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Jenna Ciszewski Date

Differences in Rotavirus Shedding by Infant Oral Rotavirus Vaccination Status in Dhaka, Bangladesh 2011-2014

By

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An abstract of
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Abstract

Differences in Rotavirus Shedding by Infant Oral Rotavirus Vaccination Status in Dhaka Bangladesh 2011-2014

By Jenna Ciszewski

Background: Oral rotavirus vaccines are less effective in low-income compared to high-income settings. In settings with lower vaccine efficacy, breakthrough rotavirus disease among vaccinated children is common. However, because few studies have examined the impact of rotavirus vaccination on fecal shedding and infectiousness, it is unknown how much these breakthrough cases contribute to transmission.

Methods: We used data from the Performance of Rotavirus and Oral Polio Vaccines in Developing Countries (PROVIDE) randomized controlled trial to examine the relationship between Rotarix® (RV1) vaccination and quantity of rotavirus shed in stool during episodes of rotavirus gastroenteritis (RVGE). We used multivariable linear regression with robust standard error to analyze 184 episodes of rotavirus diarrhea testing positive by ELISA occurring in children 10 weeks to 1 year of age. The primary outcome of interest was quantity of rotavirus shed in stool by qPCR testing.

Results: Vaccinated children had significantly lower levels of fecal viral shedding compared to unvaccinated children after controlling for age, weight-for-age, height-for-age, exclusive breastfeeding at time of episode, and time in days since symptom onset (mean difference = -0.59 log copies per gram of stool, 95% CI: -0.99, -0.19). We found no evidence of interaction by child age or disease severity (modified Vesikari score). We also found no significant interaction by time when comparing effect estimates between children 10 to 19 weeks of age (after receipt of first dose) to children 19 weeks to one year of age (2 weeks after receipt of second dose).

Conclusions: These results suggest that RV1 vaccination reduces shedding burden among breakthrough cases of RVGE, in addition to preventing RVGE cases entirely. Our results also suggest that breakthrough cases among vaccinated children may have lower transmission potential than cases among unvaccinated children, though further study is needed.

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Chapter I: Literature Review

Introduction

Rotavirus (RV) is a highly contagious, enteric pathogen that causes acute gastroenteritis (AGE) primarily in children < 5 years of age. In low-income countries, children generally acquire their first rotavirus infection before 12 months of age (1). Rotavirus infections can be symptomatic or asymptomatic, and disease ranges from mild to severe. Symptoms include watery diarrhea, vomiting, fever, abdominal pain and/or severe dehydration (2). Virus is shed in the stool of infected individuals and is spread through fecal-to-oral transmission, either directly from person-to-person or through contact with contaminated objects and surfaces (1). The incubation period is 1 -3 days, with symptoms typically lasting between 3 and 8 days (2,3).

Vaccination is one of the most important tools for preventing rotavirus diarrhea. Two live, oral, attenuated rotavirus vaccines were licensed in 2006 and have been introduced in countries around the globe (4). While vaccines have demonstrated immense impact in reducing the total global disease burden, rotavirus vaccine efficacy is appreciably lower in low- and middle- income countries (LMIC) as compared to high income countries (5–7), which poses a challenge to achieving health improvements in high-burden settings.

In most temperate high-income countries, rotavirus exhibits a seasonal pattern, with cases peaking in the dry, winter months. In contrast, rotavirus tends to circulate year-round in tropical, low-income countries in Africa and Asia, often with distinct months of peak transmission (8). Some localities have experienced changes in seasonality (smaller and/or delayed peaks) following rotavirus vaccine introduction, and more deviations from pre-vaccine patterns might occur as vaccine use becomes more widespread (9,10).

Global Burden of Disease

Rotavirus is the leading cause of diarrheal disease among children <5 years of age. Globally, it was responsible for an estimated 128,500 deaths and greater than 258,000,000 diarrhea episodes among children < 5 years in 2016 (11). Rotavirus is an important source of morbidity for children in high-, middle-, and low- income countries alike, causing roughly 1,760,113 hospitalizations in 2019 (12). However, the mortality burden resides almost exclusively in low- income countries, with over 90% of deaths occurring in Sub-Saharan Africa and South Asia (11).

