burden based on medical records are likely underestimates.[26]

1.4 Rotavirus immunology

1.4.1 Mucosal and systemic immune response to rotavirus infection

The immune response to rotavirus has been well studied in animals but remains less well understood in humans.[18] Our understanding of the human response to rotavirus infection is

primarily based on animal models supplemented with studies from adult volunteers or natural infection among children.[27]

The response to rotavirus infection begins in the small intestine where the local mucosal immune response is induced though antibody secreting cells, B cells and T cells.[18] Antibody secreting cells direct the antiviral effect.[28] When the virus infects the epithelial cells lining the small intestine, antibodies in the gut mucosa are released into the intestinal lumen.[29] Rotavirus-specific neutralizing antibodies (NAs) are produced in response to the VP4 and VP7 viral proteins on the surface of the virus.[27] These NAs are specific both to the rotavirus serotype causing infection as well as other serotypes.[30] Antibody secreting cells also stimulate the production of anti-rotavirus immunoglobulin A (IgA) and anti-rotavirus immunoglobulin G (IgG)[31] which are actively involved in neutralizing the virus locally; these antibodies bind to the virus and prevent cell entry.[18] Specifically, secretory anti-rotavirus IgA (dimeric in structure) is produced in the gut and is active at mucosal surfaces where it prevents rotavirus from binding to mucosal cells of the intestine.[32] In the days following infection, anti-rotavirus immunoglobulin M (IgM) is also triggered though its mucosal presence is relatively short-lived.[33] Memory B cells are produced in the small intestine with receptors that allow them to circulate in the blood and return to the intestine with future infections.[28] T cells are also thought to be an essential component of long-term antiviral immunity through their role in stimulating B cells to produce anti-rotavirus IgA antibodies in the intestine.

Rotavirus infection also induces a systemic immune response, which has been the focus of many human immunology studies.[18,29] Memory B cells and antibody secreting cells both contribute to the production of anti-rotavirus IgA and anti-rotavirus IgG which circulates in serum.[28] The level

of these antibodies vary widely and seem to be positively associated with child age and number of infections.[33] Importantly, levels of serum anti-rotavirus antibodies may be reflective of mucosal antibody activity.[29] Specifically, the level of serum anti-rotavirus IgA (typically monomeric in structure)[34] has been shown to be correlated with duodenal anti-rotavirus IgA, suggesting serum anti-rotavirus IgA may be approximately representative of local immunity in the gut.[35] Antibodies in the intestine have been shown to "spillover" into serum and vice versa, though the mechanisms behind this process are not fully understood.[28]

A primary rotavirus infection can elicit both homotypic (against a single serotype) and heterotypic (against multiple serotypes) NA activity which indicates cross-reactive neutralizing epitopes are present. However, this type of response may be dependent on the serotype associated with the initial infection.[36] It is likely that the immune response to an initial rotavirus infection is primarily homotypic while heterotypic responses develop with subsequent exposures.[18]

1.4.2 Correlates of protection against rotavirus infection and rotavirus gastroenteritis

Research into the association between specific components of the immune response to rotavirus and the protection provided against future infection and symptomatic illness has frequently produced conflicting results. Intestinal and serum neutralizing antibodies, intestinal and stool anti-rotavirus IgA and anti-rotavirus IgG, and serum anti-rotavirus IgA and anti-rotavirus IgG have all been explored.[27]

The primary defense against rotavirus infection occurs in the gut where anti-rotavirus specific antibodies are actively engaged.[27] Intestinal NAs and anti-rotavirus IgA antibodies specifically

targeting VP6 reflect the immune system's ability to neutralize viral particles encountered in the gut and provide mucosal protection from infection.[28,37] These antibodies likely represent the strongest correlate of protection against rotavirus infection[28] Given the difficulty in measuring intestinal antibodies, fecal anti-rotavirus IgA has been assessed as a possible representative of intestinal antibody levels. Fecal IgA has been found to be correlated with duodenal anti-rotavirus IgA, however, fecal anti-rotavirus IgA was not found to be associated with protection against rotavirus infection or gastroenteritis.[27]

There is uncertainty regarding whether serum antibodies contribute to protection against rotavirus infection and illness or whether they are correlated with other immune mechanisms more directly involved in protection against infection and illness.[38] NAs in serum have been shown to be associated with a reduced risk of rotavirus infection and illness, and, relatedly, individuals with low homotypic and heterotypic serum NAs titers are more likely to experience symptomatic rotavirus illness.[18] Two of the more promising (and most frequently assessed) indicators of protection against rotavirus gastroenteritis are serum anti-rotavirus IgA and serum anti-rotavirus IgG. Though study results have been mixed, several studies of natural rotavirus infection have found that higher levels of either antibody have been correlated with increased protection against symptomatic rotavirus infection.[28,38] A groundbreaking study of natural rotavirus infection among Mexican infants demonstrated that serum anti-rotavirus IgA and anti-rotavirus IgG provided little evidence of protection against infection, were modestly associated with protection against mild disease, and were most strongly associated with protection against severe disease. In addition, protection was shown to increase with subsequent infections.[10,39] Overall, total anti-rotavirus IgA

in serum is likely a strong correlate of intestinal anti-rotavirus IgA levels, however, the mechanisms behind this relationship are not fully understood.[28]

An important characteristic of rotavirus is its ability to induce imperfect immunity against symptomatic illness.[10,40–42] Immunity to rotavirus infection, or at least symptomatic disease, builds with each subsequent infection.[10,12,42] The level of protection against symptomatic illness afforded by previous infections varies by setting. A study of children in India estimated 79% protection against moderate to severe illness after three prior infections[12] whereas complete protection was afforded after just two infections among infants in Mexico.[10] With repeat exposure to the virus, the immune system develops full or partial immunity to rotavirus gastroenteritis for the specific strains with which an individual has been infected as well as to other strains with which the individual has not been infected (homotypic and heterotypic defenses).[43,44] Interestingly, host expression of HBGAs, the receptor carbohydrates that interact with VP8* on the surface of rotavirus, vary by host genetics and development, indicating that human susceptibility to rotavirus infection varies.[18,45–47]

1.5 Seasonality of rotavirus gastroenteritis

Distinct patterns in seasonal prevalence of rotavirus gastroenteritis can be observed in different settings. Low-income countries (perhaps driven by characteristics such as birth rate and transmission rates[48]) more typically experience year-round prevalence whereas high income countries typically have a defined rotavirus season with little detection of the virus outside the season.[49] Other factors such as climate, latitude and population density may also contribute to

seasonal patterns.[50–52] Changes in rotavirus seasonality have been observed in some countries after introduction to the rotavirus vaccine (discussed below).[53]

1.6 Rotavirus vaccines

Vaccination plays a vital role in reducing the rotavirus disease burden,[54] particularly because of the virus' high infectivity and the limited role of water, sanitation and hygiene conditions in reducing transmission.[55]

1.6.1 Vaccine safety

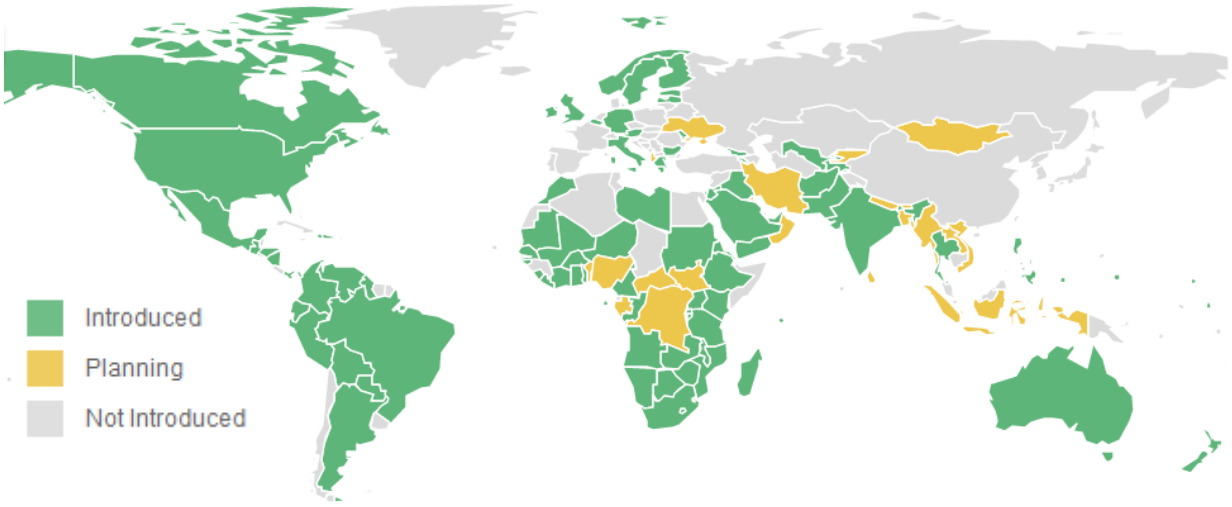
The first rotavirus vaccine, which is no longer available, was introduced in 1998 and was only administered in the United States before being pulled from use less than a year later. This vaccine, RotaShield (Wyeth Lederle Vaccines), was found to increase intussusception, a rare and very serious bowel obstruction.[56] The vaccines available today (described below) have been rigorously studied to assess the risk of intussusception as well as other possible adverse events. The World Health Organization (WHO) Global Advisory Committee on Vaccine Safety continuously monitors rotavirus vaccine safety and has affirmed that the benefits of rotavirus vaccination outweigh the small risks.[57,58]

1.6.2 Currently available vaccines

Until recently, only two vaccines were available globally: GlaxoSmithKline's (GSK's) monovalent vaccines, Rotarix, and Merck's pentavalent vaccine, RotaTeq.[59] Promising clinical

trial results from the two manufacturers were released in 2006 with the vaccines demonstrating safety and efficacy over 90% against severe rotavirus disease in some high- and middle- income settings.[60–62] Of note, these two vaccines contain rotavirus genotypes thought to cover 90% of circulating rotavirus strains[63] and provide good cross protective (heterotypic) immunity.[64] By January 2019, rotavirus vaccines had been integrated into 96 national immunization programs around the world (Figure 3).[65] Forty-six of these countries have been able to introduce the vaccines because of financial support from Gavi, The Vaccine Alliance,[66] which plays a crucial role in assisting developing countries in purchasing and increasing coverage of childhood vaccines.[66] Unfortunately, the vaccines are still not accessible to approximately 90 million infants born each year, many of whom are in low-income countries with sub-optimal access to care and the highest rotavirus disease burdens.[67,68]

Figure 3. Countries that introduced or are planning to introduce rotavirus vaccines as of January 2019[66]



GSK's Rotarix vaccine is a monovalent, live attenuated, oral rotavirus vaccine licensed in more than 100 countries.[69] A full vaccine regimen consists of two 1 mL doses, the first of which is recommended for administration at 6 weeks of age and the second after a four-week interval, but by 24 weeks of age, according to the manufacturer.[70] Vaccine efficacy from clinical trials conducted in Europe is 90% against severe rotavirus gastroenteritis and 72% for all-cause diarrheal hospitalizations.[60] Efficacy is substantially lower in many low-resource settings, such as Malawi, where the vaccine is 49% effective in preventing severe rotavirus disease.[71]

Merck's RotaTeq vaccine is integrated into the immunization programs of 22 countries.[66] This pentavalent, oral vaccine is administered in a series of three, 2 mL doses beginning when an infant is 6 to 12 weeks of age. The two subsequent doses are administered at 4 to 10 week intervals with the third dose given by 32 weeks of age.[72] As with Rotarix, RotaTeq's efficacy varies by setting. Efficacy ranges from as low as 51% in high child mortality settings to 100% in countries with low child mortality.[73,74]

In 2018, the WHO prequalified two new oral rotavirus vaccines, the Serum Institute of India's heat-stable vaccine, Rotasiil, and Bharat Biotech's monovalent and locally produced, low-cost vaccine (India), Rotavac.[54,75–77]WHO prequalification means these vaccines are now available for purchase by UN agencies and Gavi.[77] Among infants in high child mortality settings, both Rotasiil and Rotavac have demonstrated efficacy similar to Rotarix and RotaTeq, with modest efficacy of 40-67% against severe rotavirus gastroenteritis.[75,78,79] Several characteristics of the four WHO prequalified vaccines are compared in Table 1.

Table 1. Comparison of currently available rotavirus vaccines

	Manufacturer	Valency/ Antigens	Number of countries using[66,80]	Efficacy against severe rotavirus gastroenteritis[74,75,78,79] (95% CI)
Rotarix	GSK	Monovalent/ G1, P8	92	49% (11-72) (Malawi) 72% (40-88) (South Africa) 72% (54-84) (China) 81% (88-100) (Latin America) 90% (85-94) (Europe) 92% (62-99) (Japan)
RotaTeq	Merck	Pentavalent/ G1, G2, G3, G4, P8	22	51% (13-73) (Bangladesh/Vietnam) 64% (40-79) (Kenya/Ghana/Mali) 98% (88-100) (US/Finland) 100% (55-100) (Japan)
Rotasiil	Serum Institute of India	Pentavalent/ G1, G2, G3, G4, G9	1	40% (27-50) (India) 67% (50-78) (Niger)
Rotavac	Bharat Biotech International Limited, India	Monovalent/ G9, P11	1	54% (35-67) (India)

Following the introduction of rotavirus vaccines into national immunization programs, countries around the world have experienced declines in diarrhea-related morbidity along with reductions in all-cause and rotavirus-related hospitalizations among young children.[81–87] In the United States,

for example, rotavirus hospitalizations among children under 5 years of age have declined by 80%.[88] In Latin America and the Caribbean, vaccination reduced the risk of severe gastroenteritis by 50%-80% and hospitalization or emergency department visits by 85%-90%.[89] Botswana observed a 23% reduction in gastroenteritis hospitalizations among young children following vaccine introduction.[90] In Bangladesh, hospitalization from acute rotavirus diarrhea decreased by nearly one-third among children under 2 years of age after vaccine was introduced as part of a demonstration project.[91] The introduction of rotavirus vaccines has led to dramatic reductions in rotavirus risk in certain settings,[60,61,73,92,93] yet, between 128,500 and 215,000 children under 5 years of age still die from rotavirus gastroenteritis each year.[6,8,9] Moreover, in many settings, rotavirus remains the dominant cause of hospitalization from severe diarrheal disease among children under 5 years of age despite vaccine introduction.[94,95] This remaining disease burden emphasizes the critical need for ongoing research to improve rotavirus vaccine implementation and performance.

1.6.3 Correlates of immunogenicity for rotavirus vaccines

Vaccine-induced immune response against rotavirus infection and gastroenteritis has been studied as part of rotavirus vaccine development and post-licensure evaluation. As with the correlates of protection against rotavirus infection and gastroenteritis, study results have frequently been mixed and uncertainty remains.[43] Vaccination has been shown to induce serum anti-rotavirus IgA and serum anti-rotavirus NAs which have then been correlated with a reduced risk of mild/moderate and severe rotavirus gastroenteritis.[18,27,28] Other studies, however, have found anti-rotavirus NAs to be poorly associated with protection against rotavirus gastroenteritis.[43] One possibility is that

it is neither the level of anti-rotavirus IgA or anti-rotavirus IgG levels alone that dictate protection against rotavirus infection and disease, but rather a combination of the two.[43] As with natural infection, the vaccine-induced immune response has been shown to be both homotypic (stimulating an immune response to the specific vaccine strain) and heterotypic (stimulating an immune response to other strains of the virus not included in the vaccine).[43]

1.7 Rotavirus vaccine challenges

1.7.1 Vaccine performance

A central impediment to further reducing the rotavirus burden is the relatively low efficacy of the vaccine in settings where the incidence of severe disease and death is highest, such as Africa and Asia.[7,96] In high child mortality settings, vaccine efficacy against severe rotavirus gastroenteritis can be as low as 36%, as compared to over 90% in areas of low child mortality.[62,73,97] This pattern of lower efficacy in settings of lower socioeconomic development was observed under clinical trial conditions,[62,73,97] indicating weaker protection conferred by vaccination (distinct from lower vaccine uptake, i.e. lower level of vaccine coverage), and the mechanisms behind this variation in efficacy are not fully understood.[98]

Relatedly, rotavirus vaccines are substantially less immunogenic (i.e. stimulates a weaker immune response) in high child mortality settings when compared to low child mortality settings.[73,97] Rotavirus vaccine immunogenicity, represented by serum anti-rotavirus IgA, has been shown to vary by country and is inversely associated with a country's under 5 mortality rate at the aggregate level.[99] This trend has been demonstrated for two measures of vaccine immunogenicity; mean

geometric concentration (GMC) of anti-rotavirus IgA titers and anti-rotavirus seroconversion rates are substantially lower among infants in low-income countries compared to infants in high-income countries.[98] Identifying the reasons for poor immune response at the individual-level is a critical step in determining relevant factors contributing to disparate efficacy of vaccination at the population-level.

Current literature suggests host characteristics may influence rotavirus vaccine immunogenicity by reducing the effective vaccine virus titer (plaque forming units, pfu) delivered to the intestines or impairing host immune response.[98] For instance, high levels of antibodies received from breast milk may decrease the effective vaccine virus titer by binding to the vaccine virus and preventing replication.[100] Co-infection with other pathogens or malnutrition may reduce an infant's immune response to the vaccine[98] through changes in the gut microbiome (with which the vaccine virus interactions and where it replicates) or suppression of immune function.[101] Other possible explanations include differences in circulating strains of rotavirus,[22] variations in the force of infection,[73,98,102] and environmental enteropathy (chronic inflammation).[103] Table 2 summarizes potentially relevant factors for vaccine immunogenicity and efficacy at the host, household and country level.

Table 2. Factors that may compromise rotavirus vaccine efficacy or immune response [22,73,98,102–104]

Host (H)	Household (F)	Country (C)
Age at vaccination	Breastfeeding practices	Development/income level
Micronutrient malnutrition	(interval between breastfeeding	Child mortality rate
Zinc deficiency	and vaccination)	Circulating rotavirus strains
Concomitant oral polio	Socioeconomic status	
vaccinationCo-infections		
Gut flora		
Gastric acid in digestive track		
Components of breast milk		
Transplacental antibodies		
Environmental enteropathy		

Concomitant (concurrent) vaccination with oral polio vaccine (OPV) is another potential factor influencing immune response to rotavirus vaccination. Poliovirus is transmitted through the fecal-oral route and replicates in the intestines where it can lead to gastrointestinal symptoms and, in severe cases, ultimately cause neurological damage. As with rotavirus vaccine, OPV is a live, attenuated oral vaccine favored in high burden settings due to its ability to provoke a response in both the mucosal and systemic immune systems.[105] Due to the high risk of rotavirus and polio during infancy and early childhood in high child mortality settings, it is critical that both vaccines be administered during the first months of life and the two vaccines are frequently given concomitantly according to routine childhood immunization schedules.[106] Unfortunately, it has been demonstrated in several countries that concurrent OPV and rotavirus vaccination reduces

rotavirus seroconversion rates.[107–110] This interaction seems to be strongest for the first rotavirus dose[108] and there is some suggestion that staggering vaccination even by just one day might negate potential interference.[107] The mechanisms behind this interaction are poorly understood but may relate to competition between rotavirus and poliovirus for receptors on mucosal cells reducing viral entry or poliovirus causing downregulation of components of the immune system response to rotavirus.[107]

This interaction is of particular interest as changes in the polio vaccination strategy are expected over the next several years. The Global Polio Eradication Initiative is a three-decade long, worldwide effort led by the WHO to completely eradicate poliovirus.[111] As poliovirus transmission continues to be reduced, particularly in high child mortality settings, countries will likely remove OPV from their national immunization programs and replace it with a safer, inactivated polio vaccine (IPV). Importantly, IPV is a killed vaccine which is administered intramuscularly or intradermally and acts through a different mechanism than OPV to simulate the immune system.[112] Concomitant administration of IPV with rotavirus vaccine does not seem to interact with rotavirus vaccine immunogenicity.[110] The impact of removing OPV from routine use on rotavirus vaccine performance remains to be seen.

1.7.2 Identification of a correlate of protection

A correlate of protection predicts protection against clinical disease.[27,113] When used as part of a vaccine study, it can function as a substitute for clinical endpoints and predict the efficacy of a vaccine.[27,113] A reliable correlate of protection is important for a vaccine program because of its

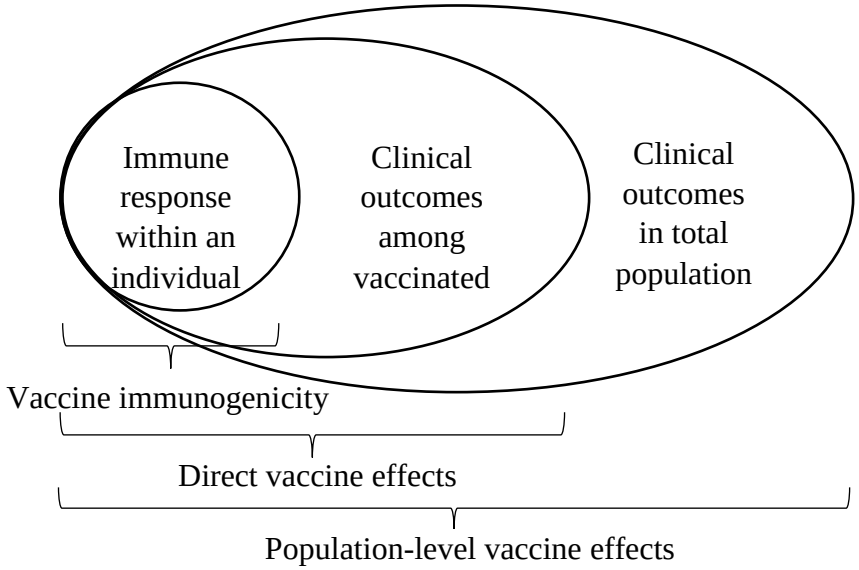
ability to predict vaccine efficacy without the need for long, large-scale trials with clinical endpoints.[27]

Reductions in rotavirus-related morbidity and mortality have been impeded by progressively slower vaccine uptake in recent years.[54] Logistic challenges including vaccine supply constraints, prohibitively high cost per dose and cold-chain requirements mean the utility of some existing vaccines are limited, particularly in settings where the vaccines are needed most.[114] Given the demand for and potential volume of new interventions for reducing the rotavirus disease burden, including new vaccine candidates in the pipeline, a simple and effective method for evaluating new vaccination strategies and vaccine candidates without the need for large-scale trials with clinical endpoints is essential.[115] Within this context, serum anti-rotavirus IgA antibodies are being explored as possible markers for protection[28] and have demonstrated value as predictors of vaccine efficacy on the aggregate level, when comparing countries by income level.[99] Research related to individual-level correlates of protection, however, has been limited.[116–119] Early investigations of individual-level correlates of protection evaluated vaccines or vaccine candidates not available for use today.[28] More recently, Cheuvart et al found vaccine-induced seropositivity measured via serum anti-rotavirus IgA to be moderately associated with reduced rotavirus gastroenteritis in a small subset of clinical trials.[119] Further insights into the value of anti-rotavirus IgA as a predictor of protection against rotavirus gastroenteritis on the individual-level may be gained by assessing this relationship among individuals from a diversity of settings.

1.8 Vaccine evaluation

The effects of a vaccine can be evaluated on several scales including (a) vaccine immunogenicity, (b) direct vaccine effects, and (c) population-level vaccine effects which include indirect, total and overall effects. These three broad categories are shown in Figure 4 and each measure is described in detail below.

Figure 4. Three broad scales for evaluating a vaccine



A fundamental measure for evaluating a vaccine relates to how the vaccine interacts with the immune system of a vaccinated individual. Immunogenicity describes "the ability of a molecule or substance to provoke an immune response" in an individual or the "magnitude of an immune response" when exposed to a substance.[120] Anti-rotavirus IgA antibodies are an integral component of the immune response to rotavirus infection.[121] For rotavirus vaccines, serum anti-rotavirus IgA is a standard measure of immunogenicity in clinical trials and observational studies. Anti-rotavirus IgA response is often measured as:[27]

1. Seroconversion: the appearance of anti-rotavirus IgA antibodies in subjects seronegative prior to the first vaccine dose
2. IgA titer: post-vaccination serum anti-rotavirus IgA concentration

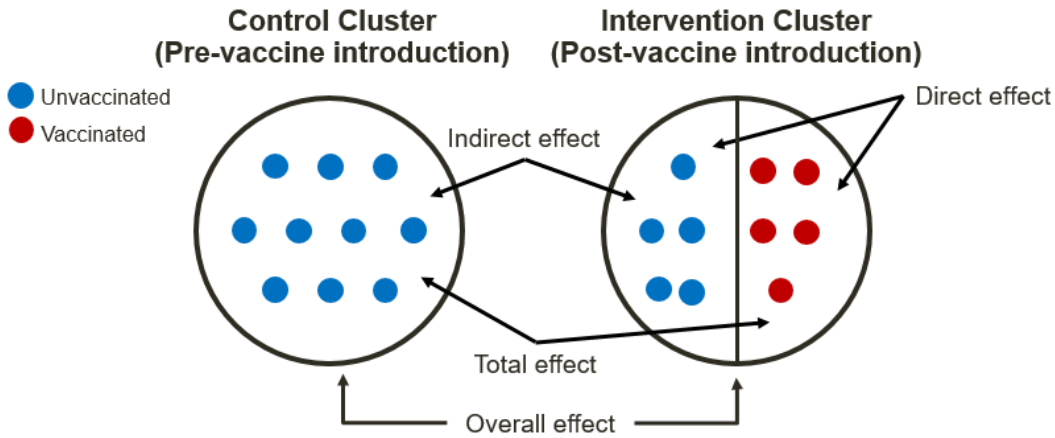
Another important scale upon which a vaccine's effects can be evaluated is how well it prevents a clinical outcome of interest among those who receive it. Direct vaccine efficacy represents the biological protection against illness obtained from vaccination at the individual level.[122] Direct vaccine efficacy is defined as the percent reduction in the risk of the outcome of interest in a vaccinated individual compared to an unvaccinated individual when both are exposed to the same sources of infection.[123] It is considered a characteristic of a vaccine that, theoretically, remains constant over time (except with waning immunity) and is not impacted by changes in vaccine coverage. Efficacy is used when describing the percentage reduction under ideal conditions, such as a in a randomized clinical trial, while vaccine effectiveness is analogously calculated under more typical, real-world conditions.

A third scale for evaluating a vaccine involves measuring the change in the prevalence/rate of the outcome of interest at the population-level. These population-level effects include (a) indirect effects or "herd protection" provided to members of the population either from reduced exposure to infected individuals or reduced infectiousness of infected individuals,[122] (b) total effects which describe the combination of biologic and indirect protection received by vaccinated individuals, and (c) the overall effects, also called "vaccine impact", which quantify the public health benefit of a vaccination program in terms of the vaccine-induced reduction in the cumulative incidence of the outcome of interest that can be attributed to the vaccination program. In contrast with direct

vaccine effects, population-level effects vary with changes in vaccine coverage levels, population immunity, and social mixing patterns.[124–126]

Measurement of vaccine effects are described in Halloran et al.'s vaccine evaluation framework.[126] Ideally, population vaccine effects would be estimated in a cluster randomized trial (shown schematically in Figure 5) with a "control cluster" and an "intervention cluster." The entire population in the control cluster has not received the intervention. For a vaccine evaluation, the control cluster would contain only unvaccinated individuals. In the intervention cluster, a portion of the population has received the intervention (i.e. are vaccinated). In practice, this type of study design is difficult to achieve and is not frequently conducted. However, "natural experiments" from populations with different vaccine coverage can be used to evaluate the same measures. To measure the effects of the rotavirus vaccine in a particular population, the pre-vaccine era represents the control cluster and the post-vaccine era, in which a portion of the population has been vaccinated, represents the intervention cluster.

Figure 5. Measurement of vaccine effects



1.9 Longer-term direct and indirect vaccine effects

Globally, introduction of the rotavirus vaccine has profoundly impacted gastroenteritis-related morbidity and mortality among children. A recent review found an overall reduction of 38% in all-cause acute gastroenteritis and a 67% reduction in rotavirus-specific gastroenteritis among children under 5 years of age since vaccine introduction.[127] High and low-income countries around the world have experienced declines in diarrhea-related morbidity along with reductions in all-cause and rotavirus-related hospitalizations among young children.[81–87] Possible indirect benefits of the vaccine to unvaccinated children have also been demonstrated in some settings.[83,85,87,128,129]

The United States was one of the first countries to introduce rotavirus vaccine nationally[130,131] with RotaTeq and Rotarix included in the routine infant vaccination schedule in 2006 and 2008, respectively, based on recommendations from the Advisory Committee on Immunization Practices.[130,132] Before vaccine introduction, rotavirus was the leading cause of severe pediatric gastroenteritis in the United States causing an estimated 55,000-70,000 hospitalizations among young children annually.[130,133] Rotavirus vaccine coverage increased rapidly in the years immediately following its introduction, however, national coverage rates have plateaued around 73% since 2013.[134,135] This is relatively modest coverage when compared with more established, routine infant immunizations such as DTaP where three-dose coverage was 94% in 2017.[135] In the decade-long post-vaccine period, there has been a dramatic decline in rotavirus-related hospitalizations,[87,129,136] ED visits[136,137] and physician office visits[87,136] among children under 5 years of age.

There is substantial evidence from the United States that demonstrates clear direct vaccine benefits among the vaccinated,[60,61] however, potential effects of the rotavirus vaccine on the unvaccinated population and longer-term trends in rotavirus gastroenteritis have been less well evaluated.[62] A recent meta-analysis on rotavirus vaccination in the United States estimates direct vaccine effectiveness is approximately 84% against rotavirus-associated hospitalizations and emergency department visits.[134] Supplementing the direct effects, the estimated reduction in rotavirus disease have exceeded vaccine coverage, which suggests unvaccinated children are experiencing indirect benefits from their vaccinated counterparts.[138] These indirect benefits may extend to newborns too young to be vaccinated as well as older children, adolescents, and adults who are age-ineligible for vaccination, as reductions in rotavirus gastroenteritis and all-cause gastroenteritis have been observed in these populations.[139] Importantly, the literature evaluating indirect effects is limited to short-term periods immediately following vaccine introduction,[139,140] projections of future disease burden[141] or restricted to select age groups.[87,129,136,142,143]

2 Study rationale, specific aims and significance

2.1 Study rationale

Despite the remarkable declines in gastroenteritis that have resulted from rotavirus vaccine introduction in countries around the world,[127] the potential of the vaccine has not been fully realized and rotavirus gastroenteritis remains a substantial threat to the health of young children.

As previously mentioned, rotavirus vaccines are substantially less immunogenic in high child mortality settings when compared to low child mortality settings.[73,99] Identifying the reasons for

poor immune response at the individual-level is a critical step for understanding the disparate efficacy of vaccination at the population-level. Conditions or factors that may contribute to poor IgA response have been hypothesized, however, the influence of these factors have typically been assessed in individual countries or in isolation from other potential confounding factors.[98] The role of these factors have not been assessed in a large, pooled analysis of individual level clinical trial data controlling for host and country-level characteristics. Identifying predictors of rotavirus vaccine immunogenicity across settings may highlight modifiable vaccine strategies or interventions for enhancing vaccine performance.

A reliable correlate of protection would benefit rotavirus vaccine programs because of its ability to predict vaccine efficacy.[27] The future of rotavirus vaccine research will inevitably require a considerable number of vaccine studies, including assessments of improved vaccination strategies and evaluation of vaccine candidates currently under development in the robust rotavirus vaccine pipeline.[144] A simple and effective method for evaluating new vaccination strategies and potential vaccine candidates without the need for large-scale, long-term trials with clinical endpoints is essential for rapidly improving rotavirus vaccine performance.[115] The value of IgA antibodies as a rotavirus vaccine correlate of protection has only been explored in limited research.[116–119] Assessing IgA as a correlate of protection among individuals from a diversity of settings will provide further insights into its value as a predictor of protection against gastroenteritis.

Lastly, a better understanding of the longer-term, population-level impacts of rotavirus vaccination is needed to fully appreciate the rotavirus disease burden across the age range. While the direct benefits of vaccination to those who receive it are well known,[60,61] questions remain about the

potential effects of the rotavirus vaccine on the unvaccinated population and longer-term trends.[62] Assessment of the indirect effects of the vaccination program among older children, adolescents and adults may reveal a considerable severe disease burden outside the pediatric age range that is preventable by infant immunization. Evaluations of longer-term data are needed to confirm and quantify these population-level effects. Settings such as the United States, where the vaccine has been in use for many years, provide a unique opportunity to assess the longer-term, population-level trends in rotavirus illness.