Considerable progress has been made in reducing the global burden of rotavirus over the last three decades. A study conducted for the WHO- Coordinated Rotavirus Surveillance Network estimated that annual deaths attributable to rotavirus among children <5 years of age decreased by 59% between 2000 (528,000 deaths) and 2013 (215,000 deaths) (13). Declines were seen across all regions, but were least pronounced in Sub-Saharan Africa (13). Vaccine introduction in 2006, as well as improvements in access to medical care and the widespread use of oral rehydration salts (ORS), are important contributors to the declines in morbidity and mortality (12).

Despite these achievements, all-cause diarrheal disease remains the third leading cause of death in children < 5 years of age as of 2019, the same ranking it occupied in 1990 (14). Given that rotavirus is responsible for approximately 29% of all diarrheal deaths in children, targeted vaccine and prevention programs are necessary to continue to reduce child morbidity and mortality worldwide (11).

Immunity

Immunity to rotavirus disease is acquired in two ways 1) via natural exposure or 2) through vaccination. However, neither vaccination nor previous infection confer complete immunity. Therefore, repeat infections in the same child and breakthrough infections in vaccination children are common (15). The scientific literature indicates that primary infection both protects against future infections and decreases the severity of subsequent disease. In a cohort study of 200 Mexican children followed from birth to age

2, having 1 previous rotavirus infection offered 87% protection against future moderate to severe RVGE, and 2 previous infections were 100% protective (16). Data from the MAL-ED study found complementary results: Across five sites without vaccination, 1, 2, and 3 prior rotavirus infections conferred 43%, 62%, and 74% protection from subsequent infection, respectively (17).

Vaccines

Two live, oral, attenuated rotavirus vaccines were licensed for use in 2006 - monovalent Rotarix (GSK Biologicals) and pentavalent RotaTeq (Merck & Co). Rotarix (RV1) is administered in a 2-dose series, given between 6 and 24 weeks of age (18). RotaTeq (RV5) follows a 3-dose schedule, given between 6 and 32 weeks of age (19). Clinical trials conducted in Europe and the Americas established that RV1 and RV5 are safe and effective, with estimates of vaccine efficacy (VE) against severe rotavirus gastroenteritis (RVGE) $\geq$ 85% (6,7). Importantly, the risk for intussusception- a rare adverse event where part of the bowel folds into a nearby part of the bowel, resulting in intestinal obstruction (20)- was found to be low (6,7). Reports of intussusception prompted a market withdrawal of the earlier RotaShield (Wyeth Laboratories, Inc.) vaccine in the United States in 1999 (21).

Successive clinical trials in Africa and Asia have demonstrated the safety and efficacy of RV1 and RV5 for use in low-income countries. Vaccine efficacy ranged from approximately 40-60%, which is substantially lower than efficacy estimates reported in high-income countries (5,22,23). In 2009, the World Health Organization (WHO) recommended that all countries introduce rotavirus vaccines into their national immunization programs (24). As of January 2022, 114 countries have introduced rotavirus vaccines into their child immunization schedules, with more planned introductions in the future (4). WHO prequalified two recently developed rotavirus vaccines in 2018 -Rotavac (Bharat Biotech International Ltd, India) and ROTASIIL (Serum Institute of India, India)- following successful clinical trials (25–27). These additional vaccines could help expand coverage and access, especially in India, which accounted for approximately 22% of global rotavirus deaths in 2013 (13).

In high-income settings, the immunity conferred by rotavirus vaccination appears to last through early childhood. In a position paper published in 2021, WHO cited evidence that RV1 and RV5 provide protection against RVGE-related emergency department visits or hospitalizations in children until at least 3 years of age, and even up to 7 years of age for RV5 (27). A meta-regression study of randomized controlled trails (RCTs) of rotavirus vaccines corroborated these findings: In low-mortality settings, pooled, cumulative vaccine efficacy (VE) estimates against severe RVGE were 94% at 12 months and 92% at 36 months after receipt of last vaccine dose (28). However, immunity may wane more quickly in low-income settings with high mortality in children under 5. In the same meta-regression study, researchers calculated a pooled, cumulative VE against severe RVGE (Vesikari score $\geq$ 11) of merely 44% at 12 months and 35% at 32 months in high-mortality settings (28).

Vaccine Impact & Effectiveness

Data from observational studies and post-licensure randomized trials have verified the real-world impact of rotavirus vaccines. Significant, post-vaccine introduction declines in child mortality from rotavirus and/or all-cause diarrhea have been documented in Latin America and Africa (29–31). Most strikingly, in Mexico, the number of annual deaths in children < 5 years of age from all-cause diarrhea has dropped by roughly 50% since routine vaccination began in 2007 (32). Incorporating vaccines into national immunization schedules has also led to sizable reductions in hospitalizations. A systematic review of data from 8 countries found that hospital admissions for laboratory-confirmed RVGE in children under 5 declined by 49 – 89% within two years of vaccine introduction (33). Evidence of substantial reductions in rotavirus cases and/or all-cause diarrheal hospitalizations in children following vaccine introduction were also reported by studies conducted in Zambia, South Africa, Ghana, and Moldova (34–37).

Furthermore, rotavirus vaccines may confer indirect protection to the unvaccinated. Studies conducted in the United States, Moldova, and Brazil identified significant declines in rotavirus and all-cause diarrhea hospitalizations among older children who were not eligible to be immunized at the time of vaccine

introduction (37–39). However, evidence of indirect protection across age cohorts is not consistent, and more data from low-resource, high-burden countries is needed (40,41).

Live, oral rotavirus vaccines have shown lower efficacy in settings with high child mortality, where rotavirus disease burden is greatest, compared to those with low child mortality. In a recent meta-analysis of studies from 32 countries, the vaccine effectiveness of Rotatrix among children < 12 months of age was 86% in low-mortality countries, 77% in medium-mortality countries, and 63% in high mortality countries. Estimates for RotaTeq were similar, 86% for low-mortality countries and 66% for high-mortality countries (42). Several factors have been postulated to explain this gradient in vaccine performance, including presence of enteric co-pathogens, malnutrition, microbiome composition, acquisition of certain maternal antibodies, and coadministration of oral polio vaccine (43). However, there is conflicting evidence regarding the significance and impact of each of these factors on vaccine effectiveness, and the biological mechanisms through which they operate are not well understood (43).

Transmission

Rotavirus is highly infectious, even at very low doses (44). The primary route of transmission is ingestion of infected feces via person-to-person contact or from contaminated objects, surfaces, or food (15). Close contacts of infected children (i.e. household members) are most at risk for secondary infection. Transmission can occur prior to symptom onset, but individuals are most infectious while symptomatic and within the first three days of recovery (15). Asymptomatic individuals can also spread infection, but likely transmit disease at lower rates than symptomatic individuals since they produce less fecal contamination in the environment (45,46). Household transmission studies from Malawi and Ecuador suggest that increasing disease severity in index cases correlates with greater risk of transmission to household contacts (45,47).

As discussed, rotavirus vaccines have produced dramatic reductions in disease incidence and hospital admissions across a range of settings. The positive association between disease severity and transmission

risk implies that vaccines have promising potential to interrupt community spread of RVGE. Additionally, vaccination could plausibly provide indirect protection to household contacts > 5 years of age by preventing rotavirus from being introduced into the home (48). However, transmission of rotavirus remains commonplace, even in populations with high vaccine coverage. For example, researchers calculated an attack rate of 55% among household contacts in Quininde, Ecuador, despite 85% of index cases having received 2 doses of vaccine (45). Likewise, a U.S. transmission study reported an acute gastroenteritis attack rate of 16% among household contacts, even though 55% of the rotavirus-positive index cases had received at least one dose of vaccine (48). Few studies of rotavirus transmission have been conducted in low-income countries with endemic infection. More research is needed to understand the extent to which vaccines prevent transmission.

Fecal Shedding

Rotavirus is shed in stool in large concentrations, around 100 billion viral particles per milliliter, which contributes to its characteristic high infectiousness (49). Two laboratory tests, enzyme immunoassays (EIA or ELISA) and semiquantitative real-time reverse transcription PCR (qRT-PCR), detect virus in fecal specimens and are used to diagnose RVGE (50,51). Peak shedding generally occurs in the first few days of symptom onset (52–54), when individuals are thought to be most infectious. Evidence shows that shedding persists well past symptom resolution, which has implications for transmission dynamics (55). Mukhopadhya et. al. reported a median shedding duration of 24 days in symptomatic children in India (54). A later study of vaccinated children in Malawi calculated a comparable median of 28 days (53).

Prior studies of unvaccinated children in India have demonstrated that children with asymptomatic infection shed smaller amounts of virus than children with symptomatic infection (54,56). Children with severe RVGE were shown to shed higher amounts of virus than children with moderate or mild disease (56). In a vaccinated community in Malawi, researchers also found that amount of viral shedding is positively associated with disease severity (53). These findings are significant because they suggest that

symptomatic individuals are most infectious and therefore might be crucial to community transmission. Few data exist on individual-level shedding episodes, which are needed to confirm this hypothesis.