The primary goal of this research is to generate insights that will help mitigate some of the major challenges preventing full global success of the rotavirus vaccine. To do so, this research will evaluate the rotavirus vaccine on three interconnected scales: (1) how host characteristics influence rotavirus vaccine immunogenicity, (2) how immune response predicts clinical disease within an individual, and (3) how rotavirus vaccination impacts severe gastrointestinal illness at the population-level. Improved understanding of the rotavirus vaccine on these three scales may provide insight into enhanced vaccination strategies, rapid and efficient methods for evaluating new interventions, and the long-term impacts of vaccination across the age range.

2.2 Aims overview

Aim 1: Identify host characteristics that contribute to rotavirus vaccine immunogenicity measured approximately 4 – 12 weeks after receipt of the last vaccine dose in high and low child mortality settings.

Individual-level data from GSK's Rotarix phase II and phase III clinical trials will be pooled and analyzed to achieve this aim. Infant characteristics such as gender, age at first dose, and nutritional status will be assessed as predictors of immune response to Rotarix immunization while controlling for country factors as covariates and potential confounders using a hierarchical model approach. Immune response will be measured as:

1. Seroconversion defined as serum anti-rotavirus IgA antibody concentrations ≥ 20 U/mL in subjects initially seronegative
2. Post-vaccine serum anti-rotavirus IgA antibody titer among infants who seroconverted

Multilevel logistic regression and linear regression of log-transformed data will be used to analyze anti-rotavirus IgA seroconversion and antibody titer, respectively. Models will first be run with data from all countries combined and then applied to each child mortality stratum individually. Child mortality is a frequently used indicator of economic development [1] and child mortality stratum will be defined based on WHO classification methods.[145]

Aim 2: Quantify a threshold of post-vaccine anti-rotavirus IgA antibody units that serves as an individual-level immune correlate of protection against mild/moderate and severe rotavirus disease among infants in high and low child mortality settings.

Individual-level data from GSK's Rotarix phase II and phase III clinical trials will be analyzed to achieve this aim. This aspect of the study will identify if there exists a cutoff value of post-vaccine serum anti-rotavirus IgA antibody, measured approximately 4 – 12 weeks after receipt of the last

vaccine dose, which is associated with dramatically reduced risk or near perfect protection against rotavirus gastroenteritis among infants. Four outcomes will be assessed:

1. At least one episode of mild/moderate rotavirus gastroenteritis through 1 year of age
2. At least one episode of mild/moderate rotavirus gastroenteritis between 1 and 2 years of age
3. At least one episode of severe rotavirus gastroenteritis through 1 year of age
4. At least one episode of severe rotavirus gastroenteritis between 1 and 2 years of age

The dichotomous outcomes of interest will be analyzed using survival analysis methods. Several anti-rotavirus IgA thresholds will be tested to evaluate their utility in predicting protection against rotavirus gastroenteritis.

Aim 3: Estimate the direct effects (among young children) and indirect effects (among all age groups) of the 2006/2007 infant rotavirus vaccine introduction in the United States on the monthly rates of acute gastroenteritis and rotavirus hospitalization.

This analysis will measure the monthly rates of all-cause gastrointestinal and rotavirus-specific hospitalizations across age groups during the pre- and post-vaccine eras to identify changes in disease burden and seasonal patterns of illness. Data from community hospitals will be used to conduct a time series analysis of monthly discharges in the pre- and post-vaccine eras for acute gastroenteritis and rotavirus from 2000 through 2013. Three comparisons will be made for each outcome to estimate rate ratios and corresponding 95% confidence intervals:

1. Combined monthly pre-vaccine rates (2000-2006) vs. combined monthly post-vaccine rates (2008-2013)
2. Combined monthly pre-vaccine rates (2000-2006) vs. combined monthly post-vaccine rates for even (2008, 2010, 2012) and odd calendar years (2009, 2011, 2013) separately to assess biennial patterns
3. Combined monthly pre-vaccine rates (2000-2006) vs. monthly post-vaccine rates (2008-2013) for each individual post-vaccine year to provide more detail on the potentially dynamic effects of vaccination over time.

Changes in hospitalization rates and patterns among young children will represent the combined direct and indirect benefits of vaccination in this population while additional indirect impacts will be represented by changes in hospitalization rates and patterns among older children, adolescents and adults.

3 Data sources

3.1 GSK clinical trials

The analyses for Aim 1 and 2 utilized individual-level data from infants enrolled in 22 of GSK's clinical trials of the Rotarix vaccine (20 individual National Clinical Trial study IDs). These data were requested from GSK through their Clinical Trial Data Request system.

Conceptualization of this project began in late 2016. In early February 2017, a formal proposal describing our planned research and requesting the data was submitted to GSK. The proposal

underwent review by GSK's Independent Review Panel, and the specifics of the data requested were discussed and clarified. Within a month and a half, GSK had approved the proposal and began formal discussions regarding methods for data sharing and legal requirements. A data sharing agreement was signed by GSK and Emory in June 2017 and data preparation officially began by GSK's Data Sharing Team at that time (patient anonymization, obfuscation of dates, compilation of raw datasets and code books, etc.). By late July 2017, the first collection of trial data were made available and additional trial data were added to the data sharing portal through September 2017. The following year was spent reviewing trial protocols in detail, becoming oriented with the available data and dataset structures, and preparing for analysis.

Each of these trials is a randomized, double-blind, placebo controlled, phase II or III clinical trial with similar study designs in terms of data collection techniques, vaccine dose and administration, immunogenicity outcome measures and follow-up protocols where applicable (Table 3). Protocol consistencies across studies enabled combination of the individual-level data for pooled analyses. The data sets provided by GSK for this analysis were comprised of carefully collected, detailed data on all aspects of the original trials, including, but not limited to, infant demographics, anthropometrics, vaccination dates and doses, serology data, medicines and vaccines received, and gastrointestinal illness occurring during follow-up.

Table 3. Similarities across GSK study protocols and definitions

Category	Study protocol and definitions
Inclusion criteria	• Male and female infants • Healthy subjects[a] free of all obvious health problems (established by medical history and physical exam)
Exclusion &/or elimination criteria	• Use of investigational or non-registered product (drug or vaccine) w/in 30 days prior to study vaccine dose • Planned administration of a vaccine not foreseen by the study protocol w/in 14 days of study vaccine dose • Chronic administration (defined as >14 days) of immunosuppressants anytime since birth • Any confirmed or suspected immune-suppressive or deficient condition based on medical history and exam • Significant history of chronic gastrointestinal disease • History of allergic reaction to any vaccine component • Acute disease, defined as the presence of a moderate or severe illness w/ or w/o fever, at the time of enrollment (warrants deferral of vaccination) • Administration of immunoglobulins and/or blood product since birth or planned administration during the study
Vaccine	• GSK RIX 4414 HRV vaccine • Vaccinated arm w/ viral suspension of $\geq 10^{6.0}$ $CCID_{50}$[b] • Doses administered 1-2 months apart
Medical exam & history	• Medical exam and history obtained at enrollment

	• Concomitant medications/vaccinations, history of medication/vaccination recorded at study visits • Anthropometric measurements obtained
Gastro-intestinal illness*	• Defined as diarrhea w/ or w/o vomiting • Diarrhea defined as ≥3 looser than normal stools in 24hr • Severity measured on Vesikari scale • Symptoms, duration, medical treatment sought recorded on a diary card provided by the study
Stool samples*	• Collected as soon as possible and no later than 7 days of severe gastrointestinal illness • Tested via ELISA, including RV strain determination
Serology	• Collected 1-2 months after final vaccine dose • Samples tested via ELISA, assay cutoff of ≥20 anti-rotavirus IgA U/mL

* Only collected in studies with follow-up for RVGE (Aim 2)

[a] Study NCT0042074 included "medically stable" preterm infants

[b] Highest viral suspensions of $10^{4.7}$ and $10^{5.8}$ median $CCID_{50}$ in NCT00385320 and NCT00425737, respectively

Because a primary objective of this study was to uncover host factors contributing to differences in vaccine immunogenicity and efficacy between low and high child mortality settings, GSK clinical trials that occurred in countries of varying levels of development were included in the combined dataset. The final dataset was comprised of infants from 33 countries/territories listed in Table 4 by child mortality stratum. Each country's mortality stratum was identified using WHO classification methods.[145] WHO divides countries into mortality strata based on under 5 mortality rates for all WHO Member Countries from the year 1999. The countries are divided into quintiles

with countries in the lowest quintile considered "very low child mortality," the second and third quintiles considered "low child mortality," and the highest two quintiles considered "high child mortality."[145] For this study, the same methods were applied to UNICEF under 5 mortality rate estimates[146] for all WHO Member Countries in 2004, the mean start year for the GSK trials. For analysis purposes, the "very low" and "low" child mortality strata were combined to represent settings with low child mortality.

Table 4. GSK study sites by mortality stratum[145–148]

Very Low	**Low**	**High**
Canada	Argentina	Bangladesh
Czech Republic	Brazil	Dominican Republic
Finland	Chile	India
France	China	Malawi
Germany	Colombia	South Africa
Hong Kong	Honduras	
Italy	Mexico	
Japan	Nicaragua	
Korea, Republic of	Panama	
Poland	Peru	
Portugal	Philippines	
Singapore	Venezuela	
Spain	Vietnam	
Taiwan		
United States		

A basic description of the number of infants and data available for the GSK trials is provided in Table 5. Data used for Aim 1 analyses were restricted to participating infants who received the rotavirus vaccine (as opposed to placebo) and from whom post-vaccination blood samples were collected approximately 4 – 12 weeks after receipt of the final vaccine dose. Of the GSK trials requested, a total of 53,292 infants received the rotavirus vaccine and post-vaccine serology data were collected from 7,300 (13.7%) of these infants.

Aim 2 used a subset of the GSK clinical trial data described for Aim 1. As with Aim 1, the data were restricted to infants who received rotavirus vaccine and for whom serology data were available. Aim 2 data were further limited to the clinical trials in which children were followed up to 1 or 2 years of age during which time data on gastroenteritis episodes were collected. A total of 5,817 infants had follow-up data. Two follow-up periods were defined for this analysis: post-vaccine IgA sample up to 1 year of age (5,817 children) and 1 year of age up to 2 years of age (4,517 children).

Table 5. Basic details of GSK clinical trials available for Aim 1 and Aim 2

Study ID (NCT#)	GSK Study ID	Study Sites	Study Phase	Age at Dose 1 (Wks)	Vacc-inated (n)	Serology (n)		Follow-Up (n)	
						Pre-Vacc	Post-Vacc	1 yr	2 yr
00480324	107625	Japan	3	6-14	492	34	34	34	34
00140673	444563/023	Argentina, Brazil, Chile, Colombia, **Dominican Republic**, Finland, Honduras, Mexico, Nicaragua, Panama, Peru, Venezuela	3	6-13	29,753	393	393	393	381
00139347	444563/024	Argentina, Brazil, Colombia, **Dominican Republic**, Honduras, Panama	3	6-12	4,234	0	176	176	56
00197210	444563/028/ 029/030	Singapore, Hong Kong, Taiwan	3	6-17	5,215	115	115	115	114
00140686	102247	Czech Republic, Finland, France, Germany, Italy, Spain	3	6-14	2,613	787	787	787	784
00241644	102248	**Malawi, South Africa**	3	5-10	2,803	221	2,297	2,295	1,577
01171963	113808	China	3	6-16	1,518	391	391	391	374
00429481	444563/007	Singapore	2	11-17	1,737	453	454	454	448

00425737	444563/004	Finland	2	6-12	249	209	209	209	204
00729001	444563/005	Canada, US	2	6-12	372	239	270	270	175
00385320	444563/006	Brazil, Mexico, Venezuela	2	6-12	1,498	427	432	432	280
00383903	444563/013	**South Africa**	2	5-10	337	262	264	261	90
00345956	105722	Vietnam	3	6-10	281	249	249		
00289172	103792	**India**	3	8-10	173	115	115		
00757770	444563/033	Columbia, Mexico, Peru	3	6-12	683	466	468		
00420745	106481	France, Poland, Portugal, Spain	3	6-14	655	147	147		
00134732	103478	Republic of Korea	3	6-12	99	48	48		
None	101555	Philippines	2	6-12	95	76	76		
00432380	109216	Philippines	2	5-10	292	240	240		
00139334	103992	**Bangladesh**	2	5-7	193	134	135		
Total					53,292	5,006	7,300	5,817	4,517

*Countries in bold are classified as high child mortality

3.2 Healthcare Cost and Utilization Project State Inpatient Database

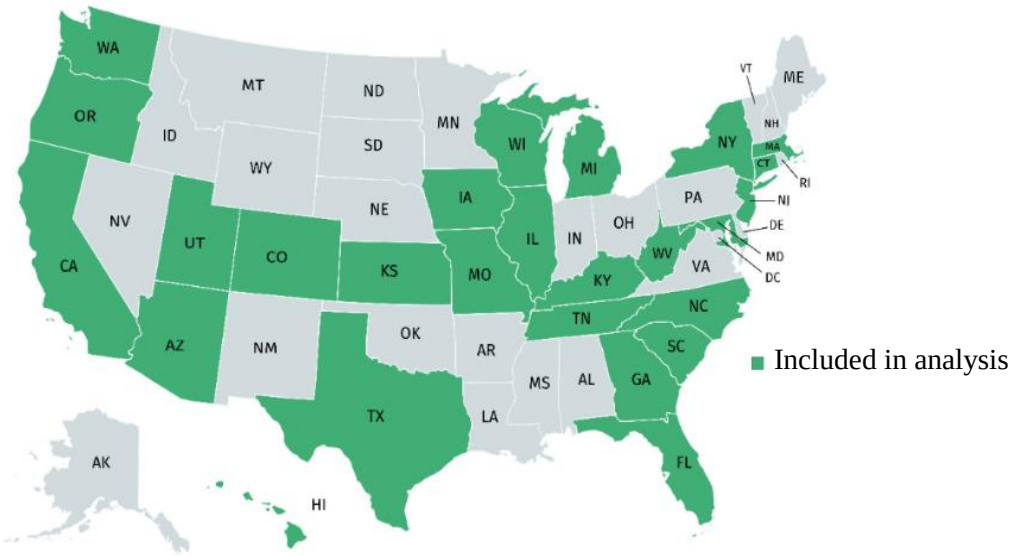
To assess Aim 3, hospitalization data from 2000 – 2013 were obtained from the Healthcare Cost and Utilization Project (HCUP) State Inpatient Database (SID),[149] sponsored by the Agency for Healthcare Research and Quality (AHRQ) within the United States Department of Health and Human Services. The data were available thanks to collaboration with the Division of Viral Diseases at the Centers for Disease Control and Prevention (CDC), National Center for Immunization and Respiratory Diseases.

The HCUP SID is a compilation of monthly discharge data from all community hospitals within participating states.[149] This analysis was restricted to the 26 states for which data were available for the entire 14 year study period: Arizona, California, Colorado, Connecticut, Florida, Georgia, Hawaii, Iowa, Illinois, Kansas, Kentucky, Maryland, Massachusetts, Michigan, Missouri, North Carolina, New Jersey, New York, Oregon, South Carolina, Tennessee, Texas, Utah, Washington, Wisconsin, and West Virginia (Figure 6). These 26 states represent approximately three-quarters (74.25%) of the total United States population in 2013.[150] Available data included the number of acute gastroenteritis and rotavirus hospitalizations by year, month, state, age group, sex and race/ethnicity.

Rotavirus or acute gastroenteritis diagnoses were extracted from discharge data. The International Classification of Diseases, Ninth Revision (ICD-9) code of 008.61 noted as the primary diagnosis or listed in any of the other diagnosis fields in a discharge record was used to identify rotavirus hospitalizations. Rotavirus-coded discharges are likely the most specific indicator of rotavirus

rates, however, restricting the analysis to only rotavirus-specified episodes may underestimate the true rotavirus burden because of limited testing for rotavirus in the clinical setting.[151] Therefore, data on all-cause acute gastroenteritis were compiled using the same method as described for rotavirus and included bacterial, parasitic, and viral gastrointestinal illness of determined cause and presumed infectious or noninfectious gastrointestinal illness of undetermined cause.[152] A total of 13,527,516 acute gastroenteritis hospitalizations and 224,099 rotavirus hospitalizations from 2000-2013 were analyzed.

Figure 6. States included in the HCUP SID analysis



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3.3 National Center for Health Statistics Bridged Race data

The SID data were combined with state population data from the National Center for Health Statistics (NCHS) Bridged Race population dataset[153] developed by the United States Census

Bureau, enabling calculation of rotavirus and acute gastroenteritis rates for use in Aim 3. These data included population numbers by state, age and race/ethnicity.

3.4 MarketScan

Data for the Aim 3 supplemental study was obtained from the IBM® MarketScan® Commercial Database. Access to MarketScan® data were again available through a collaboration with the Division of Viral Diseases at the CDC. Working closely with collaborators in the Division of Viral Diseases, we conceptualized the structure of the database and extracted data tailored specifically for this study.

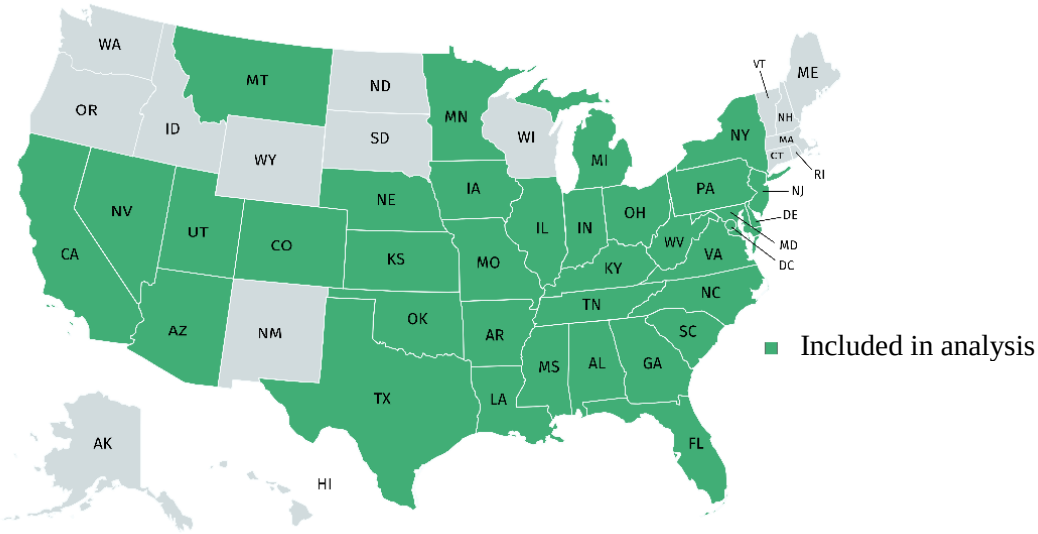
The MarketScan® Commercial Database is a collection of national medical claims and encounters data from commercially insured individuals aged 64 years and younger in the United States. The database contains individual level, de-identified information on several million individuals each year.[154] Detailed insurance enrollment, inpatient medical data and outpatient medical data are available for employees, their spouses and dependents with employer-sponsored health care insurance. A variety of health plans are contained in the dataset including, but not limited to Preferred Provider Organizations (PPOs), point-of-service (POS) plans and Health Maintenance Organizations (HMOs). Notably, claims covered by Medicaid are not available. Data are collected in all states.

A time series database was created which described monthly rotavirus gastroenteritis and all-cause acute gastroenteritis hospitalization rates for July 2001 through June 2016 (the latest available data). Monthly counts of rotavirus gastroenteritis hospitalizations included individuals with a

rotavirus International Classification of Diseases, Ninth and Tenth Revision (ICD-9 and ICD-10) code of 008.61 and A08.0, respectively. Additional data on all-cause acute gastroenteritis were also compiled to capture possible rotavirus gastroenteritis not specifically identified as rotavirus-related due to imperfect coding.[151] Records which indicated rotavirus gastroenteritis and/or all-cause acute gastroenteritis ICD-9/10 codes in one of 15 diagnosis fields from inpatient admission claims were included in the analysis.

A total of 9,211 episodes of rotavirus gastroenteritis and 726,528 episodes of all-cause acute gastroenteritis were extracted among individuals from 0 through 64 years of age. The number of enrollment member days were summed by month, year, age group, and vaccination status to provide the monthly enrolled population denominator and enable calculation of rates. Rotavirus vaccination status was tracked beginning in July 2006 when the first cohort of newborns became age-eligible for vaccination the following month.[130] Any infant who received one or more doses of either available rotavirus vaccine, RotaTeq or Rotarix, was considered vaccinated. Individuals from all states, except those residing in states with universal vaccine purchasing programs, which provide immunizations to children free of charge (Alaska, Connecticut, Idaho, Massachusetts, Maine, North Dakota, New Hampshire, New Mexico, Oregon, Rhode Island, South Dakota, Vermont, Washington, Wisconsin, and Wyoming) were included in the analysis (Figure 7). This was critical as vaccination in these states may not be recorded in insurance claim records compiled in the database.

Figure 7. States included in the MarketScan analysis



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4 Aim 1- Vaccine immunogenicity

[Manuscript 1]

Oral polio vaccine interferes with full-course rotavirus vaccine immunogenicity: an individual level analysis of pooled clinical trial data in high and low child mortality settings

Julia M. Baker, Jacqueline E. Tate, Juan Leon, Michael J. Haber, Benjamin A. Lopman

Abstract

Background: Despite the success of rotavirus (RV) vaccines over the last decade, RV remains a leading cause of severe diarrheal disease among young children. Further progress in reducing the burden of disease is inhibited, in part, by vaccine underperformance in certain settings. Individual level characteristics may influence vaccine immunogenicity by reducing the effective vaccine titer delivered to the intestines or impairing immune response to vaccination. Early trials suggest that oral polio vaccine (OPV), when administered concomitantly with RV vaccine, reduces RV seroconversion rates after the first RV dose but that RV vaccine immunogenicity is unaffected after completion of the full RV vaccine course. This study aimed to identify a range of individual-level characteristics that contribute to RV vaccine immunogenicity measured via serum anti-RV immunoglobulin A (IgA) in high and low child mortality settings controlling for individual and country level factors.

Methods: Pooled, individual level data from 22 of GlaxoSmithKline's phase II and III clinical trials of the Rotarix vaccine across 33 countries/territories were analyzed. Two standard markers for immune response were examined including seroconversion (defined as the appearance of serum anti-rotavirus IgA antibodies in subjects initially seronegative) and serum anti-rotavirus IgA titer, both collected approximately 4-12 weeks after the last administration of RV vaccine. Mixed effect logistic regression and mixed effect linear regression of log-transformed data were used to identify individual and country level predictors of seroconversion (dichotomous) and antibody titer (continuous), respectively.

Results: Data from 7,280 vaccinated infants were analyzed. A higher proportion of infants in low child mortality settings seroconverted (77%) compared to high child mortality settings (62%). Similarly, among those who seroconverted, post-vaccine anti-rotavirus IgA titers of infants in low child mortality settings (geometric mean = 240, SD = 4) were higher than that of infants in high child mortality settings (199, SD = 4). Infants who received OPV concomitantly with both their first and second doses of RV vaccine were 37% less likely to seroconvert (OR = 0.63, 95% CI = 0.47, 0.84) compared to infants who received OPV but not concomitantly with either dose. Few modifiable factors were found to be associated with IgA titer (time from last rotavirus dose to serology, $\beta = 0.92$, 95% CI = 0.90, 0.95).

Conclusions: Our findings strongly suggest that OPV given concomitantly with RV vaccine is a substantial contributor to reduced immunogenicity and this interference is apparent after the second RV dose. The eventual withdrawal of OPV from the infant immunization schedule could improve RV vaccine immunogenicity.

Background

Globally, rotavirus (RV) is the leading cause of severe diarrheal disease among infants and children under 5 years of age, estimated to cause 128,500–215,000 deaths in this age group each year.[6,7] The virus is highly infectious and improvements in water, sanitation and hygiene conditions have limited impact in reducing its spread.[55] RV is a ubiquitous infection among young children,[6] the majority of whom will experience at least one RV infection in the first two years of life.[10–12] As such, vaccination is an essential public health measure for preventing infections and reducing the severity of RV gastroenteritis.[54]

Currently, four live attenuated, oral RV vaccines administered during infancy have received World Health Organization (WHO) prequalification: GlaxoSmithKline's (GSK's) monovalent vaccine (Rotarix), Merck's pentavalent vaccine (RotaTeq), the Serum Institute of India's pentavalent vaccine (Rotasiil), and Bharat Biotech's monovalent vaccine (Rotavac).[59] Since the first clinical trial results were released in 2006 (GSK and Merck),[60–62] RV vaccines have been integrated into the national immunization programs of approximately 96 countries.[65] The introduction of these vaccines has led to dramatic reductions in RV disease in many settings.[60,61,73,92,93] Despite this success, RV remains a leading cause of severe diarrheal disease among young children[7] and continues to be the predominant cause of hospitalization for severe diarrheal disease in certain settings, even in countries where the vaccine is in use.[94,95]

A primary obstacle preventing further reductions in the RV burden is vaccine underperformance in settings where the incidence of severe disease and death is highest.[7,96] An estimated 85% of RV-

related deaths occur among children in Africa and Asia,[7] and in these high child mortality settings, vaccine efficacy against severe gastroenteritis can be as low as 36%.[155,97] In contrast, vaccine efficacy is greater than 90% in low child mortality settings.[62,73,97] Similarly, RV vaccines are substantially less immunogenic in high child mortality settings when compared to low child mortality settings.[73,97] RV vaccine immunogenicity, represented by serum anti-RV immunoglobulin A (IgA) antibodies, has been shown to vary by country and is inversely associated with a country's under 5 mortality rate at the country level.[99]

Reasons for this disparate immunogenicity and efficacy are poorly understood.[98,156] Individual level characteristics may play a central role in vaccine immunogenicity by reducing the effective vaccine virus titer delivered to the intestines or impairing immune response to vaccination.[98,156] For example, IgG antibodies received from breast milk might decrease the effective vaccine virus titer delivered to the gut while malnutrition may reduce an infant's immune response to the vaccine.[98] Additional factors that may compromise vaccine performance relate to genetic susceptibility to RV,[18,45–47] differences in force of infection,[73,98,102] and environmental enteropathy (chronic inflammation).[103]

One potentially modifiable factor is interaction with live oral polio vaccine (OPV). Polio vaccines are generally administered on the same schedule as RV. In low child mortality settings, inactivated polio vaccine (IPV) is primarily administered, whereas in high child mortality settings, OPV is used and frequently given concomitantly with RV vaccines.[106] OPV administered concomitantly with RV vaccine has been shown to reduce RV seroconversion rates.[107–110,157] Early clinical trials suggest that this inhibitory effect is largely restricted to the first RV vaccine dose and that vaccine

immunogenicity is unaffected after completion of the full RV vaccine course.[158] In contrast, there is limited evidence that interference from OPV may persist after two doses of RV vaccine.[107] If and how changes in the polio eradication strategy, including modification of viral strains included in the vaccine and the eventual global OPV withdrawal,[159] may impact RV vaccine performance remains to be seen.

Developing strategies to improve vaccine performance may first require identification of individual level factors associated with immune response. Isolating factors associated with vaccine immunogenicity across settings may highlight potentially modifiable vaccine strategies or interventions for enhancing vaccine performance and further reducing the burden of RV disease. RV vaccine clinical trials were powered to assess vaccine efficacy, however, they were not specifically designed to identify individual level factors associated with vaccine response. This study used pooled clinical trial data to identify a range of individual level characteristics that contribute to Rotarix vaccine immunogenicity measured via serum anti-rotavirus IgA in high and low child mortality settings controlling for individual and country level factors.

Methods

GSK clinical trial data

We pooled individual-level data from infants enrolled in GSK's phase II and III clinical trials of the Rotarix vaccine (Table 1). Rotarix is an oral two-dose vaccine based on a live, attenuated human rotavirus strain (G1P[8]). GSK recommends that the first dose be administered beginning

at 6 weeks of age and the second dose be given after an interval of four or more weeks and by 24 weeks of age.[70]

The 22 trials included were randomized, double-blind, placebo-controlled trials conducted in a total of 33 countries/territories including 28 low/very low child mortality and 5 high child mortality countries (Figure 1). The child mortality strata were based on 2004 under 5 mortality rates as previously described.[145,146] Research protocols across trials were similar in terms of data collection techniques, vaccine administration, and immunogenicity outcome measures (Table 2). Since the primary aim of the analysis was to examine factors associated with RV vaccine immunogenicity, data were limited to trial participants who received the Rotarix vaccine (n = 53,292). Data were further restricted to infants whose trial participation was completed according to protocol (classified by GSK) and who participated in the RV immunogenicity sub-studies of the trials (n = 8,309). Lastly, infants who had serum sample collection approximately 4-12 weeks from receipt of his/her last rotavirus vaccine dose were included (n = 7,298).

Figure 1. GSK clinical trial sites

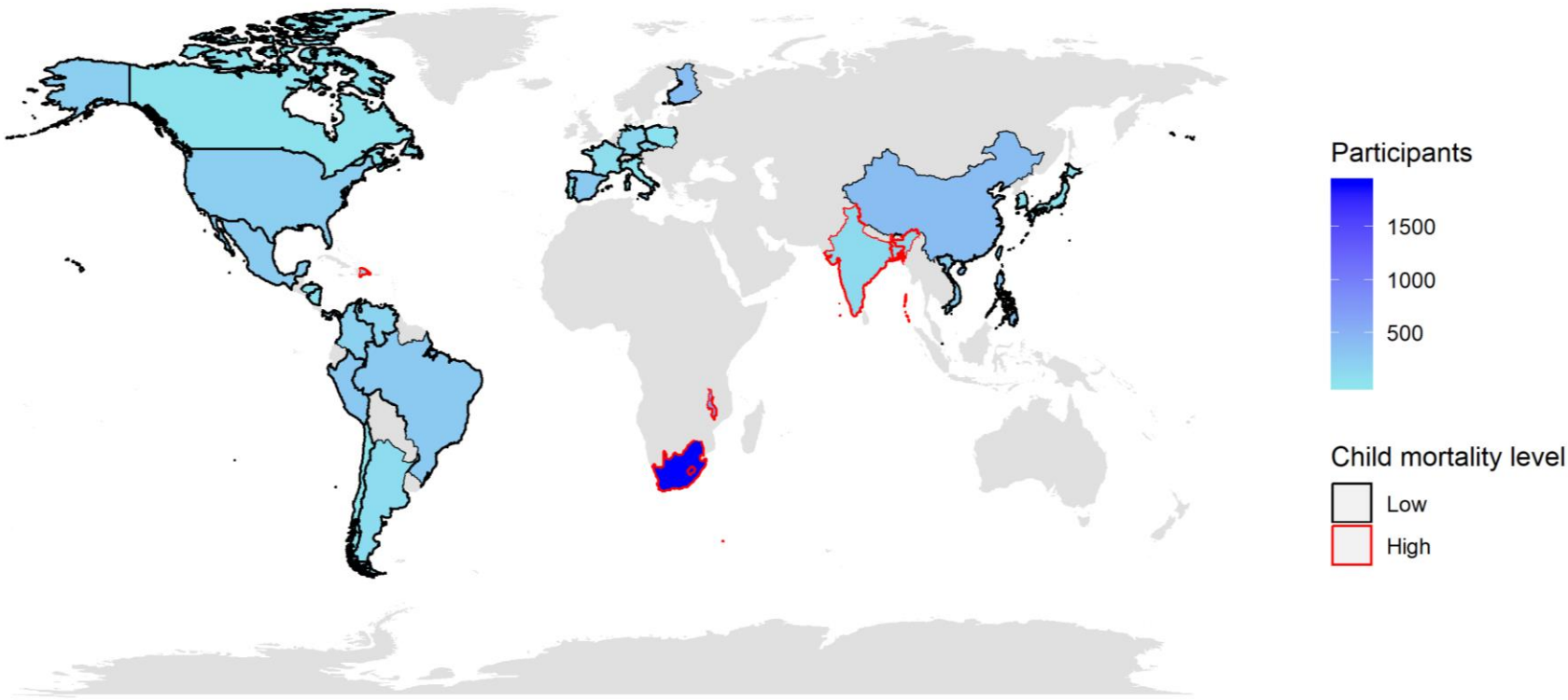


Table 1. Trial characteristics by GSK trial ID

GSK Trial Number	Study Sites	Study Phase	Age at Dose 1 (Wks)	Vacc-inated (n)	Serology (n)	
					Pre-Vacc	Post-Vacc
107625	Japan	3	6-14	492	34	34
444563/023	Argentina, Brazil, Chile, Colombia, **Dominican Republic**, Finland, Honduras, Mexico, Nicaragua, Panama, Peru, Venezuela	3	6-13	29,753	393	393
444563/024	Argentina, Brazil, Colombia, **Dominican Republic**, Honduras, Panama	3	6-12	4,234	0	176
444563/028/029/030	Singapore, Hong Kong, Taiwan	3	6-17	5,215	115	115
102247	Czech Republic, Finland, France, Germany, Italy, Spain	3	6-14	2,613	787	787
102248	**Malawi, South Africa**	3	5-10	2,803	221	2,295
113808	China	3	6-16	1,518	391	391
444563/007	Singapore	2	11-17	1,737	453	454
444563/004	Finland	2	6-12	249	209	209
444563/005	Canada, US	2	6-12	372	239	270
444563/006	Brazil, Mexico, Venezuela	2	6-12	1,498	427	432

444563/013	**South Africa**	2	5-10	337	262	264
105722	Vietnam	3	6-10	281	249	249
103792	**India**	3	8-10	173	115	115
444563/033	Columbia, Mexico, Peru	3	6-12	683	466	468
106481	France, Poland, Portugal, Spain	3	6-14	655	147	147
103478	Republic of Korea	3	6-12	99	48	48
101555	Philippines	2	6-12	95	76	76
109216	Philippines	2	5-10	292	240	240
103992	**Bangladesh**	2	5-7	193	134	135
Total				53,292	5,006	7,298

Countries in bold are classified as high child mortality

Table 2. Similarities across GSK trial protocols and definitions

Category	Study protocol and definitions
Inclusion criteria	• Male and female infants • Healthy subjects[a] free of all obvious health problems (established by medical history and physical exam)
Exclusion &/or elimination criteria	• Use of investigational or non-registered product (drug or vaccine) w/in 30 days prior to study vaccine dose • Planned administration of a vaccine not foreseen by the study protocol w/in 14 days of study vaccine dose • Chronic administration (defined as >14 days) of immunosuppressants anytime since birth • Any confirmed or suspected immune-suppressive or deficient condition based on medical history and exam • Significant history of chronic gastrointestinal disease • History of allergic reaction to any vaccine component • Acute disease, defined as the presence of a moderate or severe illness w/ or w/o fever, at the time of enrollment (warrants deferral of vaccination) • Administration of immunoglobulins and/or blood product since birth or planned administration during the study
Vaccine	• GSK RIX 4414 HRV vaccine • Vaccinated arm w/ viral suspension of $\geq 10^{6.0}$ $CCID_{50}$[b] • Doses administered 1-2 months apart
Medical exam & history	• Medical exam and history obtained at enrollment

	• Concomitant medications/vaccinations, history of medication/vaccination recorded at study visits • Anthropometric measurements obtained
Serology	• Collected 1-2 months after final vaccine dose • Samples tested via ELISA, assay cutoff of ≥ 20 anti-rotavirus IgA U/mL

[a] Study 106481 included "medically stable" preterm infants

[b] Highest viral suspensions of $10^{4.7}$ and $10^{5.8}$ median $CCID_{50}$ in 444563/004 and 444563/006, respectively

Explanatory variables, covariates and endpoints of interest

All available data for trial participants was provided by GSK and explanatory variables/covariates were selected for inclusion in the analysis based on existing literature.[98,156] GSK data were supplemented with country-level data on gross domestic product (GDP) per capita in 2004 USD[160,161] to represent a country's level of development and 2004 under 5 mortality rates.[162–164] These country level variables were considered in an effort to capture potential confounders that remained unmeasured despite the individual level factors available.

Two standard markers for immune response[165,166] were analyzed as outcomes. First, in accordance with pre-specified trial definitions, seroconversion was defined as the appearance of serum anti-rotavirus IgA antibodies (i.e. concentrations ≥ 20 U/mL) in subjects initially (prior to the first RV dose) seronegative. The second endpoint of interest was serum anti-rotavirus IgA titer among infants who seroconverted. In all trials, post-vaccine anti-rotavirus IgA data were collected

approximately 4-12 weeks after the last administration of RV vaccine and were measured using enzyme-linked immunosorbent assay (ELISA) techniques.[167]

Statistical analysis

Regression models were fit to estimate the effects of individual and country level factors on vaccine immunogenicity outcomes while controlling for potential confounders. Mixed effect logistic regression and mixed effect linear regression of log-transformed data (to create a normal distribution) were used to analyze the anti-rotavirus IgA seroconversion (dichotomous) and anti-rotavirus IgA antibody titer (continuous) outcomes, respectively. Basic formulas for each model are shown in the Supplemental Material below. Models were run using the "lme4" package in R software.

The data used for modeling anti-rotavirus IgA seroconversion included all infants who were either confirmed to be seronegative prior to vaccination via serology sample or who did not have pre-vaccine serology data available. Prior confirmed RV gastroenteritis was an exclusion criterion in a majority of the GSK trials so for the primary analysis in this study, infants without pre-vaccine serology samples were assumed to be seronegative. A sub-analysis was conducted limiting data to infants with confirmed pre-vaccine seronegative status. The data for modeling anti-rotavirus IgA titer was restricted to infants who were seropositive after vaccination.

The modeling strategy for both outcomes began with variable specification, incorporating all individual level characteristics as explanatory variables and controlling for potential country level

covariates selected based on variable distributions and existing literature. Individual level variables in the initial model included: time from last RV dose to serology sample, number of RV vaccine doses, age at first vaccine dose, vaccine concentration, sex, length-for-age z-score (LAZ) to represent nutritional status, and concomitant OPV. Country level variables in the initial model included: GDP, under 5 mortality rate, and child mortality stratum (dichotomous). Table 3 in the results section provides details on how each variable was measured. Any infants missing data were excluded from the models. Random effects were employed using a random intercept for each trial to account for potentially unmeasured differences between trial protocols or environments. The initial model included all explanatory variables of interest and incorporated interaction terms between each main effect and child mortality stratum. The model was applied to combined data from both child mortality strata and backwards elimination was then conducted using an $\alpha = 0.10$ cut-off for inclusion and maintaining a hierarchically well-formulated model throughout.

Next, models were refined by investigating alternative measures of the included variables based on possible relationships identified in bivariate analyses. For example, higher order terms for continuous variables (such as age and age-squared) were considered where appropriate and more detailed stratification of categorical variables were tested. Backwards elimination was subsequently performed after each variable was modified. Where initial models were prohibitively large and issues with model convergence were encountered, the most informative end models resulting from previous backwards elimination procedures were used as the starting point for investigation of refined measures. After all variables were explored, the most parsimonious model with all relevant variables and covariates was selected as the final model using Akaike information criterion (AIC) criteria (lower AIC indicating a better model).

A sensitivity analysis (including only children who seroconverted) was conducted by fitting the final model separately to data stratified by high and low-child mortality settings. The purpose of this sensitivity analysis was to determine if the model built with the joint data was supported within a given child mortality stratum. In other words, this sensitivity analysis aimed to demonstrate that the relationships identified using the full, combined dataset remained consistent within each mortality stratum; this would indicate that the combined data were capturing differences beyond those driven by child mortality stratum alone. Lastly, the final seroconversion model was tested using a subset of the data comprised of infants who were confirmed to be seronegative prior to vaccination This sub-analysis was conducted to confirm that the results produced using the full seroconversion dataset were consistent with results using infants with both pre- and post-vaccine serology. If true, these consistent results would indicate that inclusion of infants without pre-vaccine serology data was not causing substantial bias.

Ethical approval

All identifiers for trial participants were de-identified and all dates obfuscated by GSK prior to data sharing. This study was determined to be non-human subjects research by the Emory University Institutional Review Board.

Results

Data on 7,298 infants whose post-vaccine RV serology data were collected 4-12 weeks after receipt of the final vaccine dose (87.8% of the RV immunogenicity cohort) were available for analysis. 39.0% (n=2,849) of these infants were from high child mortality settings. A total of 18 infants (3 from high child mortality settings) were excluded from analysis due to pre-vaccine serology data indicating these children had prior RV infection (i.e. anti-rotavirus IgA titer of ≥ 20 U/mL). All other infants had either confirmed seronegative status or did not have serology data available and were assumed to be seronegative based on trial protocol. Data on 7,280 and 5,161 infants were included in the seroconversion and titer modeling, respectively.

All infants received either two or three doses of RV vaccine; nearly all infants in low child mortality settings received two doses (99%) while nearly half of infants in high child mortality settings received a third dose (45%, $p < 0.001$). In low child mortality settings, one-quarter of infants received a reduced concentration of the vaccine (viral suspension $< 10^{6.0}$ $CCID_{50}$), whereas all infants from high child mortality settings received a "standard" concentration (viral suspension $\geq 10^{6.0}$ $CCID_{50}$, $p < 0.001$) (Table 3).

Table 3. Vaccine, individual and country level characteristics of infants from 22 trials conducted in 33 countries/territories beginning in 2000-2010

	All countries, N = 7,280	Low child mortality settings, N = 4,434	High child mortality settings, N = 2,846
Vaccine characteristics, n (%)			
Standard vaccine concentration	6,208 (85)	3,362 (76)	2,846 (100)
2 RV doses (vs. 3 doses)	5,971 (82)	4,393 (99)	1,578 (55)
Individual level characteristics, n (%)			
Female	3,618 (50)	2,194 (49)	1,424 (50)
Length-for-age z-score			
Not stunted (ref)	5,604 (77)	3,433 (77)	2,171 (76)
Stunted	588 (8)	215 (5)	373 (13)
Severely stunted	397 (6)	135 (3)	262 (9)
Missing	691 (9)	651 (15)	40 (1)
OPV concomitant with RV dose			
Neither dose 1 nor dose 2 (ref)	1,835 (25)	1,614 (36)	221 (8)
Dose 1 only	14 (0.2)	3 (0.1)	11 (0.4)
Dose 2 only	26 (0.4)	18 (0.4)	8 (0.3)
Both dose 1 and dose 2	3,384 (46)	778 (18)	2,606 (92)
No OPV received	2,021 (28)	2,021 (46)	0 (0)

Individual level characteristics, median (IQR)			
Age at first RV dose (weeks)	9 (7, 11)	9 (8, 12)	9 (6, 11)
Time from last RV dose to post vaccine serology (weeks)	5 (5, 8)	8 (5, 9)	5 (5, 5)
Age at post-vaccine serology (weeks)	22 (21, 26)	24 (21, 27)	21 (20, 22)
Country level characteristics, median (IQR)			
GDP (2004, in USD)	4,745 (1,509, 15,356)	7,311 (2,448, 27,405)	4,745 (461, 4,745)
Under 5 mortality rate [a]	27 (8, 85)	19 (5, 26)	85 (85, 85)
Serology outcomes			
Seropositive after vaccination,[b] n (%)	5,161 (70)	3,411 (77)	1,750 (62)
Post-vaccine IgA titer among seroconverted, geometric mean (SD)	226 (4)	240 (4)	199 (4)

RV = rotavirus; OPV = oral polio vaccine; SD = standard deviation; IQR = interquartile range; GDP = gross domestic product

[a] Defined as deaths among children under 5 years of age per 1,000 live births

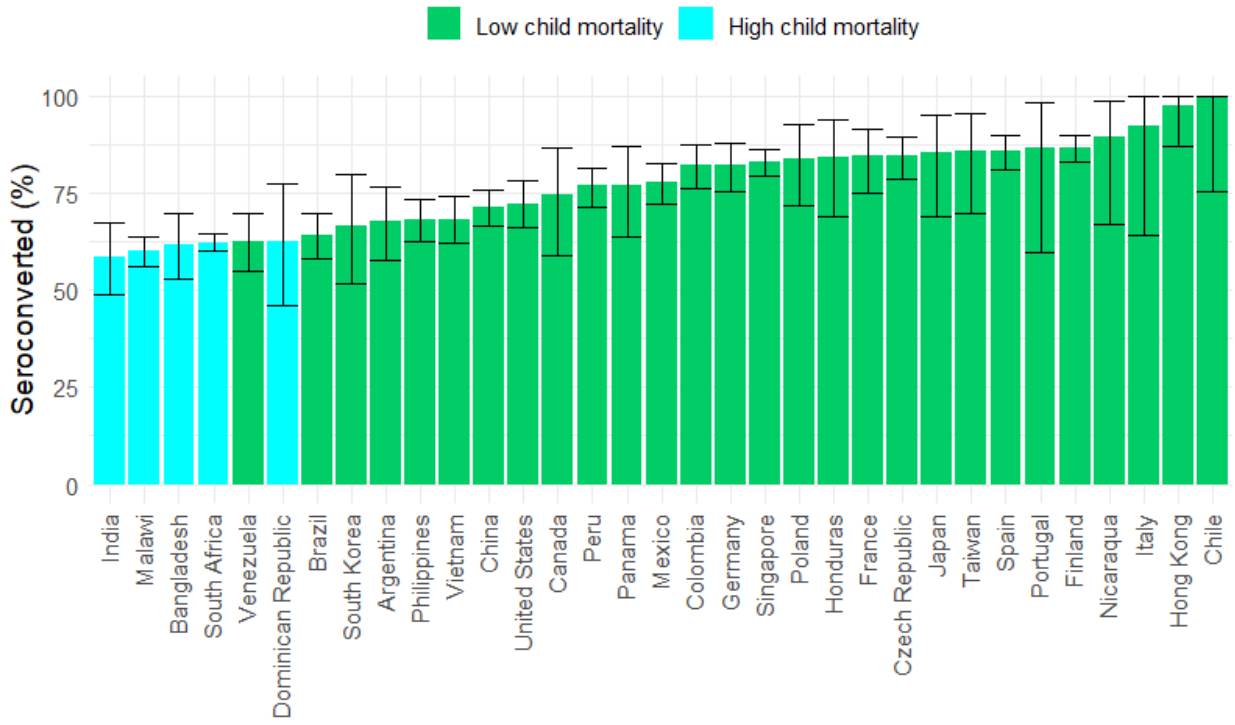
[b] Seropositive status defined as anti-rotavirus IgA titer ≥ 20 U/mL

Individual level characteristics generally differed by setting (Table 3). Over 20% of infants in high child mortality settings were stunted or severely stunted while less than 10% were in low child mortality settings ($p < 0.001$). All infants in high child mortality settings received OPV and over 90% infants received OPV concomitantly with both their first and second doses of RV vaccine. In

contrast, nearly half of infants in low child mortality settings did not receive a single dose of OPV (as IPV was more commonly administered in these settings). The median age at receipt of the first RV dose was 9 weeks (IQR = 7, 11) and infants were a median of 22 weeks of age (IQR = 21, 26) when their post-vaccine serology sample was collected. The time from receipt of the last RV dose to serology sample was slightly longer for infants in low child mortality settings (median of 8 weeks, IQR = 5, 9) compared to infants in high child mortality settings (5 weeks, IQR = 5, 5, $p < 0.001$).

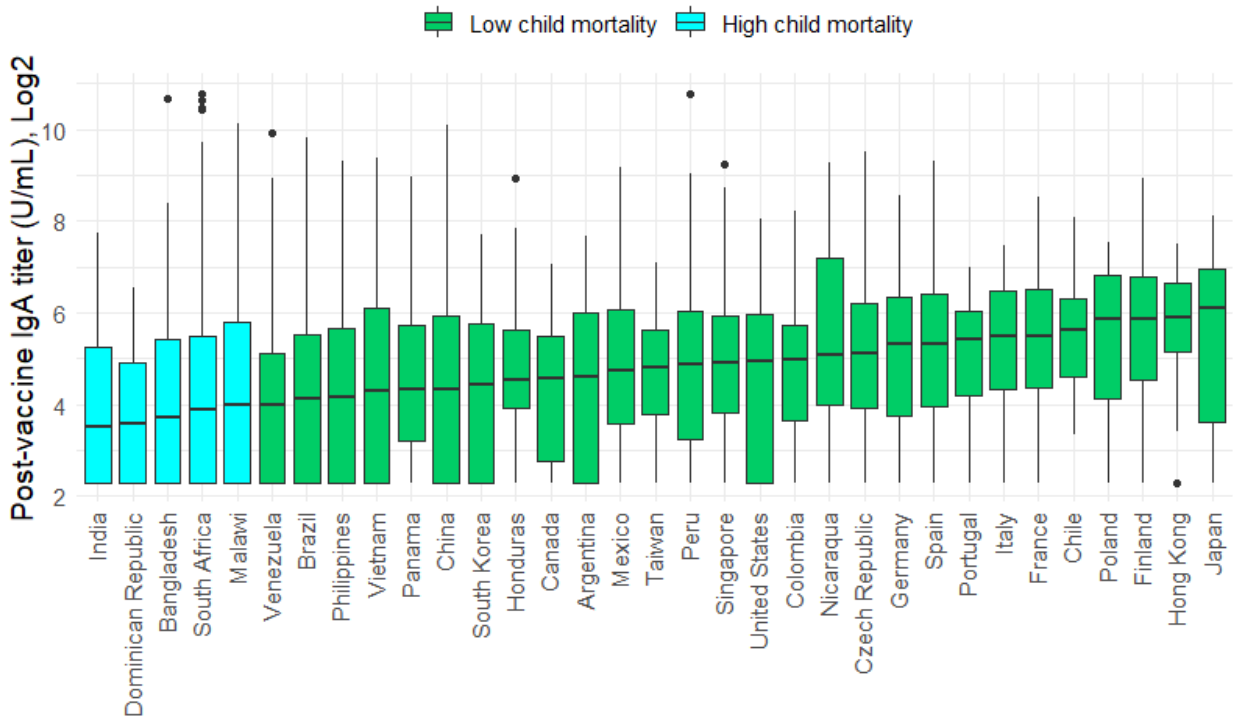
The distribution of the two outcomes of interest, anti-rotavirus IgA seroconversion and post-vaccine anti-rotavirus IgA titer, are described in Table 3 and Table 4. A majority of infants (70%) were seropositive after vaccination with a higher proportion of infants in low child mortality settings seroconverting (77%) compared to infants in high child mortality settings (62%, $p < 0.001$). Seroconversion ranged from as low as 58% in India to over 90% in Hong Kong, Italy and Chile (Figure 2a). A similar pattern was found with post-vaccine anti-rotavirus IgA titer where infants in low child mortality settings had geometric mean titers (240, SD = 4) higher than that of infants in high child mortality settings (199, SD = 4, $p < 0.001$). Anti-rotavirus IgA titer ranged from a median of 34 U/mL in India to 443 U/mL in Japan (Figure 2b).

Figure 2a. Percent of infants who seroconverted by country



Colored bars represent the percentage of infants in a given country that seroconverted, defined as an anti-rotavirus IgA titer ≥20 U/mL. Error bars indicate 95% confidence interval.

Figure 2b. Post-vaccine anti-rotavirus IgA titer (U/mL) by country



Colored bars represent median and interquartile range for post-vaccine anti-rotavirus IgA titer. Lines represent minimum and maximum values with dots representing outliers.

Table 4. Vaccine, individual and country level characteristics by anti-rotavirus IgA seroconversion and anti-rotavirus IgA titer outcomes

	Seroconversion (N = 7,280)		IgA Titer (N = 5,161)	
		Unadjusted		Unadjusted
	Seroconverted[a]	OR (95% CI)		β (95% CI)
Vaccine characteristics	**n (%)**		**mean (SD)**	
Vaccine concentration				
Standard	4,390 (71)	1 (ref)	228.5 (4)	1 (ref)
Low	771 (72)	1 (1, 1)	209.1 (3)	1 (1, 1)
Number of RV doses				
2	4,367 (73)	1.0 (ref)	229 (4)	1 (ref)
3	784 (61)	0.6 (0.5, 0.6)	208 (4)	0.6 (0.6, 0.7)
Individual level characteristics	**n (%)**		**mean (SD)**	
Sex				
Female (ref)	2,549 (70.5)	1 (ref)	233 (4)	1 (ref)
Male	2,612 (71.3)	1.0 (0.9, 1.2)	219 (4)	1 (1, 1)
Length-for-age z-score				
Not stunted (ref)	3,979 (76)	1 (ref)	227 (4)	1 (ref)
Stunted	393 (67)	0.8 (0.7, 1.0)	217 (4)	0.9 (0.7, 1.0)
Severely stunted	264 (66.5)	0.8 (0.7, 1.0)	216 (4)	0.8 (0.8, 1.0)
Missing	525 (76)	--	223 (4)	--
OPV concomitant with RV dose				
Neither dose 1 nor dose 2 (ref)	1,358 (74)	1 (ref)	203 (4)	1 (ref)
Dose 1 only	7 (50)	0.4 (0.1, 1.0)	209 (3)	0.5 (0.2, 1.3)

Dose 2 only	17 (65)	0.7 (0.3, 1.5)	1.67 (4)	0.7 (0.3, 1.4)
Both dose 1 and dose 2	2,109 (62)	0.6 (0.5, 0.7)	211 (4)	0.7 (0.7, 0.8)
No OPV received	1,670 (93)	1.7 (1.4, 2.0)	268 (4)	1.6 (1.5, 1.8)
Individual level characteristics	**median (IQR)**		**median (IQR)**	
Age at first RV dose (weeks)		1 (1, 1)	9 (7, 12)	1 (1, 1)
Seroconverted	9 (7, 12)			
Did not seroconvert	9 (7, 1)			
Time from last RV dose to post-vaccine serology (weeks)		1 (1, 1)	5 (5, 9)	1 (1, 1)
Seroconverted	5 (5, 9)			
Did not seroconvert	5 (5, 8)			
Age at post-vaccine serology (weeks)		1 (1, 1)	23 (21, 26)	1 (1, 1)
Seroconverted	23 (21, 26)			
Did not seroconvert	22 (20, 25)			
Country level characteristics	**median (IQR)**		**median (IQR)**	
Log(GDP)		1 (1, 1)	2 (0, 2)	1 (1, 1)
Seroconverted	2 (0, 2)			
Did not seroconvert	2 (1, 3)			
Log(Under 5 mortality rate)			4 (3, 4)	0.8 (0.7, 0.8)
Seroconverted	4 (3, 4)			
Did not seroconvert	3 (2, 4)			

Country level characteristics	n (%)		mean [b] (SD)	
Child mortality status				
Low child mortality	3,411 (77)	1 (ref)	115 (6)	1 (ref)
High child mortality	1,750 (62)	0.5 (0.4, 0.5)	63 (6)	0.5 (0.5, 0.6)

[a] Defined as anti-rotavirus IgA titer ≥ 20 U/mL

[b] Geometric mean

Anti-rotavirus IgA seroconversion modeling results

Results of backwards elimination and model refinement for seroconversion using data for both child mortality strata combined are shown in Table 5 with stratum-specific odds ratios for main effects with interaction terms shown at the bottom (age at first RV dose and LAZ). Older age at first RV dose and being in a country with higher GDP were both associated with increased likelihood of seroconversion. In contrast, increased time from last RV dose to serology, low vaccine concentration and concomitant receipt of OPV with the first and second rotavirus doses were each negatively associated with seroconversion. Infants who received OPV concomitantly with both their first and second doses of RV vaccine were 37% less likely (OR = 0.63, 95% CI = 0.47, 0.84)) to seroconvert compared to infants who received OPV but not concomitantly with either dose. Sex, the number of RV vaccine doses received and under 5 mortality rate were dropped from the model during backwards elimination.

Table 5. Final anti-rotavirus IgA seroconversion model developed using both child mortality stratum combined (n = 6,589)

Individual or country level factor	OR (95% CI)
Time from last RV dose to serology (weeks)	0.90 (0.86, 0.94)
Vaccine concentration $\geq 10^{6.0}$	1.00 (ref)
Vaccine concentration $< 10^{6.0}$	0.65 (0.49, 0.87)
OPV neither concomitant w/ RV dose 1 nor 2	1.00 (ref)
OPV concomitant w/ RV dose 1 & 2	0.63 (0.47, 0.84)
OPV concomitant w/ RV dose 1 only	0.36 (0.12, 1.05)
OPV concomitant w/ RV dose 2 only	0.66 (0.27, 1.57)
No OPV received	1.13 (0.76, 1.69)
Log(GDP)	1.11 (1.04, 1.19)
Age at 1st RV dose (weeks)	1.13 (1.08, 1.17)
LAZ: stunted or severely stunted	1.00 (ref)
LAZ: not stunted or severely stunted	1.24 (0.93, 1.65)
Child mortality setting- low	1.00 (ref)
Child mortality setting- high	1.62 (0.83, 3.14)
Age at 1st RV dose (weeks)*Child mortality setting	0.90 (0.86, 0.95)
LAZ: stunted or severely stunted*Child mortality setting	0.67 (0.47, 0.95)

OR = odds ratio; CI = confidence interval; RV = rotavirus; LAZ = length-for-age z-score; OPV = oral polio vaccine, GDP = gross domestic product

When the final seroconversion model was applied to each mortality stratum separately, similar results were observed (Supplementary Table S1). The negative relationship between OPV concomitant with both RV doses 1 and 2 or RV dose 1 only remained, though with less precision.

The results of the sensitivity analysis conducted using only infants confirmed to be seronegative prior to RV vaccination (Supplementary Table S2) were consistent with results of the original analysis. Infants who received OPV concomitantly with RV dose 1 and dose 2 were less likely to seroconvert than others (OR = 0.62, 95% CI = 0.46, 0.81)). The relationships between other variables and seroconversion displayed only modest changes.

Anti-rotavirus IgA titer modeling results

Very few factors were found to be significantly associated with anti-rotavirus IgA titers amongst those children who seroconverted (Table 6). Even when models were refined to explore alternative measures of various factors and when OPV was forced to remain in the model (based on its importance in seroconversion models), the results varied minimally. Increased time from last RV doses to serology sample was negatively associated with IgA titer. Concomitant OPV with rotavirus dose 1 and 2 was positively associated with IgA titer.

Table 6. Final anti-rotavirus IgA titer model developed using both child mortality stratum combined (n = 5,161)

Individual or country level factor	β (95% CI)
Time from last RV dose to serology (weeks)	0.92 (0.90, 0.95)
Sex (ref = female)	0.93 (0.87, 1.00)
OPV neither concomitant w/ RV dose 1 nor 2	1.00 (ref)
OPV concomitant w/ RV dose 1 & 2	1.28 (1.07, 1.53)
OPV concomitant w/ RV dose 1 only	1.32 (0.49, 3.58)
OPV concomitant w/ RV dose 2 only	0.92 (0.48, 1.79)
No OPV received	0.98 (0.75, 1.28)
Log(GDP)	1.16 (1.06, 1.28)
Child mortality setting- low	1.00 (ref)
Child mortality setting- high	0.76 (0.61, 0.93)
Log(GDP)*Child mortality setting	1.16 (1.06, 1.28)

CI = confidence interval; RV = rotavirus; GDP = gross domestic product (2004, in USD)

Discussion

In this study we had the unique ability to pool individual level data from 22 clinical trials and 33 countries to create a dataset that enabled assessment of RV vaccine immunogenicity across settings. Our findings strongly suggest that OPV given concomitantly with RV vaccine dramatically reduces anti-rotavirus IgA seroconversion through the second RV dose. We did not find the same modifiable characteristics to be associated with post-vaccine IgA titers among

infants who seroconverted, suggesting that such factors predict whether an infant will respond to RV vaccination, but not the intensity of the response given seroconversion.

These data provide robust evidence that infants who received OPV with both the first and second doses of RV vaccine were substantially less likely to seroconvert when compared to those not receiving OPV concomitantly or not receiving OPV at all. As expected, infants who did not receive OPV were more likely to seroconvert when compared to those who received OPV, regardless of timing. This analysis bolsters existing evidence[107–110,157] that OPV interferes with RV seroconversion and reveals that OPV can interfere with seroconversion when it is given with both the first and second RV doses. Early evidence indicates the OPV-RV interaction may be strongest for the first RV dose.[108,158] If true, our findings provide additional support for more recent, limited data[107] that suggests additional RV vaccine doses do not compensate for the reduced initial response. Applying our final seroconversion model to each mortality stratum individually and conducting a separate sensitivity analysis restricting data to only infants with confirmed pre-vaccine seronegative status provided support, as the direction of the relationship remained despite dropping approximately one-half and one-third of the study data, respectively. We are unable to assess possible mechanisms behind this relationship, however, previous literature suggests the interaction may relate to competition between rotavirus and poliovirus for receptors on mucosal cells reducing viral entry or poliovirus causing downregulation of components of the immune system response to rotavirus.[107]

Overall, these results highlight important programmatic considerations for RV vaccination and evolving OPV eradication strategies. While it may not be possible to intentionally stagger OPV

and RV immunization schedules,[107] the eventual shift from OPV to IPV may result in sizeable increases in RV vaccine performance in high child mortality settings. This is demonstrated by the dramatic difference in the seroconversion ORs among those who received both RV doses concomitantly with OPV and those who did not receive OPV at all. Data on the specific type of OPV (trivalent vs. bivalent) administered to infants in these trials was not available, though it is likely that most, if not all, individuals who received OPV received the trivalent form based on the date of administration and national immunization schedules in these settings at the time of the trials.

GDP is known to be strongly associated with RV seroconversion,[99] however, the effect of GDP is clearly not causal. Rather, GDP serves as a proxy for individual, family and community factors that are more directly influential in determining vaccine immunogenicity, a portion of which we aimed to identify in this study. We found GDP to be associated with the probability of seroconversion even after adjusting for child mortality. Comparing the adjusted OR for GDP and seroconversion developed via model selection (Table 5, OR = 1.11, 95% CI: 1.04-1.19) to their crude association (OR = 1.24, 95% CI: 1.20, 1.28) suggests that a substantial, though incomplete portion of the crude association was accounted for with our individual level factors. We were unable to identify modifiable factors associated with post-vaccine anti-rotavirus IgA titers among infants who seroconverted and the relationships between IgA titer and concomitant OPV were opposite to those expected; this suggests that intensity of a seropositive individual's immune response to RV vaccination may be more complex. For instance, concomitant OPV administration may influence whether or not an infant seroconverts, but may not dramatically influence the level of anti-rotavirus IgA among those who seroconverted. As such, anti-rotavirus IgA titers among

those who seroconverted may not serve as the ideal clinical measure for identifying the mechanisms resulting in differential vaccine immunogenicity.

This study approach has several notable strengths. Individual trials and observational studies were insufficiently powered to detect differences in vaccine performance or had little within study heterogeneity.[109,168,169] Therefore, we pooled individual level data from rigorously conducted randomized clinical trials. We are able to combine data from these clinical trials since they were conducted in a fairly standardized manner. The resulting dataset was substantially larger than that of any individual trial or other related study.[98] Further, pooling data across trials enabled the use of multilevel modeling to identify individual level factors that potentially contribute to immunogenicity while controlling for country-level factors. Including data from countries across child mortality strata facilitated the production of more generalizable results.

There are important challenges and limitations with this approach. First, trial protocols, while remarkably similar, still differed by trial location, year, and population. We attempted to account for this variability by including a random effect for trial in our models. The required adherence to study protocol and stringent monitoring necessary for a clinical trial means that the results produced from these analyses may not perfectly reflect the findings that would have occurred under more routine, real-world conditions and should, therefore, be interpreted cautiously. Relatedly, the infants included in the trials were all healthy children, potentially limiting the generalizability of the results. Second, this is a secondary analysis of data previously collected for other primary purposes. As such, we lacked data to control for genetic, maternal, socioeconomic and environmental factors that likely influence individual level immune response to vaccination. To

mitigate residual confounding from factors such as socioeconomic status or environment, proxy measures (GDP) were included in the models. Third, of the 7,280 infants included in the analysis, 2,292 (31.5%) did not have pre-vaccine serology data. The trial protocols for 2,116 (92%) of these infants indicated previous RV gastroenteritis as an exclusion criterion, providing reassurance that these infants had not previously been diagnosed with RV. We further accounted for this limitation by conducting a sensitivity analysis in which the seroconversion model was applied to only infants confirmed to be seronegative prior to vaccination and we found the effect of OPV diminished considerably. Lastly, our analysis was limited to infants who received Rotarix and it is possible that these findings may not be generalizable to the other three rotavirus vaccines prequalified by WHO.

Improving RV vaccine performance requires identification of the factors that contribute to vaccine immunogenicity on the individual level. While we explored a number of potential factors, our findings highlight the importance of concomitant OPV administration and provide encouraging evidence to suggest OPV withdrawal could improve RV vaccine performance. The ongoing efforts by the Global Polio Eradication Initiative to end OPV use creates an ideal natural experiment to confirm our results in the real-world. Vaccine immunogenicity data from infants in settings where OPV is currently in use could be compared to immunogenicity among infants after OPV withdrawal. More important still are evaluations of RV vaccine effectiveness administered before and after OPV withdrawal against the clinical endpoint of RV gastroenteritis. This research provides important programmatic considerations for improving RV vaccine immunogenicity, particularly reduction in concomitant RV vaccine and OPV administration.

Supplementary Material

Basic model formulas for mixed effect logistic regression and mixed effect linear regression of log-transformed data for seroconversion (dichotomous) and antibody titer (continuous) outcomes, respectively.