Rotavirus vaccination could conceivably curb transmission by reducing incidence of RVGE and/or reducing the infectiousness of breakthrough cases, if found to suppress viral load (53). Studies of other vaccine-preventable viral diseases have explored the relationship between vaccination and viral shedding. For example, researchers in Germany conducted a household-based study of individuals with 4 different subtypes of influenza and found comparable viral loads among vaccinated and unvaccinated cases (57). More recently, scientists have investigated the potential of vaccination to reduce transmission of SARS-CoV-2. Studies from Europe and the United States reported similar peak viral loads (Ct values) among individuals infected with the Delta variant, regardless of vaccination status (58–60). However, the impact of rotavirus vaccination on fecal shedding remains unknown. To our knowledge, no published studies have been designed to evaluate differences in viral shedding during episodes of breakthrough RVGE among vaccinated as compared to unvaccinated individuals. Addressing this gap could enhance the understanding of population-level rotavirus transmission events and inform vaccine programs and policies.

Summary

Rotavirus is the leading cause of diarrheal disease in children < 5 years of age. The introduction of rotavirus vaccines in 2006 has led to major reductions in child morbidity and mortality worldwide. Despite this success, vaccines have been found to be least effective in low-income countries in Africa and Asia, where the burden of rotavirus is greatest. The evidence suggests that rotavirus vaccines reduce incidence and disease severity in young children and provide indirect benefits to the unvaccinated, but vaccine impacts on fecal shedding and the transmission potential for breakthrough cases remain uncertain. Few data exist on individual-level shedding episodes, which are critical to understanding the transmission dynamics of rotavirus. Differences in fecal shedding (infectiousness) between vaccinated and

unvaccinated individuals may have implications for public health programs that seek to track and prevent the incidence of infection.

Chapter II: Manuscript

Introduction

Rotavirus is the leading cause of diarrheal disease in children worldwide, accounting for an estimated 128,500 deaths in 2016 (11). Roughly 90% of all deaths occur in low- and middle- income countries in Sub-Saharan Africa and South Asia (11). The introduction of two live, oral rotavirus vaccines, recommended by the WHO in 2009, has contributed to marked reductions in rotavirus-related hospitalizations and deaths (24). As of January 2022, 114 countries have introduced rotavirus vaccines into their national immunization schedules (4) . Despite these achievements, rotavirus vaccines have been shown to be less effective in low-income settings, where child mortality and rotavirus disease burden are greatest, compared to high-income settings. In a meta-analysis of 60 studies from 32 countries, the effectiveness of the Rotarix vaccine among children < 12 months of age was found to be 86% in countries with low child mortality, compared with 77% in medium-mortality countries and 63% in high mortality countries (42).

In settings with lower vaccine efficacy, breakthrough rotavirus disease among vaccinated children is common. However, because few studies have examined the impact of rotavirus vaccination on fecal shedding and infectiousness, it is unknown how much these breakthrough cases contribute to transmission. Rotavirus is shed in stool in large concentrations during episodes of rotavirus diarrhea and in the stool of individuals with asymptomatic infection (1). Shedding generally peaks in the first few days of symptom onset but persists well past symptom resolution (52–55). Previous studies in India and Malawi reported median viral shedding durations of 24 days and 28 days, respectively, among symptomatic children (53,54). In a multi-site study from the MAL-ED birth cohort, rotavirus shedding persisted for a median of 8 days post-diarrhea in children ages 0 – 24 months (61). These findings indicate that individuals remain infectious even after symptoms have resolved, which has important implications for transmission dynamics.

Evidence also suggests that disease severity may be an important predictor of quantity of fecal shedding. In a study of unvaccinated children in India, children with severe RVGE shed larger amounts of virus compared to children with moderate or mild disease (56). Similar findings were reported among a cohort of vaccinated children in Malawi: viral load was positively associated with increasing disease severity (53). Rotavirus vaccine efficacy has been shown to be greater against severe rotavirus diarrhea than against any disease (5,6,22,23). Therefore, reducing disease severity is one mechanism by which vaccination may reduce population-level shedding burden.