Logistic model: Logit $P(Y_{ij}) = b_{0j} + \beta_0 + \beta_{a1\ldots an}(H) + \beta_{b1\ldots bn}(C) + \beta_{c1\ldots cn}(H*C)$

Y_{ij} represents anti-rotavirus IgA ≥ 20 U/mL for the *i*th infant, in the *j*th trial

b_{0j} represents a random intercept for each trial

β_0 represents the intercept for each individual

$\beta_{a\text{-}c}$ represent regression coefficients for host, country and interaction term, respectively

H represents a vector of host characteristics, a_1 through a_n

C represents a vector of country factors, b_1 through b_n

H*C represents a vector of interaction terms for host/country characteristics and child mortality strata

Linear model: $Ln(Y_{ij}) = b_{0j} + \beta_0 + \beta_{a1\ldots an}(H) + \beta_{b1\ldots bn}(C) + \beta_{c1\ldots cn}(H*C) + \varepsilon$

Y_{ij} represents the anti-rotavirus IgA antibody titer for the *i*th infant, in the *j*th trial

b_{0j} represents a random intercept for each trial

β_0 represents the intercept for each individual

$\beta_{a\text{-}c}$ represent regression coefficients for host, country and interaction term, respectively

H represents a vector of host characteristics, a_1 through a_n

C represents a vector of country factors, b_1 through b_n

H*C represents a vector of interaction terms for host/country characteristics and child mortality strata

ε represents error

Table S1. Final seroconversion model applied to each child mortality stratum individually

Individual or country level factor	Low child mortality (n = 3,783) OR (95% CI)	High child mortality (n = 2,806) OR (95% CI)
Time from last RV dose to serology (weeks)	0.88 (0.84, 0.93)	0.97 (0.83, 1.13)
Age at 1st RV dose (weeks)	1.13 (1.09, 1.18)	1.02 (0.99, 1.05)
Vaccine concentration $\geq 10^{6.0}$	1.00 (ref)	1.00 (ref)
Vaccine concentration $< 10^{6.0}$ [a]	0.62 (0.45, 0.83)	--
LAZ: stunted or severely stunted	1.00 (ref)	1.00 (ref)
LAZ: not stunted	1.24 (0.93, 1.65)	0.84 (0.68, 1.03)
OPV neither concomitant w/ RV dose 1 nor 2	1.00 (ref)	1.00 (ref)
OPV concomitant w/ RV dose 1 & 2	0.61 (0.42, 0.89)	0.71 (0.40, 1.26)
OPV concomitant w/ RV dose 1 only	0.17 (0.02, 1.95)	0.49 (0.13, 1.83)
OPV concomitant w/ RV dose 2 only	0.91 (0.30, 4.44)	0.40 (0.09, 1.83)
No OPV received [b]	0.78 (0.40, 1.53)	--
Log(GDP)	1.28 (1.02, 1.60)	1.10 (1.02, 1.17)

OR = odds ratio; CI = confidence interval; RV = rotavirus; LAZ = length-for-age z-score; OPV = oral polio vaccine, GDP = gross domestic product (2004, in USD)

[a] No children in high child mortality settings received low concentration vaccines.

[b] All children in high child mortality setting received at least one dose of OPV

Table S2. Final seroconversion model applied to subset of infants with confirmed seronegative status via anti-rotavirus IgA serology sample prior to vaccination (n = 4,473)

Individual or country level factor	OR (95% CI)
Time from last RV dose to serology (weeks)	0.88 (0.84, 0.93)
Vaccine concentration $\geq 10^{6.0}$	1.00 (ref)
Vaccine concentration $< 10^{6.0}$	0.65 (0.48, 0.87)
OPV neither concomitant w/ RV dose 1 nor 2	1.00 (ref)
OPV concomitant w/ RV dose 1 & 2	0.62 (0.46, 0.81)
OPV concomitant w/ RV dose 1 only	0.36 (0.05, 2.58)
OPV concomitant w/ RV dose 2 only	0.90 (0.30, 2.70)
No OPV received	0.92 (0.56, 1.50)
Log(GDP)	1.20 (1.05, 1.38)
Age at 1st RV dose (weeks)	1.13 (1.08, 1.17)
LAZ: stunted or severely stunted	1.00 (ref)
LAZ: not stunted/severely stunted	1.22 (0.92, 1.62)
Child mortality- Low child mortality settings	1.00 (ref)
Child mortality- High child mortality settings	1.80 (0.77, 4.22)
Age at 1st RV dose (weeks)*Child mortality setting	0.89 (0.84, 0.95)
LAZ: not stunted*Child mortality setting	0.64 (0.40, 1.04)

IgA = immunoglobulin A; OR = odds ratio; CI = confidence interval; RV = rotavirus; LAZ = length-for-age z-score; OPV = oral polio vaccine, GDP = gross domestic product

5 Aim 2- Correlates of protection

[Manuscript 2]

Assessing serum anti-rotavirus immunoglobulin A as a correlate of vaccine-induced protection against rotavirus gastroenteritis in high and low child mortality settings: analysis of pooled individual-level clinical trial data

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Abstract

Background: Despite tremendous global progress, the full benefits of rotavirus vaccination have not yet been achieved. To sustain the downward trends in rotavirus morbidity and mortality, rapid and effective assessment of modified rotavirus vaccine strategies and the next generation of vaccines is essential. This study aimed to quantify a threshold of post-vaccine serum anti-rotavirus IgA antibody units that serves as an individual level immune correlate of protection against rotavirus gastroenteritis among vaccinated infants across child mortality settings.

Methods: Individual level data on infants enrolled in nine of GlaxoSmithKline's phase II and III clinical trials of the Rotarix vaccine were pooled to create the dataset for this analysis. A total of 5,074 vaccinated infants from 16 countries were include in the analysis. Cox proportional hazard models were fit to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) describing the

relationship between specific anti-rotavirus IgA thresholds and the occurrence of mild/moderate or severe rotavirus gastroenteritis. Two follow-up periods were assessed: from serum sample collection (at approximately 4-12 weeks of age) to 1 year of age and from 1 year of age to 2 years of age.

Results: In both high and low child mortality settings, seroconversion conferred substantial protection against rotavirus gastroenteritis during the first follow-up period. Among infants in low child mortality settings, seroconversion provided near perfect protection against severe rotavirus gastroenteritis (HR = 0.04, 95% CI = 0.01, 0.32). In high child mortality settings, seroconversion reduced the risk of severe rotavirus gastroenteritis by 52% (HR = 0.48, 95% CI = 0.26, 0.90). Notable patterns were observed during follow-up. The highest anti-rotavirus IgA threshold tended to have the lowest HR comparing the rate of gastroenteritis among those above that threshold to seronegative infants. Smaller HRs were observed for more severe disease indicating higher protection. Lastly, the HR for a given anti-rotavirus IgA threshold was generally higher in high child mortality settings compared to low child mortality settings. Controlling for possible confounders (such as length-for-age z-score or country gross domestic product) did not impact the HR for a given IgA threshold. No clear patterns in HRs by IgA threshold or severity of illness were observed during the second year of follow-up.

Conclusions: Serum IgA is a valuable, though imperfect, correlate of vaccine-induced protection against mild/moderate and severe rotavirus gastroenteritis up to 1 year of age. Higher anti-rotavirus IgA titer generally corresponded with increased protection against rotavirus gastroenteritis. Since a consistent anti-rotavirus IgA threshold indicating perfect protection against

rotavirus gastroenteritis was not identified and the level of protection estimated for a given threshold differed by setting, serum anti-rotavirus IgA alone may be insufficient to accurately predict an infant's risk of rotavirus gastroenteritis.

Background

Rotavirus vaccine, introduced as early as 2006 in some countries,[66] has profoundly impacted rotavirus morbidity and mortality among young children on the global scale.[127] Declines in rotavirus-related hospitalizations and death have been observed in several countries.[134,170–172] A recent meta-analysis found that rotavirus gastroenteritis has been reduced overall by 67% among children under 5 years of age in countries that have introduced the vaccine.[127] Despite this progress, the full benefits of rotavirus vaccination have not yet been achieved.[173] Rotavirus remains the leading cause of severe diarrheal disease among young children and continues to cause 128,500–215,000 deaths annually among children under 5 years of age.[6,7]

Further reduction in the rotavirus disease burden is hindered by to two important impediments faced in settings where the burden of rotavirus gastroenteritis is greatest. First, rotavirus vaccine remains inaccessible to approximately 90 million newborns (67%) each year, a majority of whom (70%) live in low-income countries of Africa and Asia.[67,68] Logistic challenges including vaccine supply constraints, cold-chain requirements, cost,[54] and co-financing arrangements with Gavi, (which assists developing countries in purchasing vaccines)[66] impede the utility of some existing vaccines[114] and may be contributing to slowed vaccine uptake in recent years. Second, rotavirus vaccine is substantially less immunogenic and less efficacious in high child mortality settings, such

as Africa and South East Asia, where the majority of the rotavirus mortality burden exists.[7,96] In some settings, despite introduction of the vaccine, rotavirus remains a leading cause of severe diarrheal disease[7] and diarrhea-related hospitalization.[94,95] Improving rotavirus vaccine performance will require ongoing evaluation of modifiable vaccination strategies for existing vaccines and evaluation of new vaccine candidates in the robust rotavirus vaccine pipeline.[144]

Currently, rotavirus studies typically assess a clinical endpoint such as rotavirus gastroenteritis or rotavirus-related hospitalization.[27] A biomarker of vaccine performance could be used to rapidly evaluate new vaccination strategies and vaccine candidates.[115] An alternative to assessing efficacy of a vaccine against clinical outcomes is to use a correlate of protection which predicts the likelihood of clinical disease.[27,113] When used as part of a vaccine study, a correlate of protection can be used as a surrogate for clinical endpoints[27,113] and reduce the need for long-term, large-scale trials following children for relatively rare clinical outcomes.[27] Indeed, correlates of protection have been found for a number of other vaccines including influenza vaccines (serum haemagglutinin antibodies,[174] meningococcal C conjugate vaccines (serum serogroup C-specific immunoglobulin G (IgG)),[175] pneumococcal conjugate vaccines (immunoglobulin G anticapsular-specific polysaccharide antibodies),[176] tetanus vaccines (serum tetanus antitoxin),[177,178] and diphtheria vaccines (serum diphtheria antitoxin).[177,179]

For rotavirus, serum anti-rotavirus immunoglobulin A (IgA) antibodies are one of the primary measures being considered as a possible marker for protection.[27,28] Other measures have been explored, including serum or intestinal rotavirus-specific neutralizing antibodies, intestinal or stool anti-rotavirus IgA and IgG antibodies, and serum anti-rotavirus IgG; these measures have

generally been found to be impractical to collect in clinical studies, inconsistently associated with protection, or difficult to measure due to short duration of detectability.[27] Vaccine-induced anti-rotavirus IgA levels have been demonstrated to predict vaccine efficacy on the aggregate (country) level,[99] however, only limited research has examined the role of anti-rotavirus IgA as a possible correlate of protection on the individual scale.[27] Much of the early research in this field relates to individual level anti-rotavirus IgA only in the context of natural infection[39,44] or assessed anti-rotavirus IgA for vaccines no longer available for use today.[116–118] More recently, a meta-analysis found individual level seropositive status (measured as anti-rotavirus IgA ≥ 20 U/mL) to be moderately associated with lower risk of gastroenteritis in a small subset of clinical trials.[119]

A rotavirus correlate of protection could improve rotavirus vaccine performance in two ways. First, it could enhance our understanding of where and why current rotavirus vaccines underperform and what modifiable vaccination strategies might improve performance. Second, a correlate of protection could help efficiently identify promising candidates in the rotavirus vaccine pipeline including new live vaccines and non-replicating vaccines. Using a correlate of protection as an endpoint could reduce trial costs and avoid ethical challenges associated with placebo controlled trials.[180] We aimed to identify a threshold of post-vaccine IgA antibody units that best predicts substantially reduced risk of rotavirus gastroenteritis among infants vaccinated infants with GlaxoSmithKline's (GSK's) Rotarix vaccine in high to low child mortality settings.

Methods

GSK clinical trial data

Individual level data on infants enrolled in GSK's phase II and III clinical trials of the Rotarix vaccine were combined to create a pooled dataset (Table 1). Rotarix is a live, attenuated oral rotavirus vaccine administered in two-doses. The first dose is recommended beginning at 6 weeks of age and the second dose following after an interval of four or more weeks and by 24 weeks of age.[70]

Data from nine trials from 16 countries were combined. Each trial was a randomized, double-blind, and placebo-controlled trial with similar protocols for the main measures of interest (Table 2). The countries in which the studies took place were categorized into child mortality strata based on WHO classification[145] using under 5 child mortality rate quintiles—the lowest quintile, second and middle quintile, and two highest quintiles were considered "very low," "low," and "high" child mortality.[146] Fourteen of the included countries were considered low/very low child mortality (combined) and two were considered high child mortality

This study was limited to infants who received the Rotarix vaccine (n = 11,619), participated in the RV immunogenicity sub-studies of the trials (i.e. had post-vaccine serology data) according to protocol (n = 6,099), and were followed up to approximately one or two years of age for rotavirus gastroenteritis. Infants who had a recorded episode of rotavirus gastroenteritis prior to collection of his/her post-vaccine serology sample (n = 59) were excluded as prior infection would impact both the anti-rotavirus IgA titer value and risk of subsequent illness; the final dataset included 5,074 infants.

Table 1. Study characteristics by trial number

GSK Trial Number	Study Sites	Study Phase	Age at Dose 1 (Wks)	Vacc-inated (n)	Follow-up	
					Year 1 (n)	Year 2 (n)
107625	Japan	3	6-14	492	34	34
102247	Czech Republic, Finland, France, Germany, Italy, Spain	3	6-14	2,613	786	783
102248	**Malawi, South Africa**	3	5-10	2,803	2,268	1,555
113808	China	3	6-16	1,518	390	373
444563/007	Singapore	2	11-17	1,737	447	441
444563/004	Finland	2	6-12	249	209	204
444563/005	Canada, US	2	6-12	372	257	168
444563/006	Brazil, Mexico, Venezuela	2	6-12	1,498	425	273
444563/013	**South Africa**	2	5-10	337	258	88
Total				11,619	5,074	3,919

Countries in bold are classified as high child mortality settings

Table 2. Similarities across trial protocols and definitions

Category	Study protocol and definitions
Inclusion criteria	• Male and female infants • Healthy subjects free of all obvious health problems (established by medical history and physical exam)
Exclusion &/or elimination criteria	• Use of investigational or non-registered product (drug or vaccine) w/in 30 days prior to study vaccine dose • Planned administration of a vaccine not foreseen by the study protocol w/in 14 days of study vaccine dose • Chronic administration (defined as >14 days) of immunosuppressants anytime since birth • Any confirmed or suspected immune-suppressive or deficient condition based on medical history and exam • Significant history of chronic gastrointestinal disease • History of allergic reaction to any vaccine component • Acute disease, defined as the presence of a moderate or severe illness w/ or w/o fever, at the time of enrollment (warrants deferral of vaccination) • Administration of immunoglobulins and/or blood product since birth or planned administration during the study
Vaccine	• GSK RIX 4414 HRV vaccine • Vaccinated arm w/ viral suspension of $\geq 10^{6.0}$ $CCID_{50}$[a] • Doses administered 1-2 months apart
Medical exam & history	• Medical exam and history obtained at enrollment

	• Concomitant medications/vaccinations, history of medication/vaccination recorded at study visits • Anthropometric measurements obtained
Gastro-intestinal illness	• Defined as diarrhea w/ or w/o vomiting • Diarrhea defined as ≥3 looser than normal stools in a 24 hour period • Severity measured on Vesikari scale • Symptoms, duration, medical treatment sought recorded on a diary card provided by the study
Stool samples	• Collected as soon as possible and no later than 7 days of severe gastrointestinal illness • Tested via EIA, including RV strain determination
Serology	• Collected 1-3 months after final vaccine dose • Samples tested via ELISA, assay cutoff of anti-rotavirus IgA ≥ 20 U/mL

[a] Highest viral suspensions of $10^{4.7}$ and $10^{5.8}$ median $CCID_{50}$ in study 444563/004 and 444563/006, respectively

All individual and study-related data for the clinical trials were provided by GSK. In this analysis we utilized data on post-vaccine serum anti-rotavirus IgA titers and rotavirus gastroenteritis episodes occurring during follow-up as the primary explanatory and endpoints of interest, respectively. In all trials, serum samples were collected approximately 4-12 weeks after receipt of the last rotavirus vaccine dose and measured using an enzyme-linked immunosorbent assay (ELISA).[181]

Infants were followed for rotavirus gastroenteritis of any severity up 1 or 2 years of age, depending on the study protocol. The onset date and severity of gastroenteritis episodes (regardless of etiology) were recorded and stool samples were tested via ELISA to assess whether each episode was rotavirus-related. Severity of gastroenteritis was defined based on the 20-point "Vesikari scale," a standard severity measure which takes into account illness symptoms including diarrhea (duration and maximum number of episodes per day), vomiting (duration and maximum number of episodes per day), fever, dehydration and required treatment.[182]

Additional data on possible confounders of the anti-rotavirus IgA titer-gastroenteritis relationship[98,104] were also considered. These variables included age at post-vaccine serology sample (weeks), sex, length-for-age z-scores (LAZ) as a proxy for nutritional status, and monthly rate of non-rotavirus gastroenteritis (calculated) to represent possible heterogeneity in susceptibility/exposure to gastroenteritis among the infants. Country level data on gross domestic product (GDP) per capita in 2004 USD[160] and 2004 under 5 mortality rates[162] supplemented the individual level trial data.

Statistical analysis

Cox proportional hazard models were fit to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) describing the relationship between specific IgA thresholds and the occurrence of rotavirus gastroenteritis. The occurrence of mild to moderate rotavirus gastroenteritis (Vesikari scores of 0-10) and severe rotavirus gastroenteritis (Vesikari score of 11 or higher) were the outcomes of interest. Anti-rotavirus IgA titer threshold was the primary explanatory variable.

Dichotomous thresholds were created by dividing the data into those who were seronegative after vaccination (defined as an anti-rotavirus IgA measure of <20 U/mL according to GSK protocol) and those above a particular anti-rotavirus IgA cut off. Eight thresholds were created, all using seronegative infants as the reference. The selected values began with 20 U/mL and were doubled to span the range of anti-rotavirus IgA titers among the infants (in U/mL): ≥20, ≥40, ≥80, ≥160, ≥320, ≥640, ≥1,280 and ≥2,560. Infant age (weeks) was the time to rotavirus event, left censored at age at serum sample collection. Clinical trial number was included in the models as a frailty component (i.e. a random intercept) to account for possible unmeasured variability between clinical trials. An example of the model formula is shown in the Supplemental Material.

Duration of follow-up was divided into two periods: 1) from post-vaccine serum sample collection up to 1 year of age and 2) from 1 year of age up to 2 years of age. Children were followed until they had an episode of rotavirus gastroenteritis (event), left the study (censored) or reached the maximum age for the follow-up period (censored), whichever occurred first. Among those who had rotavirus gastroenteritis during the follow-up period, only the first episode was considered in the analysis since so few had multiple episodes; after the first episode, an individual was censored for the remainder of the follow-up period.

Preliminary analysis began with testing the role of possible confounders of the anti-rotavirus IgA and rotavirus gastroenteritis relationship. Initial models for rotavirus gastroenteritis of any severity were created with an anti-rotavirus IgA threshold of 20 (seronegative vs. seropositive infants) as the only predictor. Next, full models were created including both the IgA threshold as the primary predictor and sex, LAZ, monthly rate of non-rotavirus gastroenteritis and GDP added as possible

confounders. Backwards elimination was conducted on the full models with an α of 0.10 as the cut-off for retaining a variable in the model. The initial, full and final models after backward elimination were evaluated to assess the proportional hazards assumption and compared to identify if controlling for possible confounders impacted the HR for anti-rotavirus IgA.

Because we found that no variables substantially changed the magnitude of the IgA relationship with rotavirus gastroenteritis, we then estimated the association between each anti-rotavirus IgA threshold alone with mild/moderate or severe rotavirus gastroenteritis during each follow-up period. Separate models were fit for low and high child mortality settings. HRs and 95% CIs were estimated for all potential anti-rotavirus IgA thresholds, severity of illness and follow-up periods. Based on findings from this analysis, a sub-analysis was conducted using the same methods to examine the value of the IgA ≥20 threshold among low child mortality countries further stratified into "very low" and "low" child mortality settings. The countries with very low child mortality included Canada, Czech Republic, Finland, France, Germany, Italy, Japan, Singapore, Spain and the United States. Low child mortality countries included Brazil, China, Mexico and Venezuela.

All modeling was conducted using the "survival" and "survminor" packages in R software.

Ethical approval

This study was reviewed by the Emory University Institutional Review Board and determined to be non-human subjects research. Trial participant data was de-identified and all dates obfuscated by GSK prior to data sharing.

Results

The final dataset included 5,074 infants, 2,526 (49.8%) of whom were from high child mortality settings (Table 3). The mean age at post-vaccine serology sample collection was 23 weeks (sd = 3) with children in low child mortality settings being, on average, approximately 4 weeks older at time of serology than children in high child mortality settings (mean in low child mortality settings = 25, sd = 4; high child mortality settings = 21, sd = 1, $p < 0.001$). 237 infants had at least one episode of rotavirus gastroenteritis during the follow-up period; only six infants (3%) had one or more subsequent episodes (which were not analyzed). A higher percentage of rotavirus gastroenteritis episodes among children in high child mortality settings were considered severe (39%, 95% CI = 32%, 47%) compared to children in low child mortality settings (28%, 95% CI = 19%, 39%, $p = 0.08$).

The median follow-up time from serum sample collection to either the first episode of rotavirus gastroenteritis or censoring was 79 weeks (Table 3). Follow-up was substantially longer for infants in low child mortality settings (median = 85 weeks, IQR = 70, 96) than infants in high child mortality settings (median = 53 weeks, IQR = 51, 92). All infants (n = 5,074) were included in the first follow-up period whereas 3,804 (80%) of infants were followed between 1 and 2 years of age.

In both settings and for both mild/moderate and severe gastroenteritis, infants who were seronegative had the highest cumulative proportion by 2 years of age (Figure 1). In contrast,

infants with the highest anti-rotavirus IgA titers (≥2,560) typically had the lowest cumulative incidence.

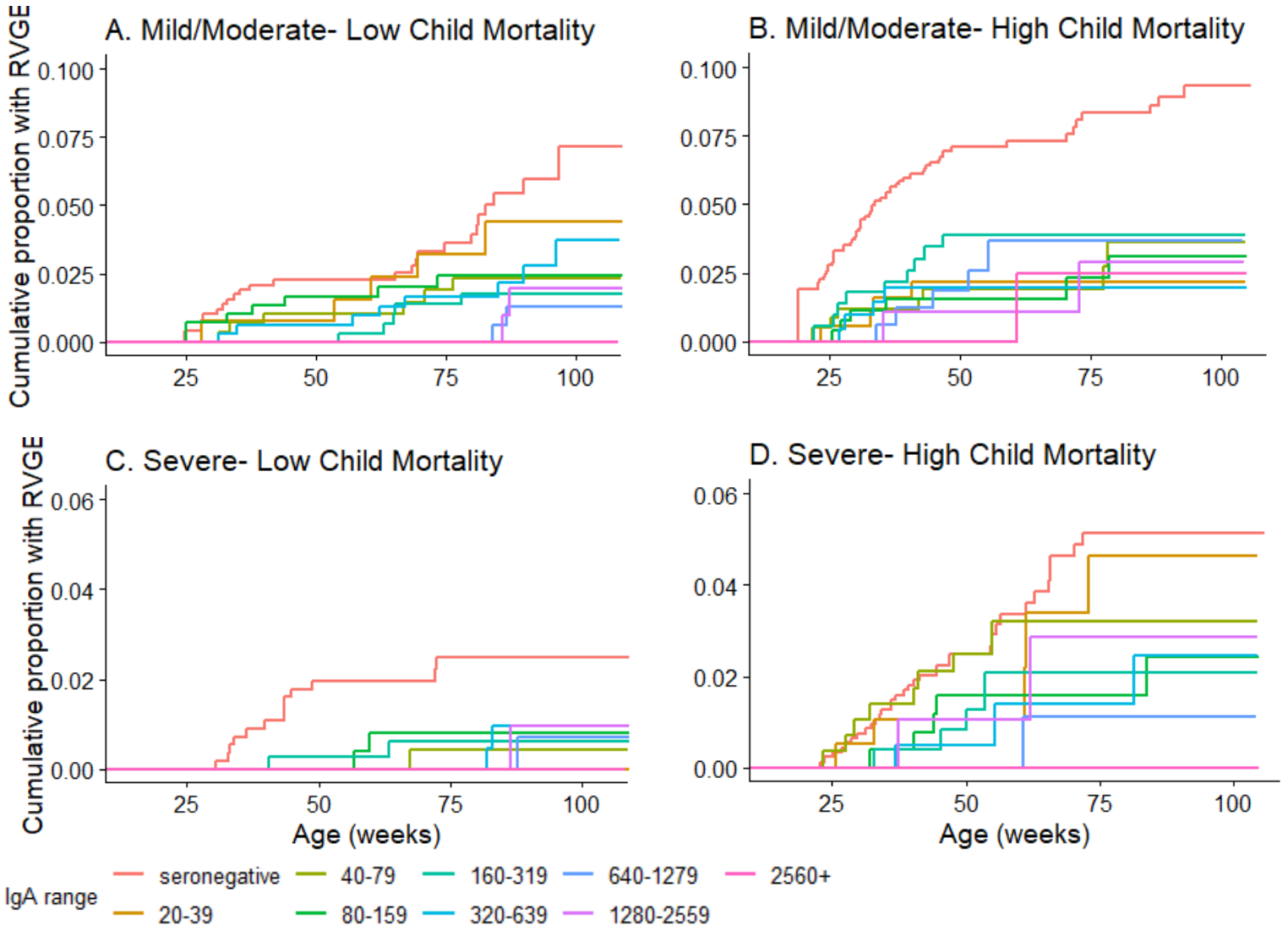
Table 3. Individual, country and follow-up characteristics of infants from 9 trials conducted in 16 countries beginning in 2000-2010

	All countries, N = 5,074	Low child mortality settings, N = 2,548	High child mortality settings, N = 2,526
Individual and country level characteristics			
Age at post-vaccine serology sample (weeks), mean (sd)	23 (3)	25 (4)	21 (1)
Female, n (%)	2,513 (50)	1,255 (49)	1,258 (50)
Stunted or severely stunted, n (%)	4,493 (89)	2,434 (96)	2,059 (82)
Rate of non-rotavirus gastroenteritis (episodes/100 months), median (IQR)	0 (0, 11)	0 (0, 7)	0 (0, 12)
Severity of first episode of rotavirus gastroenteritis during follow-up, n (%)			
Mild/moderate	153 (65)	57 (72)	96 (61)
Severe	84 (35)	22 (28)	62 (39)
GDP (2004, in USD), median (IQR)	4,745 (4,721, 27,405)	27,405 (4,271, 34,166)	4,545 (274, 4,745)

Follow-up			
Age at event/censoring (weeks), median (IQR)	79 (52, 94)	85 (70, 96)	53 (51, 92)
Time from post-vaccine serology sample to event/censoring (weeks), median (IQR)	53 (30, 72)	60 (47, 74)	33 (30, 71)
Participation in follow-up 1 period, n (%)	5,074 (100)	2,548 (100)	2,526 (100)
Duration of participation in follow-up 1 (weeks), median (IQR)	29 (25, 31)	26 (24, 29)	31 (29, 31)
Participation in follow-up 2 period, n (%)	3,804 (80)	2,248 (88)	1,556 (61)
Duration of participation in follow-up 2 (weeks), median (IQR)	36 (24, 44)	36 (26, 45)	37 (3, 43)

sd = standard deviation, IQR = interquartile range, GDP = gross domestic product in 2004 USD

Figure 1. Cumulative proportion of infants experiencing mild/moderate or severe rotavirus gastroenteritis during the entire follow-up period, by anti-rotavirus IgA antibody (U/mL), severity of rotavirus gastroenteritis and child mortality setting



Comparison of HR for anti-rotavirus IgA with and without controlling for confounders

With anti-rotavirus IgA threshold of ≥20 U/mL as the only explanatory variable (Supplemental Table S1), the HR comparing seropositive infants to seronegative infants was 0.34 (95% CI = 0.25, 0.47). Adding all available potential confounder variables to the model (full model), the HR for anti-rotavirus IgA ≥20 changed minimally (HR = 0.35, 95% CI = 0.26, 049). Similarly, after backwards elimination, no change in the HR for the anti-rotavirus IgA threshold was observed. When other anti-rotavirus IgA thresholds (≥40, ≥80, ≥160) were tested using this same process, modification of the model had very little impact on the HR for the anti-rotavirus IgA threshold. Since controlling for potential confounders did not impact the association between anti-rotavirus IgA thresholds and the HR for rotavirus gastroenteritis, subsequent modeling used the eight pre-specified IgA threshold as the only predictor.

Anti-rotavirus IgA thresholds for follow-up to 1 year of age across severity of illness

Anti-rotavirus IgA thresholds were modeled separately for low and high child mortality settings based on preliminary examination of the cumulative incidence data which suggested possible differences in HRs by setting.

Results of modeling each anti-rotavirus IgA threshold as the sole explanatory variable for mild/moderate and severe rotavirus gastroenteritis during the first follow-up period demonstrated several patterns. First, within the mild/moderate gastroenteritis models for infants in low child mortality settings (Table 4a), the HR ranged from 0.31 (95% CI = 0.13, 0.75) for the threshold of ≥20 U/mL to 0.13 (95% CI = 0.03, 0.60) for the threshold of ≥320 U/mL, above which no events

occurred. A similar pattern was observed for this same population and follow-up period when examining severe rotavirus gastroenteritis with the highest protection provided among infants with the highest levels of anti-rotavirus IgA, though the pattern was less consistent. Second, for a given threshold, the HR for protection against mild/moderate gastroenteritis was higher than that for severe gastroenteritis.

Among infants in high child mortality settings, the same general pattern of decreasing HRs as anti-rotavirus IgA thresholds increased was observed (Table 4b). For both mild/moderate and severe rotavirus gastroenteritis, the lowest HRs were observed for the highest levels of anti-rotavirus IgA. As was the case for low child mortality settings, the HR for a given anti-rotavirus IgA threshold was generally higher for mild/moderate rotavirus gastroenteritis. The HRs for infants in high child mortality settings were consistently higher than those among children in low child mortality settings.

The low child mortality setting group was further stratified into countries with "very low" and "low" child mortality to estimate HRs using the threshold of ≥20 U/mL (Table 5). Among the very low child mortality countries, the HR for mild/moderate gastroenteritis was 0.13 (95% CI = 0.03, 0.53) and was 0 (95% CI = NA) for severe gastroenteritis because no episodes occurred. Among the low child mortality settings, the HR for mild/moderate gastroenteritis (0.58, 95% CI = 0.20, 1.73) aligned more closely with that of the high child mortality settings from Table 4b than the lowest child mortality settings. In contrast, the HR for severe gastroenteritis in these countries (0.05, 95% CI = 0.01, 0.42) was more similar to that of the lowest child mortality settings.

Lastly, the HR for the ≥20 U/mL threshold was assessed for each country individually to identify if a pattern to be identified in the HR as child mortality rate increased. No pattern was identified, possibly due to small sample sizes.

Table 4a. Survival analysis results for infants in low child mortality settings during follow-up to 1 year of age

		Mild/Moderate Rotavirus Gastroenteritis				Severe Rotavirus Gastroenteritis			
IgA threshold (U/mL)	N (%)	Events n (%)	Time at risk (week)	Cum. Hazard per 100 (95% CI)	HR* (95% CI)	Events n (%)	Time at risk (week)	Cum. Hazard per 100 (95% CI)	HR* (95% CI)
Seroneg.	575 (22.6)	11 (1.9)	15322	2.28 (0.88, 3.69)	1.00 (ref)	11 (1.9)	15397	1.98 (0.81, 3.15)	1.00 (ref)
≥20	1973 (77.4)	10 (0.5)	52860	0.59 (0.21, 0.97)	0.31 (0.13, 0.75)	1 (0.1)	53030	0.05 (0.00, 0.15)	0.04 (0.01, 0.32)
≥40	1822 (71.5)	9 (0.5)	48712	0.57 (0.18, 0.96)	0.30 (0.12, 0.74)	1 (0.1)	48858	0.06 (0.00, 0.16)	0.04 (0.01, 0.35)
≥80	1519 (59.6)	6 (0.4)	40748	0.48 (0.07, 0.89)	0.22 (0.08, 0.61)	1 (0.1)	40843	0.07 (0.00, 0.20)	0.06 (0.01, 0.46)
≥160	1183 (46.4)	2 (0.2)	31909	0.17 (0.00, 0.41)	0.09 (0.02, 0.42)	1 (0.1)	31935	0.09 (0.00, 0.25)	0.08 (0.01, 0.63)
≥320	814 (31.9)	2 (0.2)	22201	0.25 (0.00, 0.60)	0.13 (0.03, 0.60)	0 (0.0)	22239	0.00 (NA)	0.00 (NA)
≥640	475 (18.6)	0 (0.0)	13044	0.00 (NA)	0.00 (NA)	0 (0.0)	13044	0.00 (NA)	0.00 (NA)
≥1280	214 (8.4)	0 (0.0)	5975	0.00 (NA)	0.00 (NA)	0 (0.0)	5975	0.00 (NA)	0.00 (NA)
≥2560	65 (2.6)	0 (0.0)	1866	0.00 (NA)	0.00 (NA)	0 (0.0)	1866	0.00 (NA)	0.00 (NA)

HR = hazard ratio; IQR = interquartile range, CI = confidence interval

*Models include study as a frailty term

Table 4b. Survival analysis results for infants in high child mortality settings during follow-up to 1 year of age

		Mild/Moderate Rotavirus Gastroenteritis				Severe Rotavirus Gastroenteritis			
IgA threshold (U/mL)	N (%)	Events n (%)	Time at risk (week)	Cum. Hazard per 100 (95% CI)	HR* (95% CI)	Events n (%)	Time at risk (week)	Cum. Hazard per 100 (95% CI)	HR* (95% CI)
Seroneg.	978 (38.7)	50 (5.1)	27550	7.33 (3.24, 11.43)	1.00 (ref)	23 (2.4)	28071	2.50 (1.48, 3.52)	1.00 (ref)
≥20	1548 (61.3)	31 (2.0)	45245	2.16 (1.39, 2.92)	0.38 (0.24, 0.60)	18 (1.2)	45536	1.22 (0.66, 1.78)	0.48 (0.26, 0.90)
≥40	1354 (53.6)	27 (2.0)	39557	2.16 (1.34, 2.98)	0.38 (0.24, 0.61)	16 (1.2)	39818	1.24 (0.63, 1.85)	0.49 (0.26, 0.93)
≥80	1058 (41.9)	22 (2.1)	30906	2.22 (1.29, 3.15)	0.40 (0.24, 0.65)	9 (0.9)	31188	0.90 (0.31, 1.48)	0.35 (0.16, 0.76)
≥160	795 (31.5)	18 (2.3)	23171	2.45 (1.31, 3.58)	0.43 (0.25, 0.74)	5 (0.6)	23408	0.67 (0.08, 1.25)	0.26 (0.10, 0.69)
≥320	546 (21.6)	9 (1.6)	15933	1.75 (0.61, 2.90)	0.31 (0.15, 0.64)	2 (0.4)	16039	0.38 (0.00, 0.91)	0.15 (0.04, 0.65)
≥640	336 (13.3)	5 (1.5)	9823	1.61 (0.20, 3.03)	0.28 (0.11, 0.71)	1 (0.3)	9861	0.31 (0.00, 0.91)	0.12 (0.02, 0.92)
≥1280	171 (6.8)	1 (0.6)	4959	0.61 (0.00, 1.81)	0.11 (0.02, 0.81)	1 (0.6)	4961	0.61 (0.00, 1.81)	0.25 (0.03, 1.83)
≥2560	73 (2.9)	0 (0.0)	2088	0.00 (NA)	0.00 (NA)	0 (0.0)	2088	0.00 (NA)	0.00 (NA)

IgA = anti-rotavirus immunoglobulin A; HR = hazard ratio; IQR = interquartile range, CI = confidence interval

*Models include study as a frailty term

Table 5. Survival analysis results for infants in low child mortality settings further stratified into very low and low child mortality during follow-up to 1 year of age

		Mild/Moderate Rotavirus Gastroenteritis				Severe Rotavirus Gastroenteritis			
IgA threshold	N (%)	Events n (%)	Time at risk (week)	Cum. Hazard per 100 (95% CI)	HR* (95% CI)	Events n (%)	Time at risk (week)	Cum. Hazard per 100 (95% CI)	HR* (95% CI)
Very low child mortality									
Seroneg.	306 (18)	5 (1.7)	7966	2.40 (0.03, 4.77)	1.00 (ref)	1 (0.3)	8059	0.33 (0.00, 0.99)	1.00 (ref)
≥20 U/mL	1427 (82)	3 (0.2)	37742	0.33 (0.00, 0.74)	0.13 (0.03, 0.53)	0 (0.0)	37805	0.0 (NA)	NA
Low child mortality									
Seroneg.	269 (33)	6 (2.2)	7356	2.43 (0.48, 4.39)	1.00 (ref)	10 (3.7)	7338	3.93 (1.49, 6.36)	1.00 (ref)
≥20 U/mL	546 (67)	7 (1.3)	15119	1.40 (0.36, 2.44)	0.58 (0.20, 1.73)	1 (0.2)	15225	0.18 (0.00, 0.54)	0.05 (0.01, 0.42)

IgA = anti-rotavirus immunoglobulin A; HR = hazard ratio; IQR = interquartile range, CI = confidence interval

*Models include study as a frailty term

Anti-rotavirus IgA thresholds for follow-up from 1 year of age to 2 years of age across severity of illness

The patterns observed during follow-up to 1 year of age were generally less apparent during the second year of life (Supplemental Table S2a). The HRs for mild/moderate gastroenteritis among infants in low child mortality settings did tend to decrease as anti-rotavirus IgA thresholds increased, however, this was not the case for severe rotavirus gastroenteritis. When comparing mild/moderate and severe gastroenteritis for the same populations and thresholds, the HR for severe disease tended to be higher. For infants in high child mortality settings, no apparent trends were observed either by anti-rotavirus IgA titer level or severity of gastroenteritis (Supplemental Table S2b).

Discussion

findings highlight characteristics of serum anti-rotavirus IgA that make it a valuable, though imperfect, surrogate endpoint for assessing rotavirus vaccine performance. First, we found that seroconversion defined as anti-rotavirus IgA ≥20 U/mL confers substantial protection against rotavirus gastroenteritis. This seroconversion threshold may represent the most informative threshold available from serum anti-rotavirus IgA titers. Second, an approximate "dose-response" relationship was identified with higher anti-rotavirus IgA levels providing better protection against the occurrence of rotavirus gastroenteritis with the strongest protection often provided against more severe disease. This pattern was generally consistent across settings. Third, the level of protection estimated for a given IgA threshold differed by child mortality setting suggesting that

serum anti-rotavirus IgA alone may be insufficient to accurately predict a child's risk of rotavirus gastroenteritis.

An ideal correlate of protection is one that indicates near perfect protection against the clinical outcome of interest.[27] However, because rotavirus is an imperfectly immunizing infection, a more practical goal for a correlate of protection in the context of rotavirus vaccination may be to identify a measure that represents substantially reduced the risk of severe gastroenteritis.[27] The results of this analysis provide encouraging evidence that serum anti-rotavirus IgA may be such a measure. We found that infants with any detectable level anti-rotavirus IgA antibodies in serum (seropositive infants) had a substantially reduced rate of rotavirus gastroenteritis up to 1 year of age in both high and low child mortality settings when compared to vaccine non-responders (seronegative infants). These findings reinforce prior research from early vaccine trials[183] and a more recent evaluation[119] that suggest the same relationship. Our findings extend beyond this, however, and provide evidence that seroconversion serves as a near perfect correlate of protection against severe rotavirus gastroenteritis in low child mortality settings, reducing the risk of rotavirus gastroenteritis by 96% (HR = 0.04, 95% CI = 0.01, 0.32) compared to infants who did not seroconvert.

While no clear IgA cutoff representing 100% protection against rotavirus gastroenteritis across settings was apparent, a characteristic of serum anti-rotavirus IgA that strengthens its utility as a surrogate end point for vaccine evaluation is the approximate "dose-response" relationship we identified between anti-rotavirus IgA titer and protection against rotavirus gastroenteritis. In both high and low child mortality settings, the highest levels of anti-rotavirus IgA were often associated

with the largest reductions in rotavirus gastroenteritis rates and, importantly, the strongest protection was typically evident for severe rotavirus gastroenteritis compared to mild/moderate gastroenteritis. Similar patterns have previously been demonstrated for anti-rotavirus IgA titers following natural infection. Velázquez et al. followed a cohort of children in Mexico for two years and found infants with IgA titers >800 U/mL had an 84% reduced risk of rotavirus gastroenteritis (risk ratio = 0.16, 95% CI = 0.04, 0.64).[39] Premkumar et al. found anti-rotavirus IgA titers ≥619 U/mL reduced the incidence rate of rotavirus diarrhea of any severity by 40% (incidence rate ratio = 0.60, 95% CI = 0.30, 2.13) in a cohort of infants in India over three years of follow-up.[184] Together, these findings support the notion that the relationship between naturally acquired immune response and rotavirus risk may extend to vaccine-induced immune response as well. This has valuable implications for future rotavirus vaccine evaluations, indicating that an improved vaccine strategy or vaccine formulation that leads to an increase in anti-rotavirus IgA titer may correspond with improved protection against rotavirus gastroenteritis.

One critical shortcoming of serum anti-rotavirus IgA confirmed by our study is its imperfect association with protection against rotavirus gastroenteritis. The occurrence of rotavirus gastroenteritis among some infants in high child mortality settings with high anti-rotavirus IgA titers and the drastically different HR estimates for a given anti-rotavirus IgA threshold in high and low child mortality settings indicate that anti-rotavirus IgA alone is not a perfect predictor of rotavirus gastroenteritis risk. This is especially true in high burden settings, in agreement with Lee et al. who found anti-rotavirus IgA seropositivity explained only a small portion of the protection provided by vaccination in Bangladesh.[45]

From the immunologic perspective, the imperfect relationship identified in our study supports the hypothesis that serum anti-rotavirus IgA is likely a "non-mechanistic" correlate of protection. In other words, it may not be causally responsible for protecting against disease.[27,185] Rather, serum anti-rotavirus IgA is likely associated with more proximal activities of the immune system, such as the development of mucosal or duodenal antibodies, that more directly confer protection.[29] Possible transfer or "spillover" of anti-rotavirus IgA from the gut into blood[28] may cause serum anti-rotavirus IgA to be correlated but not perfectly associated with immune system activities in the gut. However, serum anti-rotavirus IgA is a substantially easier to measure than possible mechanistic markers, making it a more practical tool. Further, our analysis assessing the association between anti-rotavirus IgA and rotavirus gastroenteritis while controlling for other confounding factors demonstrate that anti-rotavirus IgA in and of itself is a predictor of protection, not simply a proxy for other factors such as exposure risk or country setting.

This analysis contributes to the growing body of research on serum anti-rotavirus IgA as a possible correlate of protection against rotavirus gastroenteritis through its methodologic approach and application across settings. We pooled the relatively small immunogenicity and follow-up cohorts from carefully conducted clinical trials to create a large, individual level dataset for analysis. Combining data across settings provided increased statistical power compared to the individual trials and allowed us to take a multilevel modeling approach to assess the role of several possible confounders of the anti-rotavirus IgA and rotavirus gastroenteritis relationship.[98,104] We also made full use of available follow-up data by applying survival analysis methods, a substantially improved approach over simple Chi-square and Fisher's exact tests frequently used in previous studies.[27] Assessing two follow-up periods and different child mortality settings provided insight

into the predictive value of anti-rotavirus IgA in the approximately six months immediately following vaccination and highlighted differences in its value across settings.

This analysis took advantage of the immunogenicity and follow-up cohorts of nine clinical trials to create a robust, individual level dataset to assess post-vaccine serum anti-rotavirus IgA as a possible correlate of protection for rotavirus gastroenteritis among vaccinated infants across settings. However, important limitations in our approach should be noted. First, while the protocols were highly consistent across trials, there may be differences in study activities or measures that we were unable to identify. We attempted to account for this by including a frailty term in all survival analysis models. Second, only one post-vaccine serum anti-rotavirus IgA measure collected shortly after vaccination was consistently available across studies. Without serial anti-rotavirus IgA measurements, we were unable to identify infants who may have had asymptomatic infections during follow-up, possibly impacting their likelihood of symptomatic illness. This constraint likely contributed to the unclear association between anti-rotavirus IgA and rotavirus gastroenteritis detected in the second year of life. Infants with the highest levels of anti-rotavirus IgA at the start of follow-up were probably least likely to have rotavirus gastroenteritis by 1 year of age and were mostly likely to be included in the second year of follow-up. Third, because this was a secondary analysis of previously collected clinical trial data, we did not have access to several important potential confounders, such as breastfeeding[186] and force of infection. We aimed to account for this by including rate of non-rotavirus gastroenteritis as a proxy for force of infection and GDP as a proxy for other possible uncontrolled confounders. Lastly, this study was limited to the association between anti-rotavirus IgA and rotavirus

gastroenteritis among infants who received the Rotarix vaccine. It is possible that the association may differ for other rotavirus vaccines of which there are three with WHO pre-qualification.

Serum anti-rotavirus IgA, while not a perfect correlate with a strict threshold indicating 100% protection, is a practical and informative measure of an infant's risk of rotavirus gastroenteritis. Our findings support the hypothesis that anti-rotavirus IgA may be a non-mechanistic correlate of protection. Continued research is needed to validate this measure for use in vaccine evaluation. This could be done with additional research comparing serum anti-rotavirus IgA with more proximal measures of immune response to rotavirus infection or with vaccine efficacy measures specifically. Additionally, further investigation into why the protective levels of anti-rotavirus IgA differ in high and low child mortality settings is necessary. Nonetheless, seroresponse measured using serum anti-rotavirus IgA ≥20 U/mL is a valuable indicator of substantial protection against rotavirus gastroenteritis in both high and low child mortality settings with increased titers generally representing increased protection.

Supplementary Material

Standard hazard model used for analyses:

$$\mathbf{h_{ij}(t) = h_0(t)exp[U + \beta_{a1...an}(H) + \beta_{b1...bn}(C) + \alpha_j]}$$

$h_{ij}(t)$ represents the hazard of rotavirus gastroenteritis for the *i*th infant, in the *j*th trial during follow-up (severity/timeframe modified for alternate outcomes)

$h_0(t)$ represents baseline hazard at time t

β_{a-b} represent regression coefficients for host and country level factors, respectively

U represent anti-rotavirus IgA titer threshold (dichotomous)

H represents a vector of host characteristics, a_1 through a_n

C represents a vector of country-level characteristics, c_1 through c_n

α_j represents a random effect (random intercept) by trial and denotes the increased/decreased hazard for a given cluster (trial)

Table S1. Comparison of HR estimates for anti-rotavirus IgA threshold alone, in a full model controlling for all possible confounders, and the final model after backwards elimination

			Schoenfeld Residual		Global Schoenfeld Residual	
Description	**Predictor**	**HR (95% CI)**	**Chi-sq**	**P**	**Chi-sq**	**P**
Threshold only	IgA ≥20	0.34 (0.25, 0.47)	0.21	0.649	0.21	0.649
Threshold and all predictors	IgA ≥20	0.35 (0.26, 0.49)	0.29	0.588	8.40	0.136
	Sex (1 = male)	0.98 (0.72, 1.35)	1.44	0.230		
	LAZ (1 = not stunted)	1.01 (0.65, 1.55)	1.03	0.309		
	Non-rotavirus gastroenteritis rate (episodes/month)	5.14 (1.56, 16.94)	0.59	0.442		
	GDP	1.00 (1.00, 1.00)	5.15	0.023		
After backwards selection	IgA ≥20	0.35 (0.25, 0.49)	0.33	0.568	6.02	0.111
	Non-rotavirus gastroenteritis rate (episodes/month)	5.22 (1.59, 17.11)	0.58	0.446		
	GDP	1.00 (1.00, 1.00)	5.60	0.018		

IgA = anti-rotavirus immunoglobulin A; LAZ = length-for-age z-score; GDP = gross domestic product in 2004 USD

Table S2a. Survival analysis results for infants in low child mortality settings during follow-up from 1 year of age to 2 years of age

		Mild/Moderate Rotavirus Gastroenteritis				Severe Rotavirus Gastroenteritis			
IgA threshold (U/mL)	N (%)	Events n (%)	Time at risk (week)	Cum. Hazard per 100 (95% CI)	HR* (95% CI)	Events n (%)	Time at risk (week)	Cum. Hazard per 100 (95% CI)	HR* (95% CI)
Seroneg.	456 (20.3)	12 (2.6)	13620	5.13 (1.71, 8.55)	1.00 (ref)	2 (0.4)	13756	0.55 (0.00, 1.32)	1.00 (ref)
≥20	1792 (79.7)	24 (1.3)	60665	1.92 (1.10, 2.73)	0.53 (0.26, 1.09)	8 (0.4)	60873	0.64 (0.19, 1.09)	0.92 (0.20, 4.35)
≥40	1654 (73.6)	20 (1.2)	56267	1.77 (0.94, 2.59)	0.47 (0.22, 0.97)	8 (0.5)	56406	0.69 (0.20, 1.18)	1.00 (0.21, 4.70)
≥80	1386 (61.7)	17 (1.2)	47614	1.83 (0.91, 2.75)	0.47 (0.22, 1.01)	7 (0.5)	47746	0.73 (0.18, 1.28)	1.03 (0.21, 4.96)
≥160	1097 (48.8)	15 (1.4)	38593	2.04 (0.95, 3.12)	0.53 (0.24, 1.17)	5 (0.5)	38741	0.69 (0.08, 1.30)	0.99 (0.19, 5.18)
≥320	765 (34.0)	10 (1.3)	27804	2.06 (0.73, 3.39)	0.39 (0.17, 0.90)	4 (0.5)	27890	0.79 (0.01, 1.57)	1.21 (0.21, 6.84)
≥640	448 (19.9)	4 (0.9)	16854	1.32 (0.02, 2.62)	0.31 (0.10, 0.98)	2 (0.4)	16880	0.69 (0.00, 1.64)	0.81 (0.11, 5.74)
≥1280	204 (9.1)	2 (1.0)	8071	1.36 (0.00, 3.25)	0.35 (0.08, 1.64)	1 (0.5)	8080	0.68 (0.00, 2.02)	0.88 (0.08, 9.74)
≥2560	59 (2.6)	0 (0.0)	2362	0.00 (NA)	0.00 (0.00, Inf)	0 (0.0)	2362	0.00 (NA)	0.00 (0.00, Inf)

IgA = anti-rotavirus immunoglobulin A; HR = hazard ratio; IQR = interquartile range, CI = confidence interval

*Models include study as a frailty term

Table S2b. Survival analysis results for infants in high child mortality settings during follow-up from 1 year of age to 2 years of age

		Mild/Moderate Rotavirus Gastroenteritis				Severe Rotavirus Gastroenteritis			
IgA threshold (U/mL)	N (%)	Events n (%)	Time at risk (week)	Cum. Hazard per 100 (95% CI)	HR* (95% CI)	Events n (%)	Time at risk (week)	Cum. Hazard per 100 (95% CI)	HR* (95% CI)
Seroneg.	554 (35.6)	8 (1.4)	15286	2.54 (0.75, 4.33)	1.00 (ref)	11 (2.0)	15105	2.97 (1.21, 4.72)	1.00 (ref)
≥20	1002 (39.0)	7 (0.7)	28872	1.02 (0.26, 1.78)	0.46 (0.17, 1.26)	10 (1.0)	28767	1.42 (0.54, 2.30)	0.48 (0.20, 1.12)
≥40	880 (34.3)	7 (0.8)	25576	1.16 (0.30, 2.01)	0.52 (0.19, 1.43)	7 (0.8)	25549	1.11 (0.29, 1.94)	0.38 (0.15, 0.97)
≥80	704 (27.4)	5 (0.7)	20655	1.01 (0.12, 1.89)	0.46 (0.15, 1.40)	6 (0.9)	20650	1.19 (0.24, 2.15)	0.40 (0.15, 1.08)
≥160	529 (20.6)	3 (0.6)	15495	0.78 (0.00, 1.67)	0.37 (0.10, 1.39)	5 (0.9)	15461	1.29 (0.16, 2.42)	0.44 (0.15, 1.27)
≥320	369 (14.4)	3 (0.8)	11451	1.07 (0.00, 2.27)	0.50 (0.13, 1.89)	4 (1.1)	11430	1.44 (0.03, 2.84)	0.48 (0.15, 1.51)
≥640	225 (8.8)	3 (1.3)	7344	1.66 (0.00, 3.54)	0.78 (0.21, 2.93)	2 (0.9)	7379	1.10 (0.00, 2.62)	0.37 (0.08, 1.68)
≥1280	115 (4.5)	2 (1.7)	3856	2.18 (0.00, 5.19)	0.99 (0.21, 4.65)	1 (0.9)	3874	1.06 (0.00, 3.13)	0.36 (0.05, 2.79)
≥2560	49 (1.9)	1 (2.0)	1605	2.53 (0.00, 7.49)	1.22 (0.15, 9.76)	0 (0.0)	311	0.00 (NA)	NA

IgA = anti-rotavirus immunoglobulin A; HR = hazard ratio; IQR = interquartile range, CI = confidence interval

*Models include study as a frailty term

6 Aim 3- Longer-term direct and indirect vaccine effects

[Manuscript 3. This chapter is a pre-copyedited, author-produced version of an article accepted for publication in Clinical Infectious Diseases following peer review. The version of record (Baker JM, Tate JE, Steiner CA, Haber MJ, Parashar UD, Lopman BA. Longer-term Direct and Indirect Effects of Infant Rotavirus Vaccination Across All Ages in the United States in 2000-2013: Analysis of a Large Hospital Discharge Data Set. *Clin Infect Dis*. 2019 Mar 5;68(6):976-983.) is available online at: https://academic.oup.com/cid/article/68/6/976/5055445, DOI: https://doi.org/10.1093/cid/ciy580.]

Longer-term direct and indirect effects of infant rotavirus vaccination across all ages in the US; 2000 - 2013: analysis of a large hospital discharge dataset

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Abstract

Background: Rotavirus disease dramatically declined among children under 5 years of age since the rotavirus vaccine was introduced in 2006; population-level impacts remain to be fully elucidated.

Methods: Data from the Healthcare Cost and Utilization Project State Inpatient Database were used to conduct a time-series analysis of monthly hospital discharges across age groups for acute gastroenteritis and rotavirus from 2000-2013. Rate ratios were calculated comparing pre- and post-vaccine eras.

Results: Following vaccine introduction, a decrease in rotavirus hospitalizations occurred with a shift towards biennial patterns across all ages. The 0-4 year age group experienced the largest decrease in rotavirus hospitalizations (RR: 0.14, 95% CI: 0.09 – 0.23). The 5-19 and 20-59 year age groups experienced significant declines in rotavirus hospitalization rates overall; even post-vaccine calendar years were characterized by progressively lower rates while odd post-vaccine years were associated with reductions in rates that diminished over time. Those aged 60 years or older experienced the smallest change in rotavirus hospitalization rates overall, with significant reductions in even post-vaccine years compared to pre-vaccine years (RR: 0.51; 95% CI: 0.39 – 0.66).

Conclusions: Indirect impacts of infant rotavirus vaccination are apparent in the emergence of biennial patterns in rotavirus hospitalizations that extend to all age groups ineligible for vaccination. These observations are consistent with the notion that young children are of primary importance in disease transmission and that the initial post-vaccination period of dramatic population-wide impacts will be followed by more complex incidence patterns across the age range in the long-term.

Background

Prior to vaccine introduction in the United States (US), rotavirus was the leading cause of severe pediatric gastroenteritis, resulting in up to 70,000 hospitalizations[133] and an estimated $319 million in healthcare costs annually[187]. Following pivotal clinical trial results[188,189], two live, attenuated oral rotavirus vaccines, RotaTeq (Merck & Co.) and Rotarix (GlaxoSmithKline Biologicals) were included in the routine infant vaccination schedule in 2006 and 2008, respectively, per the Advisory Committee on Immunization Practices' recommendations[130,132]. By 2015, 73.2% of children aged 19-35 months had received a full course of rotavirus vaccine[190]. This is modest coverage compared to more established routine infant immunizations such as DTaP where coverage was 95.0% for 3 or more doses in 2015[190].

The direct effects of rotavirus vaccination have been clearly demonstrated and evidence of potential indirect effects are emerging. Among children under 5 years of age, rotavirus disease has declined dramatically in the decade following the introduction of the vaccines in the US, with reductions in hospitalizations[87,129,136], ED visits[136,137], and physician office visits[87,136]. Introduction of rotavirus vaccination has also altered epidemiological patterns, with a switch from annual to biennial patterns in disease incidence and a delay in the seasonal peak in low-incidence years[191]. Moreover, there is evidence of potential indirect benefits of the vaccine program, with reductions in hospitalizations observed among unvaccinated children, likely as a result of reduced transmission from their vaccinated counterparts[87,129,143]. In addition, early data from the US and select other early-introducing countries, suggest that these indirect benefits may extend to older

children and adults[139,140]. If true, these observations unmask a considerable severe disease burden outside the pediatric age range that may be preventable by infant immunization.

The longer-term impacts of infant rotavirus vaccination across all ages remains to be fully elucidated. Studies to date addressing these questions have been limited to relatively short-term post-vaccine time periods[139,140] or restricted to younger age groups[87,129,136,142,143]. Introduction of the rotavirus vaccine is changing epidemiologic patterns[191] and may lead to subtle shifts in circulating serotypes[192,193]. Accordingly, evaluation of longer-term trends and analysis of older age groups is needed to identify the full public health impacts of infant rotavirus vaccination. We aimed to evaluate the population-wide impact, across all ages, of infant rotavirus vaccination on gastroenteritis and rotavirus hospitalizations by comparing the pre- and post-vaccine periods in the US. We examined the total effects among young children and the indirect effects to older children and adults using a large national discharge database.

Methods

Data sources

Hospitalization data from January 2000 through December 2013 were obtained from the Healthcare Cost and Utilization Project (HCUP) State Inpatient Database (SID), sponsored by the Agency for Healthcare Research and Quality, through an active collaboration. This database is a compilation of monthly discharge data from all community hospitals (all nonfederal, short-term, general and specialty hospitals) within participating states[149]. Our analysis was restricted to 26

states for which data were available for the entire study period: Arizona, California, Colorado, Connecticut, Florida, Georgia, Hawaii, Iowa, Illinois, Kansas, Kentucky, Maryland, Massachusetts, Michigan, Missouri, North Carolina, New Jersey, New York, Oregon, South Carolina, Tennessee, Texas, Utah, Washington, Wisconsin, and West Virginia. According to the National Center for Health Statistics Bridged Race population dataset (https://www.cdc.gov/nchs/nvss/bridged_race.htm), these 26 states represent approximately three-quarters (74.2%) of the total US population in 2013. This population dataset was used to calculate rates in this analysis.

Two outcomes were separately assessed: rates of all-cause acute gastroenteritis (AGE) and rates of rotavirus specific gastroenteritis (RVGE) hospital discharges. Discharge data, coded according to the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM), were extracted from the SID database to identify AGE and RVGE codes noted as the primary diagnosis or listed in one of 15 other possible diagnosis fields. Applicable ICD-9-CM codes for AGE are detailed elsewhere[152] and included bacterial, parasitic, and viral gastrointestinal illness of determined etiology and presumed infectious or non-infectious gastrointestinal illness of undetermined etiology. We defined a RVGE hospitalization as any discharge with ICD-9-CM diagnosis code 008.61. Rotavirus coded discharge data were expected to provide the most specific indicator of rotavirus rates. However, restricting the analysis to only these episodes may underestimate the true rotavirus burden due to limited testing for rotavirus in medical settings[25,26]. Therefore, the broader classification of all-cause AGE was also analyzed to capture RVGE not identified as being rotavirus-related due to incomplete detection or miscoding.

Statistical analysis

The impact of vaccination was estimated by comparing AGE and RVGE age-specific hospitalization rates per 10,000 prior to and after vaccine introduction using regression models. Monthly counts of AGE and RVGE hospitalizations were modeled using negative binomial regression with a categorical variable representing post-vaccine year and adjustment for changing population size using an offset of the log of population[194]. Analyses were performed using R software version 3.5.0.

Three comparisons were made for each outcome to estimate rate ratios (RRs) and corresponding 95% confidence intervals (CIs). Monthly pre-vaccine rates overall (2000-2006) were compared to: (1) monthly post-vaccine rates overall (2008-2013), (2) monthly post-vaccine rates for even (2008, 2010, 2012) and odd post-vaccine calendar years (2009, 2011, 2013) separately to assess biennial patterns, and (3) monthly post-vaccine rates for each individual post-vaccine calendar year to provide more detail on the potentially dynamic effects of vaccination over time. The year 2006 was included in the pre-vaccine period because the vaccine was not recommended until August of that year and vaccine coverage was initially low.[195] The year 2007 was considered a transition period and excluded from analyses. All analyses were performed separately for the age groups 0-4 years, 5-19 years, 20-59 years, and 60 years and older to assess the total and indirect effects of the vaccine across age groups. Age groups were selected by combining those with similar disease patterns and trends to increase power for the analyses. For AGE, we modeled data from the historic rotavirus season in the US (January-June) to improve the model's specificity for AGE cases that may be rotavirus related; rotavirus models included year-round data.

We aimed to adjust for potential exogenous secular trends (trends over time) with the inclusion of a sequential (continuous) time variable. The AGE model results were sensitive to the inclusion of time trends, so we further considered second and third order time variables. Based on the Akaike information criterion values, including higher order time variables did not substantially improve model fit. RVGE hospitalization model results were not sensitive to the addition of a sequential time variable, so it was excluded. Rotavirus models controlled for the period 2000-2003 using an indicator variable to account for the increase in RVGE hospitalization rates that occurred just prior to 2004 among all age groups. The cause of this increase in RVGE hospitalization rates is uncertain but may be a result of increased rotavirus testing in anticipation of vaccine introduction. Because we sought to identify the long-term impact of vaccine exposure on AGE and RVGE hospitalization rates, rather than short-term deviations from an "underlying pattern", we chose to account for secular trends (where appropriate) and not consider autocorrelation.

Ethical approval

This study was not subject to Institutional Review Board approval at Emory University or the Centers for Disease Control and Prevention because it involved de-identified, aggregate data.

Results

A total of 13,527,516 AGE hospitalizations from 2000-2013 were analyzed, including 224,099 (1.7%) specified as RVGE.

AGE patterns

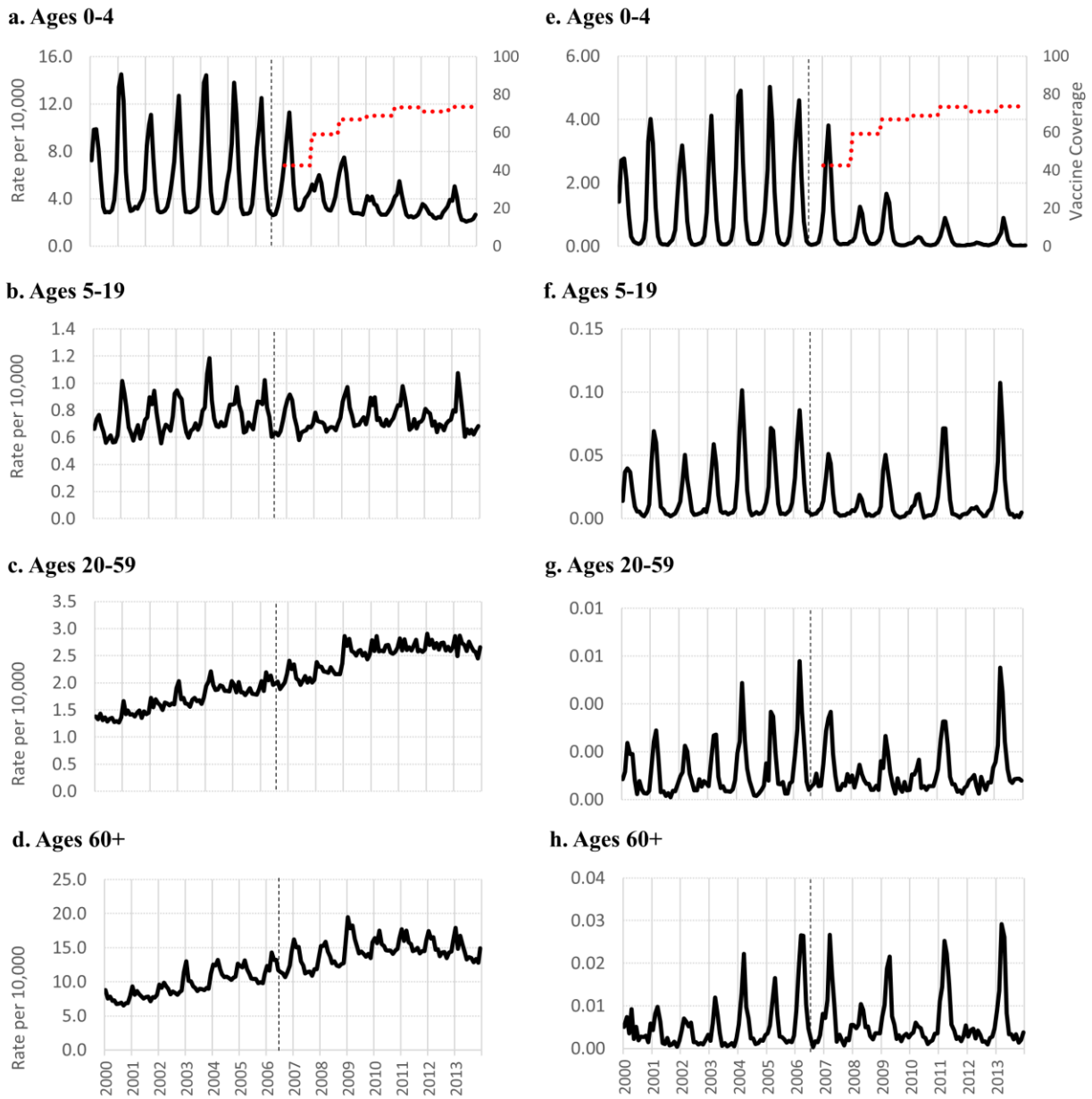
Using unrestricted (full-year) data, the highest rates of AGE hospitalization were among those aged 60 years and older with an average monthly rate of 10.2 hospitalizations per 10,000 age-specific population (unadjusted rate range of 3.3–24.3) during the pre-vaccine period, increasing to 15.7 hospitalizations per 10,000 age-specific population (unadjusted rate range of 6.1–33.3) during the post-vaccine period. A secular increasing trend began during the pre-vaccine period for this age group as well as among those 20-59 years (Figure 1c-d). In contrast, we observed no clear evidence of a secular trend in the 0-4 or 5-19 year age groups (Figure 1a-b).

During the historic rotavirus season (Jan.–Jun.), we observed significant reductions in AGE hospitalizations for the 0-4 year age group in the post-vaccine compared with the pre-vaccine periods (Table 1a; RR: 0.54, 95% CI: 0.34–0.85), with slightly greater reductions in even calendar years than odd calendar years (even year RR: 0.50, 95% CI: 0.32–0.78; odd year RR: 0.58, 95% CI: 0.35–0.95) and generally decreasing RRs, punctuated by a subtle biennial pattern (Figure 2a). Similar patterns with smaller and non-significant reductions were observed among the 5-19 age group (Figure 2b). No significant reductions in rates of AGE hospitalizations were observed for the 20-59 or 60+ age groups (Figure 2c-d). Similar patterns with more diluted impacts were observed in the unrestricted (full-year) comparative analyses (Supplementary Table 1).

Figure 1a-h. Monthly all-cause acute gastroenteritis (AGE) and rotavirus gastroenteritis (RVGE) hospitalizations per 10,000 age-specific population; United States, 2000-2013.[a,b, c]

Figure 1a-d. AGE

Figure 1e-h. RVGE



[a] Data from 26 states from the State Inpatient Data (SID) from the Healthcare Cost and Utilization Project (HCUP) included: AZ, CA, CO, CT, FL, GA, HI, IA, IL, KS, KY, MD, MA, MI, MO, NC, NJ, NY, OR, SC, TN, TX, UT, WA, WI, WV.

[b] Data are full-year (not restricted to January – June) for the purpose of aiding visualization of the patterns in hospitalization rates.

[c] Red dotted line represents up-to-date vaccine coverage among children ages 19-35 months (2 or 3 doses depending on vaccine manufacturer); the vertical black dashed line represents rotavirus vaccine introduction.

Table 1. Monthly all-cause acute gastroenteritis (AGE) hospitalization rates and adjusted rate ratios restricted to the rotavirus season (January-June); United States 2000-2013.

Table 1a. Comparing the pre-vaccine period to the post-vaccine period overall and in even and odd calendar years [a, b]

	Pre-Vaccine		**Post**		**Post-Even Years**		**Post-Odd Years**	
Age Group	**Rate[c]**	**Range**	**Rate[c]**	**RR[d] (95% CI)**	**Rate[c]**	**RR[d] (95% CI)**	**Rate[c]**	**RR[d] (95% CI)**
0-4	8.04	2.89 – 14.49	4.19	0.54* (0.34 – 0.85)	3.92	0.50* (0.32 – 0.78)	4.47	0.58* (0.35 – 0.95)
5-19	0.79	0.43 – 1.84	0.79	0.84 (0.68 – 1.06)	0.75	0.81 (0.65 – 1.01)	0.83	0.88 (0.69 – 1.12)
20-59	1.72	0.78 – 3.43	2.62	0.93 (0.74 – 1.18)	2.55	0.94 (0.75 – 1.18)	2.69	0.93 (0.72 – 1.20)
60+	10.24	3.34 – 24.33	15.74	0.82 (0.58 – 1.17)	15.33	0.83 (0.59 – 1.18)	16.16	0.81 (0.55 – 1.19)

[a] Data from 26 states from the State Inpatient Data (SID) from the Healthcare Cost and Utilization Project (HCUP) included: AZ, CA, CO, CT, FL, GA, HI, IA, IL, KS, KY, MD, MA, MI, MO, NC, NJ, NY, OR, SC, TN, TX, UT, WA, WI, WV. [b] Adjusted rate ratios from negative binomial regression comparing the pre-vaccine period (2000-2006) to post-vaccine period (2008-2013) overall and by even and odd calendar years, controlling for time and restricted to the rotavirus season. [c] Unadjusted rate per 10,000 age-specific population. [d] Rate ratio, compared to pre-vaccine era. * Significant at $\alpha = 0.05$ level.

Table 1b. Comparing the pre-vaccine period to the post-vaccine period by year [a, b]

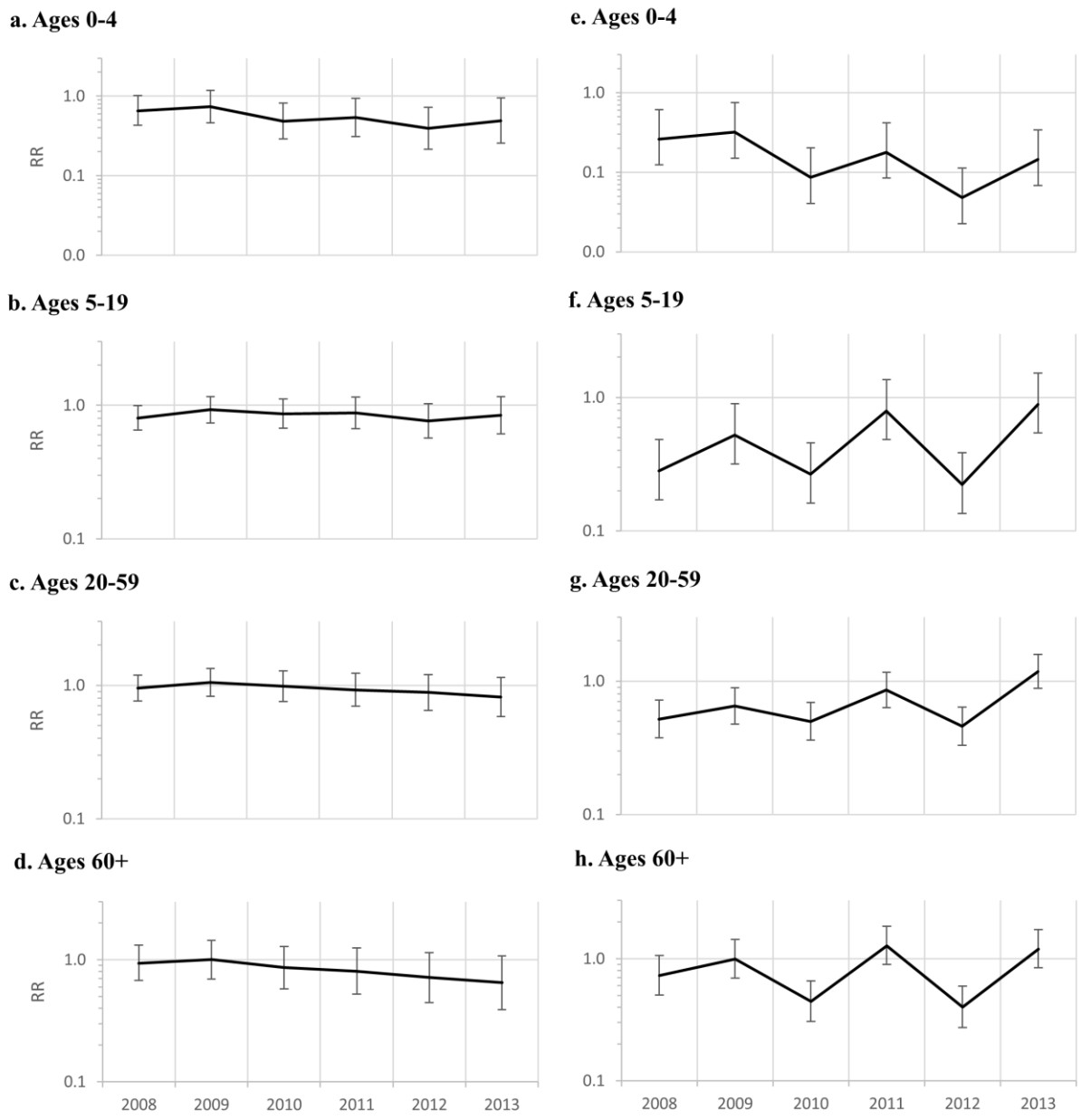
	2008		2009		2010		2011		2012		2013	
Age Group	**Rate[c]**	**RR[d] (95% CI)**	**Rate[c]**	**RR[d] (95% CI)**	**Rate[c]**	**RR[d] (95% CI)**	**Rate[c]**	**RR[d] (95% CI)**	**Rate[c]**	**RR[d] (95% CI)**	**Rate[c]**	**RR[d] (95% CI)**
0-4	5.08	0.66 (0.43 – 1.01)	5.67	0.74 (0.46 – 1.18)	3.70	0.49* (0.29 – 0.81)	4.08	0.54* (0.3 – 0.94)	2.97	0.40* (0.22 – 0.72)	3.66	0.49* (0.26 – 0.94)
5-19	0.71	0.81* (0.65 – 0.99)	0.84	0.93 (0.74 – 1.17)	0.80	0.86 (0.67 – 1.11)	0.83	0.88 (0.67 – 1.16)	0.74	0.76 (0.57 – 1.03)	0.83	0.84 (0.61 – 1.16)
20-59	2.26	0.95 (0.77 – 1.19)	2.67	1.05 (0.83 – 1.34)	2.66	0.98 (0.76 – 1.28)	2.68	0.93 (0.69 – 1.23)	2.73	0.88 (0.65 – 1.20)	2.71	0.82 (0.58 – 1.15)
60+	14.55	0.94 (0.68 – 1.32)	16.87	1.00 (0.70 – 1.44)	15.82	0.86 (0.58 – 1.28)	16.18	0.81 (0.52 – 1.25)	15.60	0.72 (0.45 – 1.14)	15.43	0.65 (0.39 – 1.08)

[a] Data from 26 states from the State Inpatient Data (SID) from the Healthcare Cost and Utilization Project (HCUP) included: AZ, CA, CO, CT, FL, GA, HI, IA, IL, KS, KY, MD, MA, MI, MO, NC, NJ, NY, OR, SC, TN, TX, UT, WA, WI, WV. [b] Adjusted rate ratios from negative binomial regression comparing the pre-vaccine period (2000-2006) to post-vaccine period (2008-2013) by year, controlling for time and restricted to the rotavirus season. [c] Unadjusted rate per 10,000 age-specific population. [d] Rate ratio, compared to pre-vaccine era. * Significant at $\alpha = 0.05$ level.

Figure 2a-h. Monthly all-cause acute gastroenteritis (AGE) and rotavirus gastroenteritis (RVGE) hospitalization rate ratios comparing the pre-vaccine period (2000-2006) to post-vaccine period (2008-2013) by year; United States 2000 to 2013.[a]

Figure 2a-d. AGE[b]

Figure 2e-h. RVGE[c]



[a] Data from 26 states from the State Inpatient Data (SID) from the Healthcare Cost and Utilization Project

(HCUP) included: AZ, CA, CO, CT, FL, GA, HI, IA, IL, KS, KY, MD, MA, MI, MO, NC, NJ, NY, OR, SC, TN, TX, UT, WA, WI, WV.

[b] Negative binomial regression using a sequential (continuous) time variable to control for potential exogenous secular trends, restricted to the rotavirus season (January-June).

[c] Negative binomial regression controlling for the pre-2004 period using an indicator variable to account for the increase in RVGE hospitalization rates that occurred just prior to 2004, including all months.

RVGE patterns

Following vaccine introduction, a decrease in RVGE hospitalization rates occurred across all age groups (Table 2a), with parallel trends and a shift towards biennial patterns (Figure 1e-h) across all ages. The 0-4 age group experienced the largest overall decrease in RVGE hospitalization rates comparing the pre- and post-vaccine periods.

After introduction of the infant rotavirus vaccine, RVGE hospitalization rates among those aged 0-4 years declined by more than 85% (Table 2a, RR: 0.14, 95% CI: 0.09–0.23). A biennial pattern in RVGE hospitalization rates was apparent for this age group, with larger declines in the rate of RVGE hospitalization in even calendar years (RR: 0.10, 95% CI: 0.06–0.18) compared to odd calendar years (RR: 0.20, 95% CI: 0.12–0.35). This pattern was further characterized by consistently decreasing rates over time for both the even and odd post-vaccination calendar years (Figure 2e).

The 5-19 and 20-59 age groups both experienced a significant decline in RVGE hospitalization rates overall, primarily due to the decline in RVGE hospitalizations that occurred in even calendar

years after introduction of the vaccines (Table 2a). No significant changes in RVGE hospitalization rates were observed for either age group when comparing pre-vaccination rates with odd calendar years post-vaccination. Of note, analysis of RRs by individual year post-vaccine revealed that even years were characterized by progressively lower rates while odd years were typically associated with reductions in rates that diminished over time or modest, non-significant increases (Figure 2f-g).

Among those 60 and older, patterns similar to those observed for the 5-19 and 20-59 age groups were seen, including overall declines in hospitalization rates in the post-vaccine period compared to pre-vaccine years (Table 2a RR: 0.76; 95% CI: 0.61–0.96) and significant declines in even post-vaccine years (RR: 0.51; 95% CI: 0.39–0.66). In the odd post-vaccine years, we observed no change in RVGE rates in 2009 (Table 2b, RR: 0.99; 95% CI: 0.70–1.44) and non-significant increases in rates in 2011 and 2013 (RR: 1.28; 95% CI: 0.90–1.85 and RR: 1.20; 95% CI: 0.84–1.73, respectively) compared to the pre-vaccine period (Figure 2h).

Table 2. Monthly rotavirus specific gastroenteritis (RVGE) hospitalization rates and adjusted rate ratios; United States 2000 to 2013.

Table 2a. Comparing the pre-vaccine period to the post-vaccine period overall and in even and odd calendar years [a, b]

	Pre-Vaccine		**Post**		**Post-Even Years**		**Post-Odd Years**	
Age Group	**Rate[c]**	**Range**	**Rate[c]**	**RR[d] (95% CI)**	**Rate[c]**	**RR[d] (95% CI)**	**Rate[c]**	**RR[d] (95% CI)**
0-4	1.205	(0.045 – 5.023)	0.237	0.14* (0.09 – 0.23)	0.181	0.10* (0.06 – 0.18)	0.294	0.20* (0.12 – 0.35)
5-19	0.023	(0.000 – 0.257)	0.013	0.43* (0.31 – 0.59)	0.007	0.26* (0.18 – 0.37)	0.019	0.72 (0.50 – 1.03)
20-59	0.001	(0.000 – 0.009)	0.001	0.65* (0.54 – 0.79)	0.001	0.49* (0.39 – 0.62)	0.001	0.87 (0.70 – 1.08)
60+	0.005	(0.000 – 0.042)	0.006	0.76* (0.61 – 0.96)	0.004	0.51* (0.39 – 0.66)	0.008	1.15 (0.89 – 1.48)

[a] Data from 26 states from the State Inpatient Data (SID) from the Healthcare Cost and Utilization Project (HCUP) included: AZ, CA, CO, CT, FL, GA, HI, IA, IL, KS, KY, MD, MA, MI, MO, NC, NJ, NY, OR, SC, TN, TX, UT, WA, WI, WV. [b] Adjusted rate ratios from negative binomial regression comparing the pre-vaccine period (2000-2006) to post-vaccine period (2008-2013) overall and in even and odd calendar years, controlling for the pre-2004 period. [c] Unadjusted rate per 10,000 age-specific population. [d] Rate ratio, compared to pre-vaccine era. * Significant at $\alpha = 0.05$ level.

Table 2b. Comparing the pre-vaccine period to the post-vaccine period by year [a, b]

	2008		2009		2010		2011		2012		2013	
Age Group	**Rate[c]**	**RR[d] (95% CI)**	**Rate[c]**	**RR[d] (95% CI)**	**Rate[c]**	**RR[d] (95% CI)**	**Rate[c]**	**RR[d] (95% CI)**	**Rate[c]**	**RR[d] (95% CI)**	**Rate[c]**	**RR[d] (95% CI)**
0-4	0.359	0.26* (0.12 – 0.61)	0.438	0.32* (0.15 – 0.75)	0.118	0.09* (0.04 – 0.20)	0.245	0.18* (0.08 – 0.42)	0.066	0.05* (0.02 – 0.11)	0.198	0.14* (0.07 – 0.34)
5-19	0.007	0.28* (0.17 – 0.48)	0.014	0.52* (0.32 – 0.89)	0.007	0.27* (0.16 – 0.46)	0.021	0.79 (0.48 – 1.36)	0.006	0.22* (0.13 – 0.38)	0.023	0.89 (0.54 – 1.52)
20-59	0.001	0.52* (0.38 – 0.72)	0.001	0.65* (0.48 – 0.89)	0.001	0.50* (0.36 – 0.69)	0.001	0.86 (0.63 – 1.16)	0.001	0.46* (0.33 – 0.64)	0.002	1.18 (0.88 – 1.59)
60+	0.005	0.73 (0.50 – 1.06)	0.007	0.99 (0.70 – 1.44)	0.003	0.45* (0.30 – 0.66)	0.009	1.28 (0.90 – 1.85)	0.003	0.40* (0.27 – 0.59)	0.008	1.20 (0.84 – 1.73)

[a] Data from 26 states from the State Inpatient Data (SID) from the Healthcare Cost and Utilization Project (HCUP) included: AZ, CA, CO, CT, FL, GA, HI, IA, IL, KS, KY, MD, MA, MI, MO, NC, NJ, NY, OR, SC, TN, TX, UT, WA, WI, WV. [b] Adjusted rate ratios from negative binomial regression comparing the pre-vaccine period (2000-2006) to post-vaccine period (2008-2013) by year, controlling for the pre-2004 period. [c] Unadjusted rate per 10,000 age-specific population. [d] Rate ratio, compared to pre-vaccine era. * Significant at $\alpha = 0.05$ level.

Discussion

Our analysis of six years of post-vaccination data offers several insights into the complex longer-term population-level effects of infant rotavirus vaccination. Rotavirus vaccination had a substantial impact on RVGE hospitalizations across age groups during the six year period, highlighting the role of infants as drivers of infection transmission. These indirect effects are evidenced by parallel trends in the incidence of RVGE hospitalizations whereby biennial temporal patterns emerged among all age groups. Overall reductions in the incidence of RVGE hospitalizations were observed for children, adolescents, and adults during the post-vaccine period with diminished indirect impacts as age group increased. Remarkably, by 2013, there was a suggestion in the data that RVGE hospitalization rates among the 20-59 and 60 years and older age groups may have slightly exceeded pre-vaccine levels. Infant vaccination programs increase the average age of infection; further years' data will be need to determine whether there is an absolute increase in disease rates in older age groups.

The US was one of the first countries to introduce a rotavirus vaccine nationally[130,131] and we were, therefore, uniquely positioned to assess the longer-term temporal trends in RVGE hospitalization rates. A central strength of this study is the use of national AGE and RVGE hospital discharge data spanning six years after the introduction of the rotavirus vaccine and including data on older children, adolescents and adults. This is the longest age stratified time-series analysis of the rotavirus vaccine era trends to date. The 26 states included in the analysis represent 74.2% of the national population and include all cases occurring in community hospitals (all nonfederal, short-term, general and specialty hospitals) in these states. The time-series methodology enabled us to

assess the dynamic effects of the vaccine while accounting for background secular trends. These results extend findings from previous studies that suggested possible indirect impacts of infant vaccination among older children and adults using data from the first few years after vaccine introduction[139,140,196–198]. This study also provides a more detailed look at temporal patterns by analyzing both pre- and post-vaccination rates overall but also for individual years, enabling the identification of biennial patterns previously noted[140,191].

Potentially confounding our analysis were secular changes in RVGE and AGE rates, particularly among older adults, that may have been unrelated to vaccine introduction. We aimed to control for these trends by including time variables in our models, a technique used in some[139,140] but not all[129,138] previous studies. Depending on the direction of the secular trend, not accounting for time confounding may lead to an over- or under-estimation of vaccine impact. Without genotype data, we are unable to determine if the new patterns of RVGE hospitalizations during the vaccination era are associated with previously circulating rotavirus strains or the emergence of less commonly observed strains—a question raised by recent evaluations[192,193,199]. The role of vaccine induced pressures on circulating strains should be considered cautiously as vaccination has been shown to induce cross-protective immunity[43] and there is evidence of strain diversity by geography and over time unrelated to vaccination[200].

Important biases may arise from rotavirus testing in the clinical setting. It is well recognized that not all AGE hospitalization cases are tested for rotavirus, even among young children where the burden is most appreciated[25,26]. The ICD-9 coding for rotavirus has demonstrated high specificity (97%) but low sensitivity (estimated between 25-47%) for children under 5 years of age during

the pre-vaccine era[151] and there is limited information about the sensitivity and specificity of rotavirus coding among older age groups[14,201] with trends in testing practices and coding validity largely unknown. We are also unable to assess how seasonality of rotavirus (i.e. changes in rotavirus prevalence) may impact the positive and negative predictive value of rotavirus tests. However, only confounders that vary over time, such as a change in testing practices, would bias our results; we have no evidence to suggest such a change did or did not occur. The percentage of AGE cases that tested positive for rotavirus has declined sharply among children since introduction of the rotavirus vaccine[143,191] so the decline in rotavirus cases after vaccine introduction cannot be attributed to reduced testing alone. Nonetheless, any potential changes in testing practices are unlikely to account for the biennial pattern observed. Differences in the patterns between AGE and RVGE hospitalization rates are likely due to the non-specific nature of the AGE outcome which captures gastroenteritis cases from diverse etiologies.

This study reveals the important role that infants play as drivers of rotavirus infection among all age groups, the indirect effects of infant vaccinations among older children, adolescents, and adults, and the emergent biennial patterns of RVGE hospitalization across age ranges. The biennial pattern in RVGE hospitalization rates could be a result of changing rates of susceptibles within the population. Vaccine coverage combined with acquired immunity could be high enough to transiently raise herd immunity such that transmission is low and allow for a short-term accumulation of susceptibles, particularly among young children, spurring biennial epidemics that spread across age groups[143,202]. The indirect effects of infant vaccination are apparent in the reduced incidence of RVGE among older age groups observed immediately after introduction of the vaccine. These findings are likely a result of higher transmissibility from children and social

contact patterns that result in children being the primary drivers of infection[203]. A related pattern observed in the data is the reduced relative effect of infant RVGE vaccination on unvaccinated individuals as age group among the unvaccinated increases and over time. Seasonal patterns among older age groups are likely to be closely linked to incidence among young children, however, population immunity and transmission within the age group may play an increasing role[204].

Further understanding of the long-term impacts of rotavirus vaccination across the age range could be gained with additional data from the post-vaccine period and data from comparable early-introducing countries. Data from 2014 and beyond would enable us to determine if the relationships identified in this study continue in future years. Indeed, infant vaccination can create immunity gaps in older ages, as has been observed for varicella[205] and rubella[206]. While our analysis shows the substantial influence of infant immunization across the age range, the source of infection among adults and their role in rotavirus transmission is not clear and should be the subject of future studies. Since nearly all adults in our study would have acquired rotavirus antibodies as children in the pre-vaccination era[130], our findings support the notion that immunity is not life-long[201]. Data on vaccine effectiveness by year could provide further insight into these trends.

Funding: This work received no dedicated funding.

Acknowledgements: The authors are grateful to Dr. Juan Leon of the Emory University Rollins School of Public Health for his review of this manuscript. In addition, we sincerely thank and

acknowledge the HCUP state partners for their active support of this evaluation (https://www.hcup-us.ahrq.gov/sidoverview.jsp)

Potential conflicts of interests: BAL has received personal fees from Takeda Pharmaceuticals for service on their Norovirus Advisory Board outside the submitted work. JMB, JET, CAS, MJH and UDP have no conflicts of interest.

Supplementary File

Supplementary Table 1. Monthly all-cause acute gastroenteritis (AGE) hospitalization rates and adjusted rate ratios using unrestricted (January-December) data; United States 2000 to 2013.

Supplementary Table 1a. Comparing the pre-vaccine period to the post-vaccine period overall and by even and odd calendar years [a, b]

	Pre-Vaccine		**Post-Vaccine**		**Post-Even Years**		**Post-Odd Years**	
Age Group	**Rate[c]**	**Range**	**Rate[c]**	**RR[d] (95% CI)**	**Rate[c]**	**RR[d] (95% CI)**	**Rate[c]**	**RR[d] (95% CI)**
0-4	5.91	2.63 – 14.49	3.25	0.79 (0.55 – 1.15)	3.25	0.78 (0.54 – 1.12)	3.25	0.81 (0.54 – 1.21)
5-19	0.76	0.43 – 1.84	0.70	0.92 (0.80 – 1.05)	0.69	0.90 (0.79 – 1.03)	0.70	0.93 (0.80 – 1.09)
20-59	1.74	0.75 – 4.33	2.45	0.95 (0.81 – 1.12)	2.43	0.96 (0.81 – 1.12)	2.46	0.94 (0.79 – 1.13)
60+	10.13	3.26 – 32.52	14.07	0.85 (0.67 – 1.09)	14.13	0.87 (0.69 – 1.11)	14.02	0.83 (0.64 – 1.09)

[a] Data from 26 states from the State Inpatient Data (SID) from the Healthcare Cost and Utilization Project (HCUP) included: AZ, CA, CO, CT, FL, GA, HI, IA, IL, KS, KY, MD, MA, MI, MO, NC, NJ, NY, OR, SC, TN, TX, UT, WA, WI, WV. [b] Adjusted rate ratios from negative binomial regression comparing the pre-

vaccine period (2000-2006) to post-vaccine period (2008-2013) overall and in even and odd calendar years, controlling for time. [c] Unadjusted rate per 10,000 age-specific population [d] Rate ratio, compared to pre-vaccine era. * Significant at $\alpha = 0.05$ level

Supplementary Table 1b. Comparing the pre-vaccine period to the post-vaccine period by year [a, b]

	2008		2009		2010		2011		2012		2013	
Age Group	**Rate[c]**	**RR[d] (95% CI)**	**Rate[c]**	**RR[d] (95% CI)**	**Rate[c]**	**RR[d] (95% CI)**	**Rate[c]**	**RR[d] (95% CI)**	**Rate[c]**	**RR[d] (95% CI)**	**Rate[c]**	**RR[d] (95% CI)**
0-4	4.06	0.91 (0.65 – 1.30)	3.94	0.92 (0.63 – 1.34)	3.06	0.74 (0.49 – 1.13)	3.08	0.78 (0.49 – 1.22)	2.64	0.69 (0.42 – 1.13)	2.73	0.74 (0.44 – 1.26)
5-19	0.71	0.89 (0.78 – 1.01)	0.72	0.98 (0.85 – 1.13)	0.71	0.95 (0.81 – 1.10)	0.71	0.94 (0.80 – 1.12)	0.66	0.87 (0.72 – 1.04)	0.68	0.89 (0.73 – 1.08)
20-59	2.33	0.97 (0.83 – 1.13)	2.42	1.05 (0.89 – 1.25)	2.46	1.00 (0.83 – 1.21)	2.47	0.95 (0.78 – 1.16)	2.51	0.90 (0.73 – 1.12)	2.47	0.84 (0.66 – 1.06)
60+	14.31	0.96 (0.77 – 1.21)	14.39	1.00 (0.78 – 1.29)	14.17	0.91 (0.69 – 1.20)	14.31	0.85 (0.63 – 1.15)	13.89	0.76 (0.55 – 1.05)	13.36	0.68* (0.48 – 0.96)

[a] Data from 26 states from the State Inpatient Data (SID) from the Healthcare Cost and Utilization Project (HCUP) included: AZ, CA, CO, CT, FL, GA, HI, IA, IL, KS, KY, MD, MA, MI, MO, NC, NJ, NY, OR, SC, TN, TX, UT, WA, WI, WV. [b] Adjusted rate ratios from negative binomial regression comparing the pre-vaccine period (2000-2006) to post-vaccine period (2008-2013) by year, controlling for time. [c] Unadjusted rate per 10,000 age-specific population. [d] Rate ratio, compared to pre-vaccine era. * Significant at $\alpha = 0.05$ level.

7 Aim 3- Supplemental Study

[Manuscript 4. This chapter appears as accepted for publication in *BMC Infectious Diseases* by Oxford University Press (Baker JM, Dhal RM, Cubilo J, Parashar UD, Lopman BA. Effects of the rotavirus vaccine program across age groups in the United States: analysis of national claims data, 2001–2016. *BMC Infect Dis*. 2019;19:186. Available online at: https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-019-3816-7, DOI: 10.1186/s12879-019-3816-7.]

Effects of the rotavirus vaccine program across age groups in the United States: analysis of national claims data, 2001-2016

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Abstract

Background: The direct effectiveness of infant rotavirus vaccination implemented in 2006 in the United States has been evaluated extensively, however, understanding of population-level vaccine effectiveness (VE) is still incomplete.

Methods: We analyzed time series data on rotavirus gastroenteritis (RVGE) and all-cause acute gastroenteritis (AGE) hospitalization rates in the United States from the MarketScan® Research

Databases for July 2001–June 2016. Individuals were grouped into ages 0-4, 5-9, 10-14, 15-24, 25-44, and 45-64 years. Negative binomial regression models were fitted to monthly RVGE and AGE data to estimate the direct, indirect, overall, and total VE.

Results: A total of 9,211 RVGE and 726,528 AGE hospitalizations were analyzed. Children 0-4 years of age had the largest declines in RVGE hospitalizations with direct VE of 87% (95% CI: 83%, 90%). Substantial indirect effects were observed across age groups and generally declined in each older group. Overall VE against RVGE hospitalizations for all ages combined was 69% (95% CI: 62%, 76%). Total VE was highest among young children; a vaccinated child in the post-vaccine era has a 95% reduced risk of RVGE hospitalization compared to a child in the pre-vaccine era**.** We observed higher direct VE in odd post-vaccine years and an opposite pattern for indirect VE.

Conclusions: Vaccine benefits extended to unvaccinated individuals in all age groups, suggesting infants are important drivers of disease transmission across the population. Imperfect disease classification and changing disease incidence may lead to bias in observed direct VE.

Trial registration: Not applicable.

Background

The United States (US) was one of the first countries to introduce infant rotavirus vaccination nationally[131] and dramatic changes in the rotavirus disease burden and epidemiologic patterns of diarrheal disease have followed.[53] Prior to vaccine introduction in 2006,[130] rotavirus was estimated to cause 55,000-70,000 hospitalizations and over 600,000 emergency room and outpatient/office visits among children under 5 years of age in the US annually.[130,133] Consistent annual peaks in disease incidence occurred in winter and early spring.[133] Early evaluations of rotavirus seasonality in the post-vaccine era identified substantial alterations of disease patterns, including a reduced magnitude, delayed onset, and shorter duration of the rotavirus season.[53] Further, there has been a distinct shift from annual to biennial peaks in disease incidence among children under 5 years of age,[53,138,207] a pattern not observed in some other high-income countries that have introduced the vaccine.[208–210]

The direct vaccine effectiveness (VE) of rotavirus vaccine has been evaluated extensively while understanding of indirect vaccine effects is still incomplete. Substantial vaccine impacts are evidenced by 50-90% reductions in rotavirus hospitalizations among young, vaccine-eligible children.[211] A recent meta-analysis estimated a direct VE of 84% against rotavirus-associated hospitalizations or emergency department visits in the US.[134] Notably, this estimate is limited by imperfect rotavirus diagnostics[151] largely due to incomplete testing for rotavirus in the clinical setting.[25] In addition to the remarkable direct effects, reductions in rotavirus disease have exceeded vaccine coverage, suggesting indirect benefits to unvaccinated children.[138] These indirect benefits may extend to children too young to be vaccinated, age-ineligible older children, adolescents, and

adults among whom reductions in rotavirus gastroenteritis (RVGE) and all-cause gastroenteritis (AGE) have been observed.[139]

The long-term impact of a vaccine program will be governed by the direct effects of vaccinating children together with the transmission-modulating consequences of vaccination as described by Halloran et al.[126] Theoretically, direct effects, which represent the biological protection obtained from vaccination at the individual level,[122] are a vaccine characteristic that remains constant over time (except with waning immunity) and are independent of vaccine coverage. Conversely, population-level effects can vary with changes in vaccine coverage, population immunity, and social mixing patterns.[124–126] These population-level effects include (a) indirect effects or "herd protection" provided to unvaccinated individuals, (b) total effects which describe the combination of biologic and indirect protection received by vaccinated individuals, and (c) the overall effects which quantify the public health benefit of a vaccination program by weighting the total effects among the vaccinated and indirect effects among the unvaccinated populations.[126,212]

Given the relative novelty of the rotavirus vaccine in the US, there have been few evaluations of how vaccine effects may change during the post-vaccine era and their relationship with disease patterns. In order to quantify the full, population-wide impacts of infant rotavirus vaccination, longer-term evaluations of vaccine effects across age groups are needed. Understanding these phenomena could lead to strategies that maximize the program's benefits and anticipate future healthcare resource needs (e.g. biennial versus annual epidemics). This study aimed to quantify the direct, indirect, overall, and total effectiveness of infant rotavirus vaccination on hospitalization

for RVGE and AGE across age groups and their annual variation during the post-vaccine era in the US.

Methods

Data source and study period

We analyzed data from the IBM® MarketScan® Commercial Database, a collection of national medical claims and encounters data from commercially insured individuals under 65 years of age in the US. The database includes de-identified, individual-level enrollment, inpatient, and outpatient medical data on employees, their spouses and dependents with employer-sponsored health care insurance in all US states. This encompasses a variety of health plans such as PPOs, POS plans and HMOs but does not include claims covered by Medicaid. The database contains information on several million individuals each year.[154]

We analyzed time series data on monthly RVGE and AGE hospitalization rates for July 2001–June 2016. Study years were defined from July through June of the following calendar year and identified by the year in which the rotavirus season occurred (e.g. July 2007–June 2008 was identified as "2008").

Identification of RVGE and AGE hospitalizations

Monthly counts of RVGE hospitalizations included individuals with a rotavirus International Classification of Diseases, Ninth and Tenth Revision (ICD-9 and ICD-10) code (008.61 and A08.0, respectively). Given the incomplete detection of rotavirus using ICD coding,[151] data on all-cause AGE was also compiled in order to represent possible RVGE not identified as rotavirus-related. Applicable ICD-9/10 codes for AGE include bacterial, parasitic, and viral gastrointestinal illness of determined etiology and presumed infectious or non-infectious gastrointestinal illness of undetermined etiology.[152] For both RVGE and AGE, the ICD-9/10 codes were identified in one of 15 diagnosis fields from inpatient admission claims.

All RVGE and AGE inpatient claims among children, adolescents, and adults not age-eligible to receive the rotavirus vaccine during the study period were included in the analysis and were considered unvaccinated. For children less than 10 years of age who were age-eligible for the vaccine, only those who were continuously enrolled from birth through 6 months of age were included in the analysis. This continuous enrollment requirement aimed to reduce misclassification of vaccination status by helping ensure that rotavirus vaccination occurring within the CDC recommended schedule (at 2, 4 and 6 months of age)[213] was captured in the insurance claim records. Children age-eligible for vaccination but without continuous follow-up were excluded from the analysis because of their unknown vaccination status.

Individuals were grouped into ages 0-4, 5-9, 10-14, 15-24, 25-44, and 45-64 years (data on adults aged 65 years and older are not available in the MarketScan® Commercial Database and were therefore excluded). Children under 5 were additionally categorized into one-year age groups and children under 10 were stratified by vaccination status. The number of enrollment member days

were summed by month, year, age group, and vaccination status to provide the enrolled population denominator for each month of the study period and enable calculation of rates.

Vaccination status

For this analysis, child vaccination status was tracked beginning in July 2006 when the first cohort of newborns became age-eligible for vaccination the following month, coinciding with the Centers for Disease Control and Prevention Advisory Committee on Immunization Practices' August announcement[130] recommending the vaccine. Children who received at least one dose of either available rotavirus vaccine, RotaTeq (RV5) or Rotarix (RV1) were considered vaccinated. Current Procedural Terminology (CPT) was used to define receipt of RV5 or RV1 based on CPT codes, 90680 and 90681, respectively. In order to further reduce misclassification of vaccination status, all individuals (children and adults) residing in states with universal vaccine purchasing programs, which provide immunizations to children free of charge, were excluded from the analysis throughout the study period as vaccination in these states may not be recorded in insurance claim records (Alaska, Connecticut, Idaho, Massachusetts, Maine, North Dakota, New Hampshire, New Mexico, Oregon, Rhode Island, South Dakota, Vermont, Washington, Wisconsin, and Wyoming).

Statistical analysis

Negative binomial regression models were fitted to monthly RVGE and AGE count data to estimate rate ratios (RR) and 95% confidence intervals (CIs) from which vaccine effects were calculated. Negative binomial models were chosen after overdispersion was identified in

preliminary models using Poisson regression. Using the framework proposed by Halloran,[126] we estimated the (a) direct effectiveness of the vaccine by comparing the rates in vaccinated and unvaccinated groups in the post-vaccine era, (b) indirect effectiveness by comparing rates among unvaccinated groups in the post-vaccine era to the pre-vaccine era, (c) overall effectiveness by comparing average rates in the post- and pre-vaccine eras, and (d) total effectiveness by comparing rates in the vaccinated groups in the post-vaccine era to the corresponding groups (all unvaccinated) in the pre-vaccine era. The count of RVGE and AGE cases was modeled using the glm.nb package in R with adjustment for changes in person-years of follow-up using an offset of the log of person-years. Each model included one dichotomous predictor to differentiate the comparison groups: vaccination status (direct), pre- vs. post-vaccine era (indirect and overall), and vaccinated and post-vaccine era vs. pre-vaccine era (total). VE was calculated as 1-RR.

Models were fitted separately for each age group. Direct and total VE was estimated for all age groups eligible for rotavirus vaccination by the end of the study period, with children under 1 year of age vaccine eligible during the entire post-vaccine period (July 2007–June 2016) and children 9 years of age only eligible during the last year of study data (July 2015–June 2016). Indirect and overall VE were estimated for all age groups, though these values were equal for groups ineligible for vaccination throughout the study period because vaccination coverage equaled zero. For the 0-4 age group, direct, indirect, total, and overall effects were additionally calculated for each individual post-vaccine year beginning in 2008 to estimate annual changes in VE. For all age groups, indirect VE was estimated for individual post-vaccine years.

RVGE models were fitted using full-year data. To improve model specificity, AGE models were restricted to the historic rotavirus season of January–June. The year immediately following vaccine introduction, July 2006–June 2007, was excluded as a transition period for all models. The inclusion of a continuous time variable was considered for all models in an effort to adjust for potential secular trends unrelated to vaccination that may have impacted rates. None of the RVGE model results were sensitive to the addition of the time variable based on Akaike information criterion (AIC) and the variable was therefore excluded. Analyses were performed using R software.

Investigation into annual variation in direct VE

Preliminary estimates of direct vaccine effects among the 0–4 age group appeared to vary annually. Given that direct VE should not change over time (in the absence of waning), we performed calculations to evaluate whether this observed variation could be explained by the combination of imperfect coding of rotavirus and annual variation in disease incidence leading to different magnitudes of misclassification of cases/non-cases in post-vaccine years. In other words, even with constant sensitivity and specificity of ICD-9/10 codes, the number of rotavirus positive/negative cases that are misclassified (and which are ultimately used in calculations of VE) will vary based on the incidence of disease. In years with higher disease incidence, there may be a larger number of individuals misclassified and vice versa. We began with a hypothetical population and applied input parameters of true VE, vaccine coverage, and rotavirus incidence in the unvaccinated population from 2010–2016; this enabled estimation of the number of 'true' RVGE cases among the vaccinated and unvaccinated children. We then applied realistic values for

rotavirus ICD-9 code sensitivity (0.5) and specificity (0.99)[151] to this 'true' data to estimate 'projected' RVGE cases. From these values, we calculated a 'projected' VE and compared this to our 'observed' VE estimated via regression analysis and 'true' VE (an input parameter).

Role of the funding source

This study received no dedicated funding. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

From July 2001–June 2016, there were a total of 9,211 RVGE hospitalizations and 726,528 AGE hospitalizations across all age groups (Supplementary Table 1). Over 91% of RVGE hospitalizations (91.4%) and over half of AGE hospitalizations (54.0%) occurred during the rotavirus season of January–June.

RVGE time series

Young children, 0-9 years of age

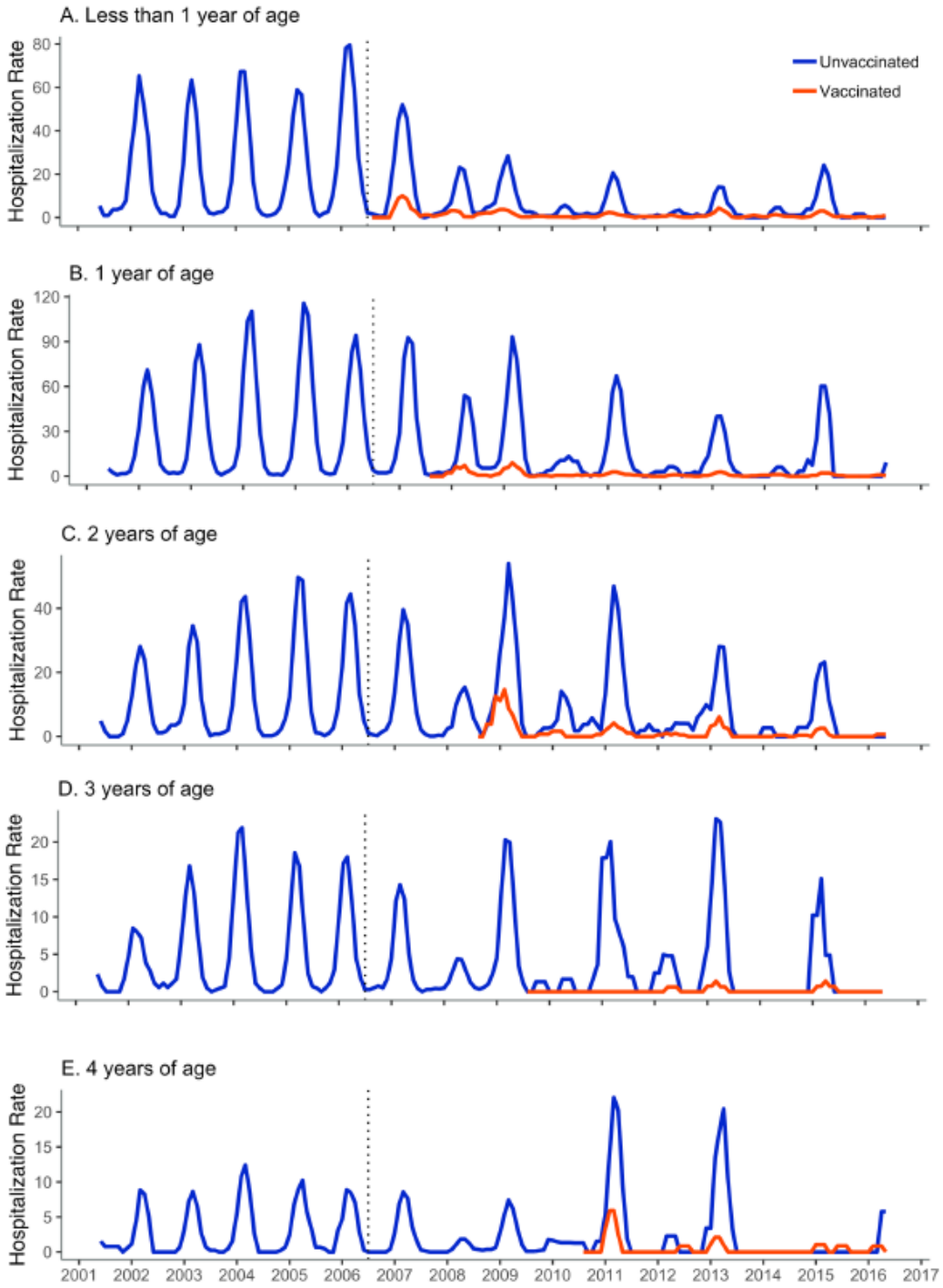
Among children 0-9 years of age, the pre-vaccine period displayed a consistent pattern of rotavirus illness with single annual peaks in hospitalization rates during the winter/spring months (Figures 1 and 2). Rates declined dramatically for child age groups after introduction of the vaccine and

both the 0-4 and 5-9 groups settled into a biennial pattern with the highest rates observed in odd post-vaccine years. When the 0-4 group was further examined by single year and vaccinated/unvaccinated cohorts, the biennial pattern became more apparent among the unvaccinated and younger children. Among the vaccinated children, the biennial pattern was clearest among those 2 years of age and younger. Notably, rates among older, unvaccinated children returned to levels similar to those seen prior to vaccine introduction after initial declines in the post-vaccine period. In contrast, the rates of RVGE remained dramatically lower in the post-vaccine period among vaccinated children.

Older children, adolescents and adults

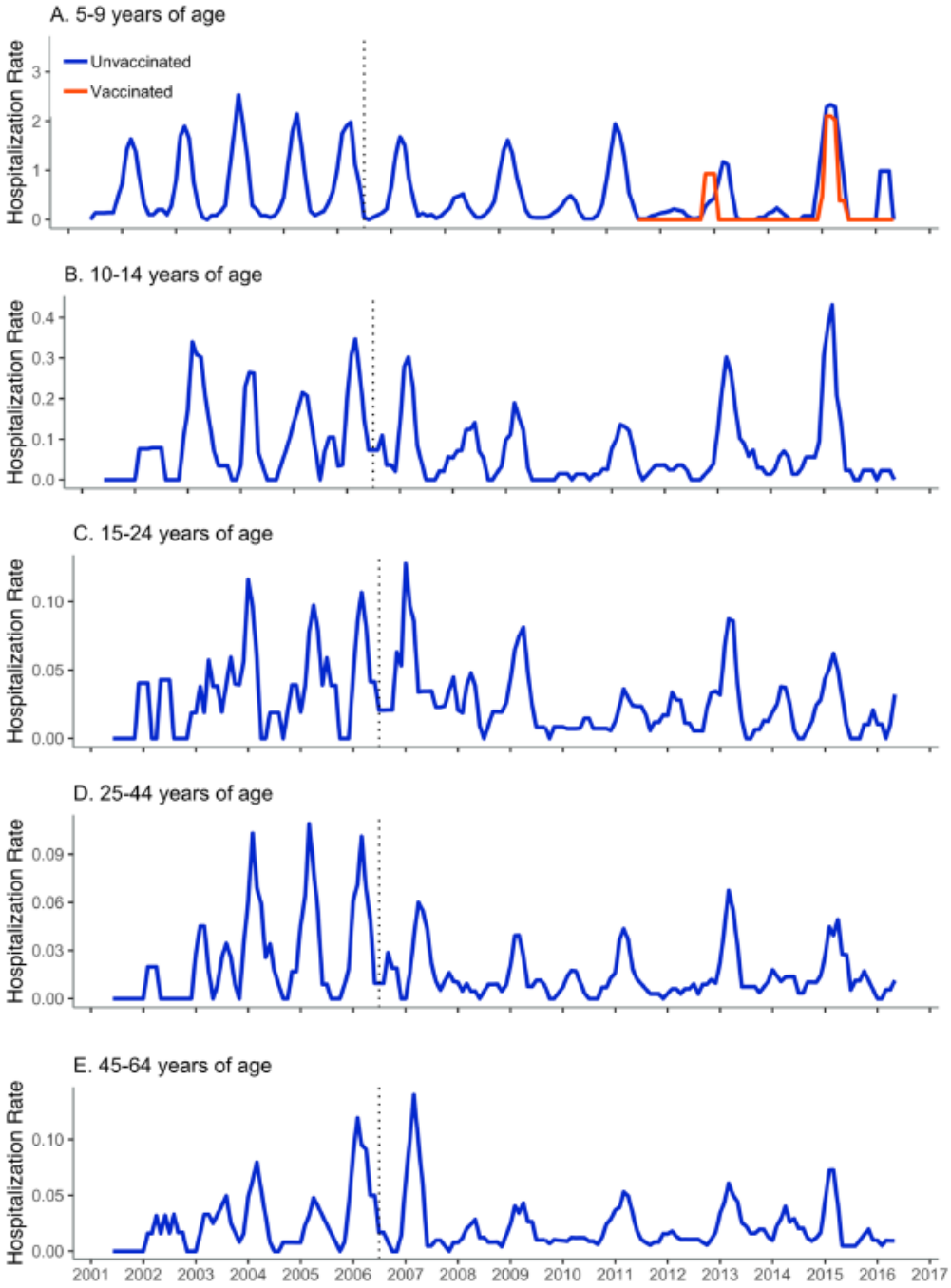
RVGE rates among older children, adolescents, and adults during the pre-vaccine era did not display the same distinctive pattern observed among young children (Figure 2). Rather, the pre-vaccine period for these groups was characterized by frequent, irregular spikes in rates. After vaccine introduction, the sporadic pattern continued, however, at a lower rate and now punctuated with biennial peaks corresponding to those seen among young children.

Figure 1. Monthly inpatient RVGE rates per 10,000 person-years, United States, July 2001-June 2016, children 0-4 years.[a]



[a] Time series includes all years (including 2007 transition year) and all months (not restricted to the historic rotavirus season), Vertical dashed line represents July 2006 (time of vaccine introduction)

Figure 2. Monthly inpatient RVGE rates per 10,000 person-years by age group, United States, July 2001-June 2016.[a]



[a] Time series includes all years (including 2007 transition year) and all months (not restricted to the historic rotavirus season); Vertical dashed line represents July 2006 (time of vaccine introduction)

RVGE VE for entire post-vaccine period

The largest declines in RVGE hospitalizations were observed among the youngest children (Table 1). Direct VE was 87% (95% CI: 83%, 90%) among children 0-4 years of age.

Substantial indirect effects were observed across age groups and these effects generally declined in each older group. Indirect VE against RVGE hospitalization among unvaccinated children under 1 year of age was 79% (95% CI: 66%, 87%) compared to adults 45-64 years of age among whom indirect VE was 35% (95% CI: 9%, 53%). One exception to this general trend was the greater indirect VE in adults 25-44 years of age (indirect VE: 56%; 95% CI: 36%, 70%).

Overall VE against RVGE hospitalizations for the entire study population (all ages) combined was 69 % (95% CI: 62%, 76%). Significant reductions in hospitalization rates were observed across all ages and the overall vaccine effectiveness generally declined in each older group (Table 1).

Total vaccine effects mirrored the pattern seen in direct and indirect effects, with the largest total VE observed in the youngest children (Table 1, total VE for children 0-4 years of age: 95%, 95% CI: 93%, 96%).

Table 1. Vaccine effectiveness against RVGE hospitalization during the post-vaccine period by age group

Age Group	**Direct VE, % (95% CI)**	**Indirect VE, % (95% CI)**	**Overall VE, % (95% CI)**	**Total VE, % (95% CI)**
<1	80* (70, 87)	79* (66, 87)	88* (82, 92)	96* (93, 97)
1	92* (87, 95)	59* (33, 76)	79* (65, 88)	97* (95, 98)
2	87* (76, 93)	43* (4, 67)	68* (44, 83)	93* (86, 96)
3	96* (89, 99)	42 (-2, 68)	71* (47, 84)	97* (93, 99)
4	81* (53, 93)	36 (-25, 68)	59* (22, 79)	88* (70, 96)
0-4	87* (83, 90)	60* (48, 69)	78* (71, 83)	95* (93, 96)
5-9	47 (-12, 79)	48* (30, 61)	50* (34, 63)	72* (42, 89)
10-14		46* (14, 67)		
15-24		42* (10, 62)	Equivalent to indirect VE[a]	
25-44		56* (36, 70)		
45-64		35* (9, 53)		
All ages			69* (62, 76)	

*Represents significance at the alpha = 0.05 level

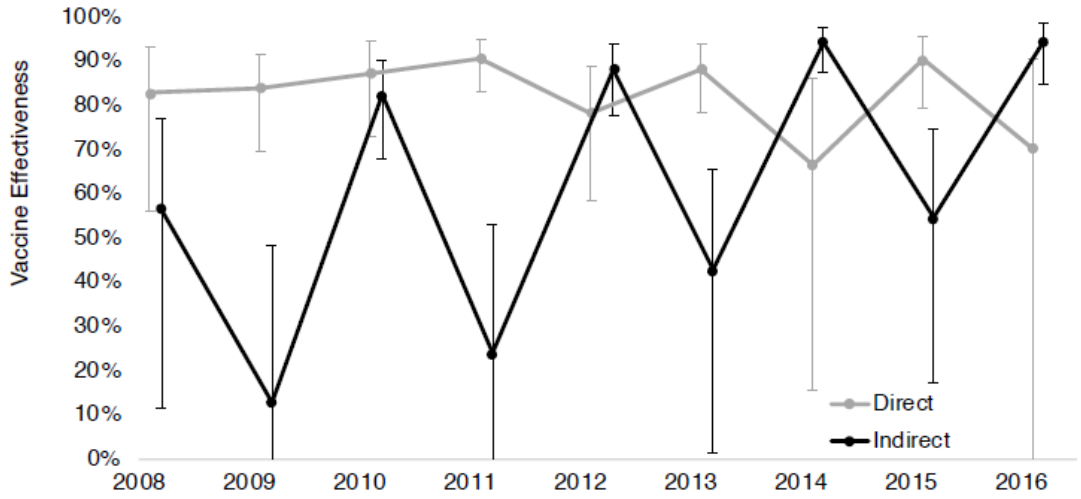
[a] Indirect and overall VE are equivalent for children, adolescents, and adults over 9 years of age because there are no vaccinated individuals in these age groups.

RVGE direct and indirect VE by post-vaccine year

After relatively consistent direct VE immediately following vaccine introduction, we observed a possible alternating pattern in estimated direct VE among children 0-4 years of age, with the

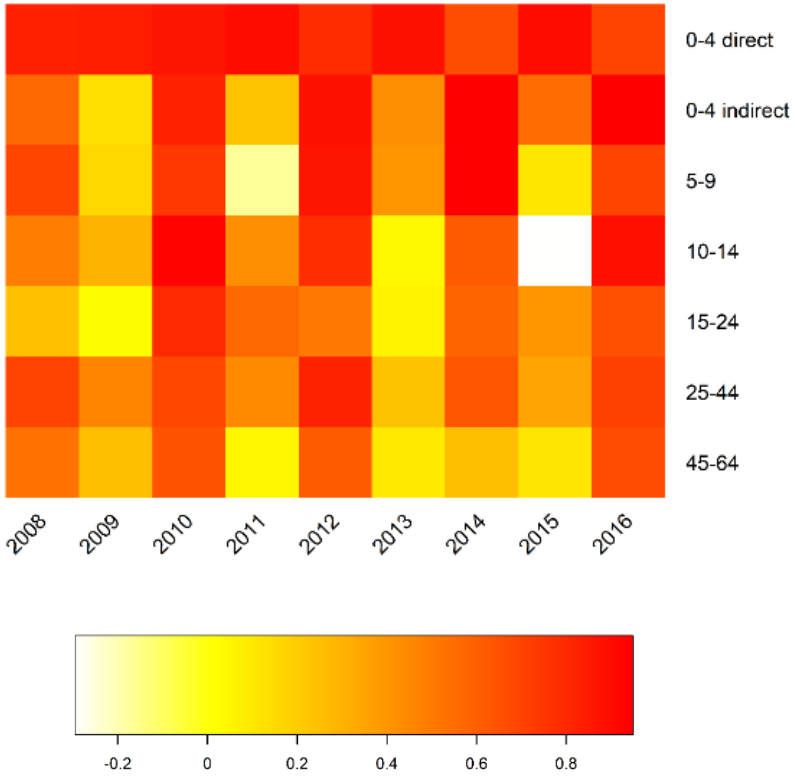
slightly higher direct VE in odd post-vaccine years (Figure 3). A more extreme and opposite pattern was apparent for indirect VE for this group, with higher indirect VE during even post-vaccine years compared to odd post-vaccine years. This pattern extended to all ages (Figure 4). Both VE measures displayed wide CIs due to small numbers of cases.

Figure 3. Direct and indirect VE against RVGE by post-vaccine year,[a] United States, children aged 0-4 years[b]



[a] Post-vaccine years defined as the 12-month period from July through June of the following year. (e.g. "2008" represents July 2007-June 2008) [b] VE calculated based on all children in the age group, regardless of age eligibility for rotavirus vaccination; Bars represent 95% confidence limits; Axis truncated at 0%

Figure 4. Direct and indirect VE against RVGE for each post-vaccine year,[a] United States, by age group[b]



[a] Post-vaccine years defined as the 12-month period from July through June of the following year. (e.g. "2008" represents July 2007-June 2008) [b] VE calculated based on all children in the age group, regardless of age eligibility for rotavirus vaccination

Calculations to evaluate how variations in disease incidence impacted direct VE revealed a pattern in projected direct VE similar to that of our observed direct VE (Table 2). Our calculations used an population of1,000,000, vaccine coverage of 50%, true VE of 95%, sensitivity of 0.5, specificity of 0.99, and estimated RVGE incidence among unvaccinated children for each year from 2010–2016 from the MarketScan® Commercial Database.

Table 2. Projected direct VE calculated in a hypothetical population compared with true and observed direct VE

Direct VE	**2010 %, (95% CI)**	**2011 %, (95% CI)**	**2012 %, (95% CI)**	**2013 %, (95% CI)**	**2014 %, (95% CI)**	**2015 %, (95% CI)**	**2016 %, (95% CI)**
True[a]	95 (90,98)	95 (93, 97)	95 (87, 98)	95 (92, 97)	95 (82, 99)	95 (92, 97)	95 (80, 99)
Observed[b]	87 (73, 94)	91 (83, 95)	78 (59, 89)	88 (78, 94)	67 (15, 86)	90 (79, 96)	70 (-10, 90)
Projected[c]	84 (71, 91)	91 (87, 94)	77 (56, 88)	90 (84, 94)	67 (35, 83)	89 (82, 93)	64 (29, 82)

[a] True VE is a set value used in the calculations

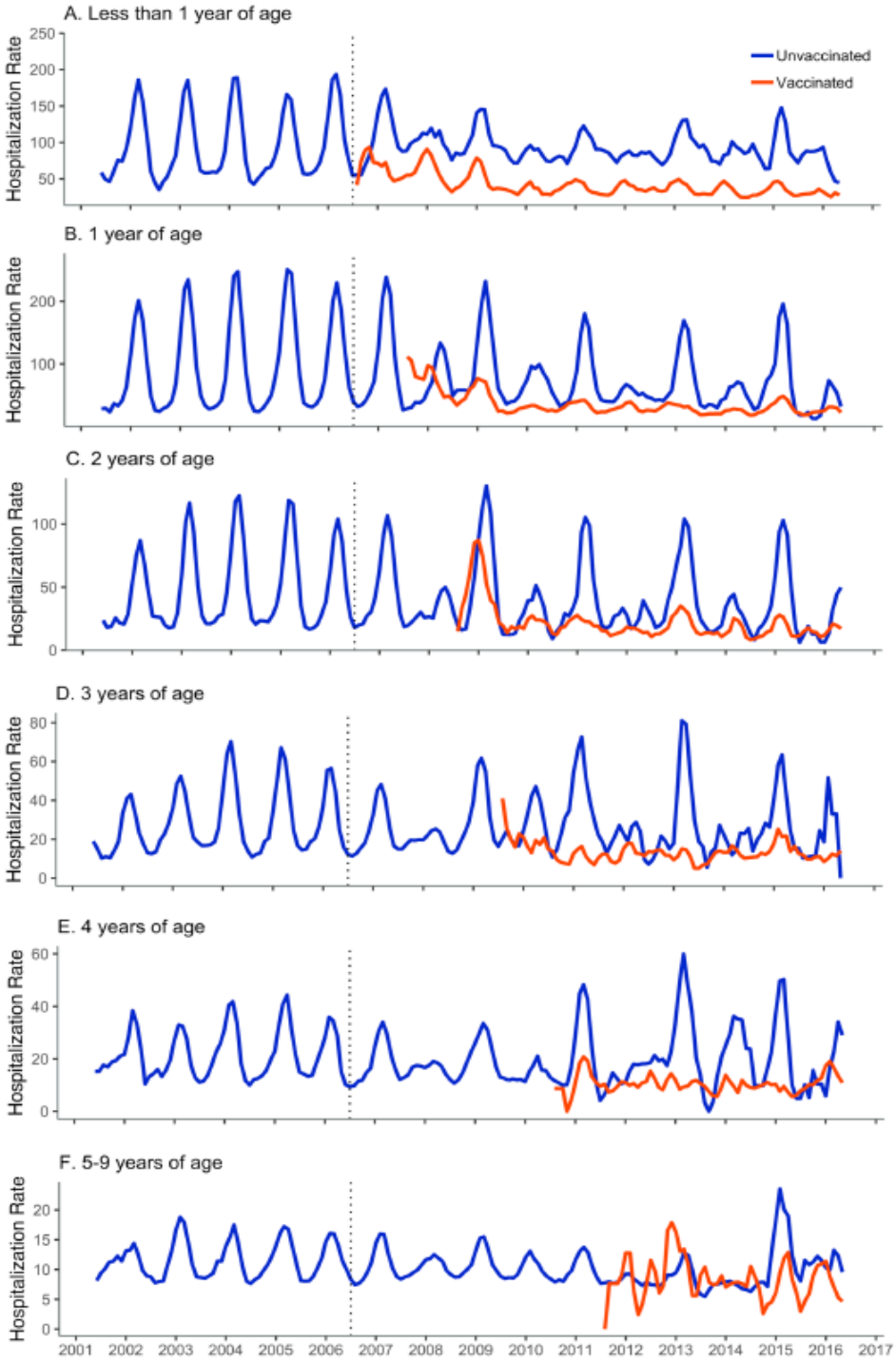
[b] Observed VE is direct VE estimated using the MarketScan® Commercial Database data

[c] Projected VE is the direct VE calculated in a hypothetical population of 1,000,000with vaccine coverage of 50%, true VE of 95%, sensitivity of 0.5, specificity of 0.99, and estimated RVGE incidence among unvaccinated children based on the MarketScan data for each year from 2010-2016.

AGE time series

The observations for RVGE rates among children were consistent with those observed for AGE rates in the same population, though the patterns were often less distinct. The pre-vaccine era was characterized by consistent, annual peaks that shifted towards a biennial pattern in the post vaccine era among young children; the biennial patterns were most apparent among the youngest and the unvaccinated children (Figures 5). Among older children, adolescents, and adults, the pre-vaccine era displayed relatively erratic patterns in AGE rates (Supplementary Figure 1). Unlike RVGE rates, the post-vaccine period was not punctuated by clear biennial peaks in these age groups and a slight increasing trend in rates was observed among those age 10 and older.

Figure 5. Time series of monthly inpatient AGE rates per 10,000 person-years, United States, July 2001-June 2016.[a]



[a] Timeseries includes all years (including 2007 transition year) and all months (not restricted to the historic rotavirus season); Vertical dashed line represents July 2006 (time of vaccine introduction)

AGE VE for entire post-vaccine period

Significant direct, indirect, overall, and total VE was observed among both 0-4 and 5-9 year age groups with the largest impacts among the youngest children (Table 3). Among older age groups, VE estimates were highly sensitive to the method used to control for time. Several methods were considered, including use of a continuous time variable as well as higher order terms. Estimates of VE and their statistical significance varied based on the method used, however, no single model was found to be markedly superior to the rest based on AIC values. Given the uncertainty of these models and output, these results are not presented.

Table 3. Vaccine effectiveness against AGE hospitalization during the post-vaccine period by age group

Age Group	**Direct VE, % (95% CI)**	**Indirect VE, % (95% CI)**	**Overall VE, % (95% CI)**	**Total VE, % (95% CI)**
<1	59* (54, 64)	25* (12, 36)	49* (37, 58)	69* (63, 74)
1	63* (55, 69)	38* (20, 52)	59* (46, 68)	77* (71, 81)
2	57* (44, 66)	28* (5, 46)	49* (33, 62)	69* (59, 76)
3	60* (49, 68)	16 (-7, 34)	42* (27, 55)	67* (58, 74)
4	54* (42, 64)	11 (-13, 29)	33* (16, 46)	60* (47, 70)
0-4	56* (50, 61)	27* (15, 38)	48* (40, 55)	68* (63, 72)
5-9	33* (19, 45)	16* (8, 24)	20* (12, 27)	44* (31, 55)

*Represents significance at the alpha = 0.05 level

Discussion

Vaccines may have impacts that go beyond their direct, immunological effects. We observed that for rotavirus vaccination in the US, the individual and population-level effects are considerable and complex. First, rotavirus vaccination led to a 95% reduction in RVGE hospitalizations among vaccinated 0-4 year olds. Second, introduction of the vaccine provided 35-60% protection against RVGE hospitalizations to unvaccinated individuals across age groups; this protection was generally limited to even post-vaccine years. Parallel patterns in indirect effects observed across all ages highlight the underrecognized burden of rotavirus outside the pediatric age range and emphasize the importance of infants in disease transmission. Lastly and surprisingly, estimates of direct VE varied annually, but we demonstrated that this observation is consistent with biases resulting from ICD-9/10 misclassification combined with biennial incidence patterns rather than variable vaccine performance.

The decade-long post-vaccine period in the US provides a unique opportunity to assess the longer-term impacts of rotavirus vaccination across age groups and to quantify specific vaccine effects. A central strength of this study was the compilation and analysis of 15 years of national data from the MarketScan® Commercial Database. The large size, comprehensive information on vaccination status and consistent coding[154] enabled detailed analysis of nine years of post-vaccine data including age- and year-specific vaccine effects. Previous studies assessing the impacts of the rotavirus vaccine are limited to short-term post-vaccine periods, limited geographic ranges, or pediatric age groups.[139,212,143,142] This study contributes to existing literature on the effects of

rotavirus vaccination across the age range[214] and is the first to estimate annual variation in vaccine effects over the nearly decade long post-vaccine period.

Overall, among all age groups combined, rotavirus hospitalization rates declined by nearly 70% after introduction of the vaccine. The youngest children were impacted most, however, the effects of the vaccine program were also felt outside the pediatric age range, bolstering existing evidence of indirect vaccine effects in the more immediate period following vaccine introduction.[134,214] Population-wide indirect benefits of infant vaccination were demonstrated by reductions in RVGE hospitalization rates across age groups coupled with the emergence of biennial peaks in rates corresponding to those seen among vaccinated children. These findings reinforce the notion that infants are primary drivers of rotavirus infection across age groups. This theory is further supported by the increase in indirect benefits suggested among adults 25-44 years of age, a population likely to have close contact with young children.[203] Yet, the general decline in indirect vaccine effects in older age groups indicate that rotavirus infection among these unvaccinated populations are not solely driven by infants.

The biennial pattern in disease incidence may have influenced our estimation of direct vaccine effectiveness. Imperfect classification results in a bias in vaccine effectiveness; if incidence is changing, the magnitude of the bias will vary because the number of cases to which the sensitivity and specificity of the codes are being applied changes. Indeed, we found that the annual variation in observed direct VE is entirely consistent with a vaccine with constant true effectiveness, imperfect sensitivity/specificity of hospital coding, and varying incidence. In other words, variation in direct effectiveness may be due to the biennial patterns in disease incidence rather than

true changes in vaccine effects. This bias could potentially arise in other estimates of direct vaccine effectiveness measured in the context of varying disease incidence and imperfect disease classification.

Cycles are a well-documented[134] feature of many infectious diseases. When host immunity combines with some seasonal factor (e.g. school terms or weather) season cycles may emerge.[215] Vaccination, which serves to reduce the number of individuals susceptible, may perturb these patterns and increase the inter-epidemic cycle. Indeed, this was predicted to occur for rotavirus under some epidemiological and vaccine-coverage scenarios.[141,202,216] Our study adds to the empirical data supporting this idea, but also extends it by documenting these effects ripple across the age range. During even (low incidence) years, indirect VE was high across the age range while in odd (high incidence) years, there was little-to-no indirect VE.

Important limitations should be noted. We relied on ICD-9/10 codes which imperfectly capture RVGE. Not all individuals hospitalized for AGE are tested for rotavirus; evaluations among children during the pre-vaccine era have demonstrated that rotavirus ICD-9 coding has high specificity (97%) and low sensitivity (less than 50%).[151] Little is known about the specificity and sensitivity of the coding in the post-vaccine era, possible misclassification or incomplete coding in the MarketScan® Commercial Database, frequency of testing among adults,[14] or temporal changes in testing practices since vaccine introduction. One approach to address this limitation was to assess disease patterns in AGE rates, which have been shown to be valuable in assessing the burden of severe RVGE.[25] While we observed consistent patterns in RVGE and AGE in young age groups, vaccine impacts were not clear in older age groups, perhaps because an effect was

overwhelmed by an increasing secular trend in AGE among older individuals (see additional materials).

A primary concern in analysis of time series data is time varying confounders. We aimed to adjust for potential unknown temporal trends by testing the sensitivity of all models to the inclusion of a sequential time variable; none of the RVGE models were found to be sensitive to this variable. National coverage levels for the rotavirus vaccine increased in the years immediately following its introduction though have plateaued around 73% since 2013[134,217]; coverage may indirectly contribute to the variation in direct and indirect effectiveness observed by impacting the number of susceptibles in the population. There is evidence of changes in prevalence of circulating rotavirus strains in the US since vaccine introduction though no consistent pattern has been observed[218], making this unlikely to be the driver of the distinct patterns observed for RVGE rates and VE. It is possible that increased frequency of testing[191] and improved laboratory techniques[219] may impact the number of RVGE cases over the post-vaccine period. If these changes have occurred, they would likely result in an underestimation of the VE measures. Finally, this study may have limited generalizability as the data used did not include the under-insured, individuals on Medicaid, and individuals aged 65 years and older. We are unable to draw conclusions about specific patterns of illness or VE among populations not included in the dataset, such as the underinsured who may have different levels of vaccine coverage.[190] Nonetheless, the effects observed are a function of the wider US population, not just those captured in the dataset.

This study provides new evidence of the individual and population-wide impacts of the rotavirus vaccine and highlights an important potential for bias in direct VE estimation, not previously

investigated for rotavirus vaccination. Measurements of direct rotavirus VE may be prone to downward bias in the post-vaccine era due to reductions in disease incidence resulting in lower and changing predictive value of diagnosis. A vaccinated child in the post-vaccine era has a 95% reduced risk of RVGE hospitalization compared to a child in the pre-vaccine era. Vaccine benefits extended to unvaccinated individuals across the age range and demonstrate the important role of infants in rotavirus transmission.

Conclusions

This comprehensive estimation of the range of vaccine effects provides new evidence of the individual and population-wide impacts of infant rotavirus vaccination and highlights an important potential for bias in direct vaccine effectiveness estimation. Our findings demonstrate the high direct effectiveness of infant rotavirus vaccination and suggest that the impacts of the vaccine program can be felt population-wide, including among adults and unvaccinated children. A novel finding was that imperfect disease classification combined with changing disease incidence during the post-vaccine period may lead to downward bias in the estimated direct vaccine effectiveness. This bias should be considered in other estimates of direct vaccine effectiveness in the context of varying disease incidence and imperfect case classification.

Declarations

Ethics approval and consent to participate

This study involved de-identified, aggregate data and was not subject to Institutional Review Board approval at Emory University or the Centers for Disease Control and Prevention.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from the IBM® MarketScan® Research Databases but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission from IBM® Watson Health™.

Competing interests

BAL has received personal fees from Takeda Pharmaceuticals for service on their Norovirus Advisory Board outside the submitted work. JMB, RMD, JC and UDP have no conflicts of interest.

Funding

This work received no dedicated funding.

Authors' contributions

The study was conceptualized by BAL with input from JMB and RMD and supervisory support from UDP. RMD designed and conducted data extraction from the IBM® MarketScan® Research Databases with input from JMB and BAL. JMB led the main study analysis with support from

BAL. JC, JMB and BAL conducted the sub-analysis examining variations in direct vaccine effects. JMB drafted the manuscript with supervisory input from BAL. All authors reviewed, edited, and approved the manuscript.

Acknowledgements

Not applicable.

Authors' information

Not applicable.

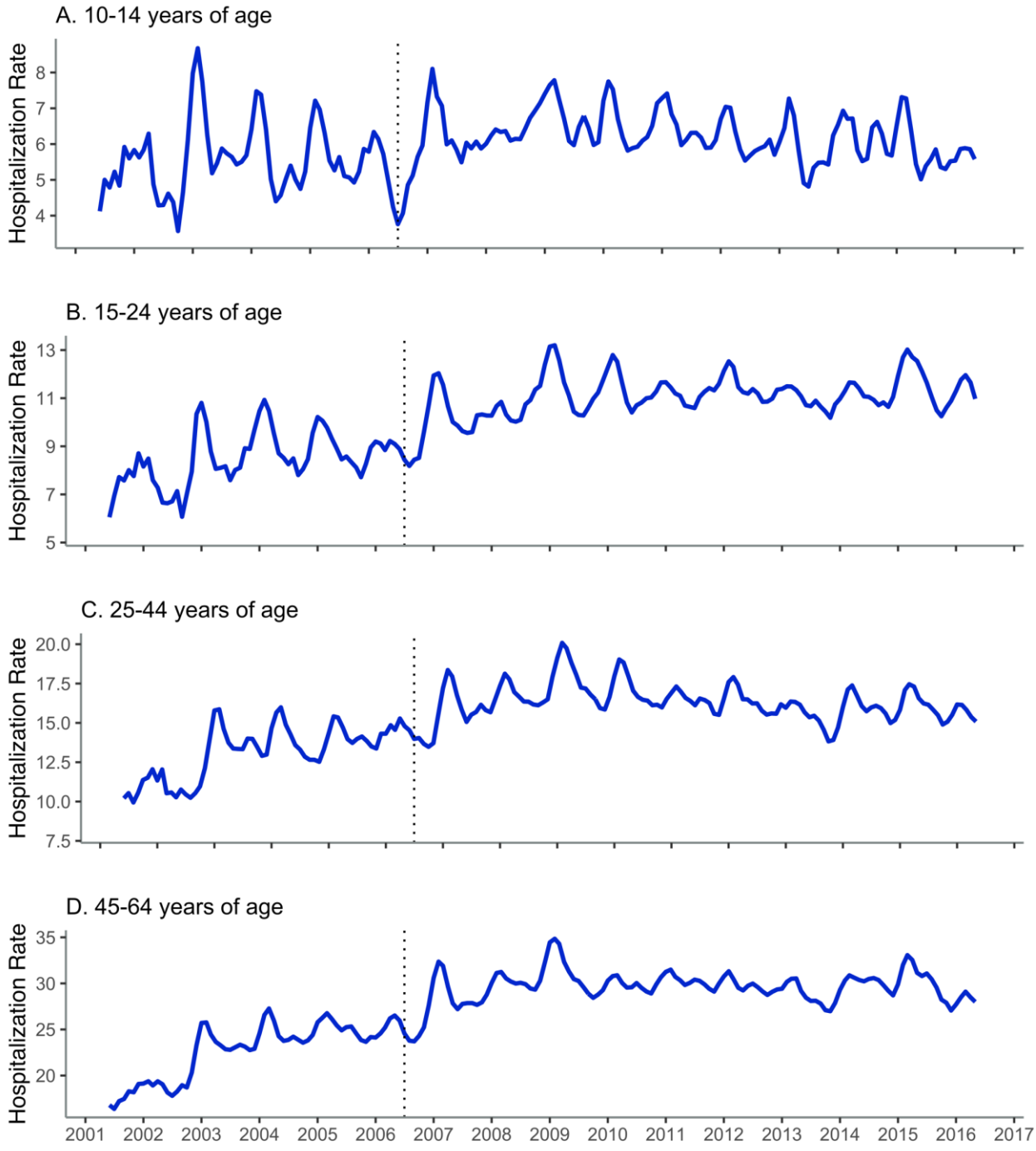
Additional material

Supplemental Table 1. Number of RVGE and AGE cases by age group, United States, July 2001- June 2016.[a]

Age Group	RVGE	AGE
<1	1,908	15,647
1	2,879	11,959
2	1,545	6,857
3	689	4,624
4	477	4,169
0-4	7,498	43,256
5-9	865	18,469
10-14	206	18,474
15-24	152	64,326
25-44	190	189,900
45-64	300	392,103
All ages	9,211	726,528

[a] Includes all years (including 2007 transition year) and all months (not restricted to the historic rotavirus season)

Supplementary Figure 1. Time series of monthly inpatient AGE rates per 10,000 person-years by age group, United States, July 2001- June 2016.[a]



[a] Timeseries includes all years (including 2007 transition year) and all months (not restricted to the historic rotavirus season)

Vertical dashed line represents July 2006 (time of vaccine introduction)

8 Public Health Impact

8.1 Overview

Rotavirus vaccine has contributed to remarkable declines in gastroenteritis around the world,[127] yet the full potential of the vaccine has not been realized. Several challenges remain, including disparate efficacy of the vaccine in different settings, lack of a strong correlate of protection for assessing improved vaccine strategies or vaccine candidates, and limited understanding of the longer-term, population level effects of the vaccine. The primary goal of this dissertation research was to contribute to the knowledge base necessary for mitigating these major challenges and improving the public health benefits of vaccination. Specifically, this research investigated rotavirus vaccination on three interconnected scales: (1) how host characteristics influence rotavirus vaccine immunogenicity, (2) how immune response to rotavirus vaccination predicts clinical disease risk within an individual, and (3) how rotavirus vaccination impacts severe gastrointestinal illness at the population-level. This analysis provided insight into rotavirus vaccine performance and highlighted several avenues for further research, described for each aim below.

8.2 Contribution of each specific aim

8.2.1 Aim 1- Immunogenicity

The disparate immunogenicity and efficacy of currently available rotavirus vaccines mean the infants who are at the greatest risk of severe disease are provided the least protection through

vaccination.[73,99] Through Aim 1, we contribute to the growing body of research into the determinants of vaccine underperformance in high child mortality settings by identifying factors associated with rotavirus vaccine immunogenicity across settings. Ultimately, this information can be used to identify modifiable vaccine strategies or interventions for enhancing vaccine performance.

In Aim 1, we had the unique ability to examine both individual and country level factors that contribute to Rotarix vaccine immunogenicity in high and low child mortality countries. The most salient finding was the rather dramatic effect that OPV has on rotavirus vaccine immunogenicity when the vaccines were administered concomitantly. Our analysis supports existing evidence[107–110,157] that OPV interferes with serum anti-rotavirus IgA seroconversion and, critically, reveals that reduced seroconversion is apparent when OPV is given concomitantly with both the first and the second rotavirus dose. This latter result contrasts with early evidence suggesting that the rotavirus and OPV interaction is primarily limited to the first dose.[108,158] Limited research[107] has suggested that interference from OPV may extend beyond the first dose of rotavirus vaccine and our findings provide robust support for this theory. Essentially, if it is true that the primary interference between rotavirus vaccine and OPV occurs with the first doses, our results suggest that a second rotavirus dose does not enable an infant to overcome the initial reduced immune response induced by the first concomitant doses.

Considering these findings when developing or modifying rotavirus and polio vaccine programs could influence the public health benefits of rotavirus vaccination. Currently, rotavirus vaccines are typically administered on the same schedule as polio vaccines. Concomitant vaccination is not

of concern in low child mortality settings where IPV is primarily administered, however, this schedule has substantial public health implications in high child mortality settings where OPV is still in use.[106] While it may not be feasible to stagger OPV and rotavirus immunization schedules at this time,[107] there will eventually be a shift from OPV to IPV as polio eradication nears. This shift has the potential, based on our results, to lead to a sizeable increase in rotavirus vaccine performance in high child mortality settings.

This possibility will ideally motivate further research into the rotavirus and OPV interaction to better identify its causes and provide insight into how future changes in vaccine strategies may impact the burden of rotavirus gastroenteritis. The current efforts by the Global Polio Eradication Initiative to reduce and eventually end OPV use creates an opportunity to confirm our results in the real-world setting. In this type of natural experiment, vaccine immunogenicity data from infants in settings where OPV is currently administered could be compared to immunogenicity among infants after OPV withdrawal. Even more impactful will be evaluations of rotavirus vaccine effectiveness before and after OPV withdrawal against the clinical endpoint of rotavirus gastroenteritis.

The substantial impact of GDP as an explanatory factor for seroconversion, and our inability to identify modifiable factors for predicting anti-rotavirus IgA titer, provide an important reminder that there is still a great deal more about rotavirus vaccine immunogenicity that we have yet to understand. Similarly structured analyses to the one conducted in this dissertation, but with more detailed data on factors such as breastfeeding and household risk factors, would help address some of the shortcomings of our research. Another critical avenue for future research is the role of

previous rotavirus infection on vaccine immunogenicity. This is likely an important factor in high child mortality settings where early infections may be more common and where improvements in vaccine performance are most essential.

8.2.2 Aim 2- Correlates of protection

The vaccine challenges described in Aim 1 combined with logistic constraints of the currently available vaccines[114] mean the full potential benefits of global rotavirus vaccination are not being realized. To address both of these challenges, ongoing evaluations of modified vaccination strategies and new vaccine candidates in the rotavirus vaccine pipeline[144] will likely take place over the next several years. The findings from Aim 2 provide insight into how serum anti-rotavirus IgA may be a valuable measure for rapidly assessing vaccine strategies and vaccine performance in these future studies. Overall, our research highlights that serum anti-rotavirus IgA may be an informative, though suboptimal, correlate of vaccine-inducted protection against rotavirus gastroenteritis. Despite its limitations, using serum anti-rotavirus IgA as a correlate of vaccine-induced protection against rotavirus gastroenteritis has several advantages over previously studied measures[27] and has beneficial implications for rotavirus vaccine programs and vaccine research.

We found that seroconversion, indicated by the presence of serum anti-rotavirus IgA antibodies ≥20 U/mL, confers substantial protection against rotavirus gastroenteritis both in high and low child mortality settings in the months following vaccination and up to 1 year of age. For instance, in both high and low child mortality settings, the risk of mild/moderate and severe rotavirus gastroenteritis among infants who seroconverted was less than half the risk among those who were seronegative. This provides encouraging evidence that any infant who responds to vaccination,

regardless of their level of response, is likely receiving some degree of protection during the critical period when their risk of severe disease is highest.[13] From a public health perspective, this lends additional support for WHO's recommendation that all counties include rotavirus vaccines in their national immunization programs,[106] even those where immunogenicity may be less than ideal. Though the vaccines available today have been demonstrated to be less immunogenic and efficacious in high child mortality settings,[7,96] it is in these settings where even a small reduction in the risk of rotavirus gastroenteritis on an individual level can contribute to substantial reductions in disease at the population level. Our analysis of serum anti-rotavirus IgA indicates that the largest increase in protection is generally achieved when an individual shifts from anti-rotavirus IgA seronegative to seropositive status. Additional protection is generally afforded by higher titer values, however, dramatic reductions in gastroenteritis risk can be provided by seroconversion alone.

A practical example of the value of serum anti-rotavirus IgA thresholds can be applied using results from Aim 1 and Aim 2 together. In our assessment of rotavirus vaccine immunogenicity, we found that anti-rotavirus IgA seroconversion among infants who received OPV with both rotavirus dose one and two were nearly half as likely to seroconvert compared to infants who did not receive OPV at all. The eventual shift from OPV to IPV in low income settings could result in twice as many infants seroconverting after rotavirus vaccination. The HR identified for the anti-rotavirus IgA threshold of ≥20 in high child mortality settings (Aim 2) then provides a possible indication of the degree of protection these seroconverted infants might receive. Based on our results, we could anticipate the risk of mild/moderate rotavirus gastroenteritis among these infants would be

reduced by over 60% and their risk of severe rotavirus gastroenteritis reduced by about half through one year of age.

Relatedly, we found an approximate "dose-response" relationship between anti-rotavirus IgA thresholds and the occurrence of rotavirus gastroenteritis through one year of age across settings. While a definitive level of anti-rotavirus IgA that provides perfect protection against rotavirus gastroenteritis was not identified, anti-rotavirus IgA itself is informative. Generally, infants with the highest levels of anti-rotavirus IgA have the highest protection against rotavirus gastroenteritis. The public health and policy implications of this relationship are two-fold. First, activities that may increase serum anti-rotavirus IgA titer levels induced by the existing vaccines, such as a shift in age at first vaccination or possibly a booster dose, should be explored. If these activities are found to increase serum anti-rotavirus IgA titer levels, it is possible that a corresponding reduction in the risk of rotavirus gastroenteritis will be observed. Second, anti-rotavirus IgA is an informative and relatively easily collected measure, even if imperfect, for assessing vaccine performance. This makes it an invaluable tool for the development of the next generation of rotavirus vaccines and vaccination strategies. A serum anti-rotavirus IgA cutoff of ≥20 U/mL can be used in future studies as an efficient alternative to clinical endpoints and serum anti-rotavirus IgA provides a scale to upon which to compare vaccines since placebo controlled trials are no longer ethical for rotavirus.[180]

From a public health standpoint, the potential value of serum anti-rotavirus IgA is both encouraging, as described above, but also less than ideal. When our findings are considered in the context of existing literature in the field, it seems that serum anti-rotavirus IgA may be a non-

mechanistic correlate of protection—one that is related to but not directly on the causal path between rotavirus vaccination and protection against rotavirus gastroenteritis.[27,185] Serum anti-rotavirus IgA threshold alone, while informative, is insufficient to accurately predict one's risk of rotavirus gastroenteritis. This further emphasizes our Aim 1 conclusion that serum anti-rotavirus IgA levels are complex and not well understood. From a programmatic standpoint, this means that assessments of anti-rotavirus IgA titers following a vaccine campaign (to assess who did or did not meet the anti-rotavirus IgA threshold), for instance, would be of little benefit. Additionally, without a strong correlate of protection, we are unable unquestionably identify high-risk populations based on serum anti-rotavirus IgA titers. Continued research is needed to identify other factors that, when combined with anti-rotavirus IgA, may strengthen the validity and utility of IgA as a possible correlate of protection on the individual or population-level. Simultaneously, attention should be given to identifying a potentially stronger correlate of protection, possibly looking at more proximal measures of immune response that may directly dictate disease protection, when possible.

8.2.3 Aim 3- Direct and indirect vaccine effects

The United States was one of the first countries to introduce infant rotavirus vaccination nationally[131] and, therefore, provided a unique opportunity to assess the longer-term and population-wide impacts of infant rotavirus vaccination. In Aim 3, we quantify the full, population-wide impacts of infant rotavirus vaccination across age groups, describe shifts in disease patterns, and highlight an important potential for bias in estimating direct vaccine effectiveness in context of varying disease incidence and imperfect case classification. To our

knowledge, we conducted the longest age-stratified time series analysis of the rotavirus vaccine in the United States, and possibly any country, to date.

Comparison of objectives and methods for HCUP and MarketScan analyses

The HCUP and MarketScan analyses that comprise Aim 3 together address questions regarding the longer-term and population-level effects of rotavirus vaccination in the United States. The HCUP analysis provided initial insight into patterns in disease rates, including overall and indirect effects, using the simple counts of rotavirus and gastroenteritis hospitalizations that occurred monthly in the United States. Using the similarly structured MarketScan dataset with additional stratification by vaccination status, we were afforded more granular insight into population-wide patterns. We estimated the direct, indirect, overall and total vaccine effectiveness across age groups with this information.

Both analyses assessed time series data on rotavirus gastroenteritis and all-cause gastroenteritis hospitalization rates across age group. The analysis approaches were similar. Time series figures were created to visually assess patterns in the rates of illness by age group. Next, monthly counts of rotavirus and gastroenteritis hospitalizations were modeled using negative binomial regression to estimate rate ratios and 95% confidence intervals. Models were adjusted for changing population size using an offset of the log of population and were run separately by age group.

In the HCUP regressions, monthly pre-vaccine rates of rotavirus/acute gastroenteritis overall (2000-2006) were compared to:

1. monthly post-vaccine rates of rotavirus/acute gastroenteritis overall (2008-2013) to assess overall and indirect effects
2. monthly post-vaccine rates of rotavirus/acute gastroenteritis for even (2008, 2010, 2012) and odd post-vaccine calendar years (2009, 2011, 2013) separately to assess biennial patterns, and
3. monthly post-vaccine rates of rotavirus/acute gastroenteritis for each individual post-vaccine calendar year to provide more detail on the potentially dynamic effects of vaccination over time

The MarketScan analysis built upon the modeling techniques used in the HCUP analysis and modified these to assess more detailed comparisons. Four measures of vaccine effectiveness were estimated for rotavirus/acute gastroenteritis:

1. direct effectiveness of the vaccine by comparing the rates of rotavirus/acute gastroenteritis in vaccinated and unvaccinated groups in the post-vaccine era,
2. indirect effectiveness by comparing rates of rotavirus/acute gastroenteritis among unvaccinated groups in the post-vaccine era to the pre-vaccine era,
3. overall effectiveness by comparing average rates of rotavirus/acute gastroenteritis in the post- and pre-vaccine eras, and
4. total effectiveness by comparing rates of rotavirus/acute gastroenteritis in the vaccinated groups in the post-vaccine era to the corresponding groups (all unvaccinated) in the pre-vaccine era

Comparison of results for HCUP and MarketScan analyses

Visual inspection of hospitalization patterns in the HCUP data demonstrated a rapid decline in the rates of rotavirus gastroenteritis after introduction of the vaccine and the emergence of a strong biennial pattern with higher rates in odd post-vaccine calendar years. This pattern was most strongly observed in young children but extended to older individuals as well. Children under 5 years of age experienced an 86% reduction in rotavirus gastroenteritis (95% CI: 77%-91%) while reductions were also apparent among older children, adolescents and adults. These findings provided robust evidence of the indirect effects of infant rotavirus vaccination beyond the pediatric age range. Adults over the age of 60 years experienced the smallest declines, suggesting disease transmission in this age group is impacted by infants, but likely also driven by other factors.

In the MarketScan analysis, similar biennial patterns were observed. The largest effects of vaccination were seen among young children 0-4 years of age (direct vaccine effectiveness = 87%, 95% CI: 83%, 90%). As with the HCUP results, substantial indirect effects of infant vaccination were experienced across age groups with a general decline in effects in each older group. These results corroborated those found in the HCUP dataset. Overall vaccine effectiveness against rotavirus gastroenteritis hospitalizations for all ages combined was 69% (95% CI: 62%, 76%). Remarkably, we found that a vaccinated child in the post-vaccine era has a 95% reduced risk of rotavirus gastroenteritis hospitalization compared to a child in the pre-vaccine era**.**

Public health implications

In both the HCUP and MarketScan analyses, we revealed the emergence of a biennial pattern in rotavirus gastroenteritis hospitalization rates across age groups. The importance of this pattern is two-fold. First, it could be suggestive of changing rates of susceptibles within the population. One possible hypothesis is that vaccine coverage and acquired immunity could be high enough to transiently raise herd immunity such that rotavirus transmission is low for a short period of time. This would allow for the accumulation of susceptibles as new infants are born into the population and as other young children temporarily avoid infection, ultimately spurring biennial epidemics that spread across age groups[143,202] These patterns have not been seen among most other high-income countries,[208–210] though one study found this type of pattern may be emerging in the Netherlands where the vaccine was recently introduced.[220] This pattern inevitably raises the question of its cause. Some evidence suggests relatively low vaccine coverage may be a contributing factor—an area in need of additional research. Identifying and further researching these trends could aid in identifying strategies that maximize the rotavirus vaccine program's benefits (such as increasing coverage among certain populations or vaccinating other age groups) and help in anticipating future healthcare resource needs.

Second, and relatedly, *parallel* biennial patterns in rotavirus hospitalizations across age groups were observed in both studies. This visual synchrony was reinforced by estimates of indirect vaccine effects in each age group. Children under 5 years of age experienced the largest reductions in rotavirus gastroenteritis rates while substantial reductions were also apparent among older children, adolescents and adults. Notably, the overall vaccine effectiveness against rotavirus gastroenteritis generally declined in each older group. Older adults experienced the smallest change in the incidence of rotavirus hospitalizations, suggesting disease transmission in this age

group is likely very closely linked to that among young children, however, not solely driven by infants. Combined, these results provided robust evidence of the previously under-recognized rotavirus burden outside the pediatric age range while emphasizing the importance of infants in rotavirus disease transmission. Longer-term evaluations of rotavirus vaccination have inherently been limited to early introducing countries. Whether or not similar patterns and population-wide indirect effects will be seen among other countries, particularly high child mortality settings, is unknown. Opportunities to assess longer-term trends in these settings will arise as additional years of data become available. This type of analysis requires time series data, ideally including vaccination status, emphasizing the importance of sufficient data collection now and into the future.

The MarketScan analysis identified an important bias that may impact estimates of direct vaccine effectiveness in the context of variable disease incidence and imperfect disease classification. Our estimates of direct vaccine effectiveness varied annually with higher direct effects observed in odd (high incidence) post-vaccine years. However, we don't believe this is true variation in vaccine performance. Rather, we demonstrated that this variation could be the result of bias from ICD-9/10 misclassification combined with biennial incidence patterns. Essentially, imperfect disease classification (imperfect sensitivity and specificity) can results in a bias in vaccine effectiveness. If the incidence of disease is changing over time, the magnitude of the bias will vary because the number of cases to which the sensitivity and specificity of the codes are being applied changes. While we found this specifically in our investigation of rotavirus vaccination, it is possible that this bias could arise in other estimates of direct vaccine effectiveness measured in similar contexts and should be considered in future assessments.

Overall, both studies provide similar and complementary evidence of dramatic direct and indirect effects of infant rotavirus vaccination in the United States. The longer-term patterns in disease incidence are complex, but provide strong evidence of population-level effects. The two data sources provide an ideal opportunity to continue evaluating rotavirus patterns into the future. The impacts of possible changes in vaccine coverage, rotavirus testing trends, or vaccination strategies over that may arise over the coming years could be assessed using these valuable data sources. Beyond the US, similar analysis approaches could be used to detect longer-term impacts of vaccine introduction across age groups to identify how and why differences occur.

8.3 Summary

Collectively, the results of this dissertation research contribute to the body of literature on the rotavirus vaccine both in the United States and globally. Identifying factors associated with rotavirus vaccine immunogenicity across child mortality settings brings us one step closer to recognizing modifiable strategies for improving vaccine performance for the children most at risk of the virus' severe effects. With the knowledge we can gain from serum anti-rotavirus IgA titer data, even if imperfect, we can more rapidly screen potential new vaccine candidates and strategies, eventually making rotavirus vaccination more accessible and effective globally. Lastly, understanding the considerable and complex long-term impacts of rotavirus vaccination at the individual and population levels will enable us to better understand, interpret and anticipate disease patterns.

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10 Appendix

Abbreviations

AGE	Acute gastroenteritis
AHRQ	Agency for Healthcare Research and Quality
AIC	Akaike information criterion
CI	Confidence interval
CDC	Centers for Disease Control and Prevention
ELISA	Enzyme-linked immunosorbent assay
GDP	Gross Domestic Product
GMC	Geometric mean concentration
GMT	Geometric mean titer
GSK	GlaxoSmithKline
HCUP	Healthcare Cost and Utilization Project
HIV	Human Immunodeficiency Virus
HMO	Health maintenance organization
HR	Hazard ratio
ICD-9	International Classification of Disease, Ninth Revision
ICD-10	International Classification of Disease, Tenth Revision
ID	Identification
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IQR	Interquartile range

IPV	Inactivated polio vaccine
LAZ	Length-for-age z-score
N	Number
NA	Not applicable
NAs	Neutralizing antibodies
NCHS	National Center for Health Statistics
OPV	Oral polio vaccine
OR	Odds ratio
POS	Point-of-service
PPO	Preferred provider organization
RR	Rate ratio
RV	Rotavirus
RVGE	Rotavirus gastroenteritis
SD	Standard deviation
SDI	Socio-demographic index
SID	State Inpatient Database
U5MR	Under 5 mortality rate
U/mL	Units per millileter
VE	Vaccine effectiveness
VP	Viral protein
WHO	World Health Organization