There are two primary mechanisms through which vaccination may impact quantity of rotavirus shedding and, by extension, infectiousness. First, vaccines reduce total shedding burden by preventing cases of RVGE from occurring. Post-vaccination declines in severe rotavirus diarrhea have been well documented worldwide (31,32,40,62). Furthermore, a systematic review of data from 8 countries found that hospital admissions for laboratory-confirmed RVGE in children under five declined by 49 – 89% within two years of vaccine introduction (33). Second, vaccination could conceivably suppress viral load. This would reduce the infectiousness of vaccinated individuals experiencing breakthrough cases of RVGE relative to unvaccinated individuals, though further evidence is needed to confirm this hypothesis.

Few data on individual-level shedding episodes exist, and the impact of rotavirus vaccination on fecal shedding among breakthrough cases remains largely unknown. Addressing this gap could enhance our understanding of rotavirus transmission dynamics, expose potential impacts of vaccination on transmission, and inform rotavirus prevention programs in low-resources settings where vaccine effectiveness is reduced. In this study, we examined the relationship between vaccination status and quantity of rotavirus shed during episodes of RVGE among infants in a randomized controlled trail conducted in Bangladesh, a country that has not yet introduced rotavirus into its routine vaccination schedule (4).

Methods

Data Sources & Study Sample

Data for this study came from the Performance of Rotavirus and Oral Polio Vaccines in Developing Countries (PROVIDE) randomized controlled clinical trial conducted in the Mirpur slum of Dhaka, Bangladesh (63). The study methods for PROVIDE have been previously described in the published literature (63–65). To summarize, PROVIDE was conducted from May 2011 to November 2014. 700 infants and their mothers were enrolled into a birth cohort in the first week of life and followed until they reached 2 years of age. The children were randomized to receive either two doses of RV1 at 10 and 17 weeks of age or no RV1 (non-placebo controlled). Informed consent was obtained for all participants. Human subjects research approval was obtained from the Ethics Review Committee at the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) and from the IRBs at the University of Virginia and University of Vermont. PROVIDE was registered at Clinicaltrails.gov (NCT01375647). Per the Emory Non-human Subjects Research Determination Tool, the current study did not require Emory University IRB review.

Bangladeshi field research assistants conducted twice weekly home visits to identify episodes of diarrhea in enrolled children. Diarrhea was defined as "three or more abnormally loose stools in 24 hours according to the mother" (63). Diarrhea episodes separated by 72 diarrhea-free hours were counted as discrete episodes. Fecal samples for each diarrhea episode were collected and tested for the presence of rotavirus by PosSpecT enzyme-linked immunosorbent assay (ELISA; Oxoid Ltd, Hampshire, United Kingdom). Primary outcomes used to estimate RV1 vaccine efficacy were defined by ELISA positivity (64). The fecal samples were then reanalyzed with real-time reverse transcription-quantitative polymerase change reaction (qPCR) using the TaqMan Array Card platform (ThermoFisher, Carlsbad, CA, USA). Missing diarrheal stool samples were presumed negative for rotavirus (64). Per protocol vaccine efficacy for PROVIDE was 51% against all rotavirus diarrhea and 73.5% against severe rotavirus diarrhea (64).

Data & Definitions

For the present study, an episode of RVGE was defined as testing rotavirus positive by ELISA. The outcome of interest was quantity of rotavirus (viral load) shed in stool during episodes of RVGE. The primary exposure of interest, infant vaccination status, was determined by RV1 randomization arm and analyzed per intention-to-treat (ITT) protocol. Fecal viral load was measured in log-copy numbers per gram of stool based on the cycle threshold (Ct) values obtained by qPCR. Stool samples testing negative by qPCR were set to half the limit of detection (1.8495 log-copy numbers per gram of stool). The data were restricted to first year of life during which diarrhea samples were tested by qPCR. Additionally, only episodes of RVGE occurring in children between the ages of 10 weeks and one year were included, since children randomized to receive RV1 were not administered their first dose of vaccine until the tenth week of life.

Other covariates of interest were selected a priori from the PROVIDE dataset based on a causal framework constructed following a review of the literature. Variables chosen for inclusion were disease severity, child age, nutritional status, number of days of exclusive breastfeeding, and time (days) from symptom onset to stool collection. RVD severity was classified using the 20-point modified Vesikari scale, with a score of ≥ 11 indicating severe disease (63). Child age reflects age in days at the onset of each diarrheal episode, measured by the appearance of symptoms. Nutritional status was evaluated using anthropometric measures from study visits to calculate weight-for-age (WAZ) and weight-for-height (HAZ) z-scores (63). We classified children as 'stunted' (HAZ) or 'underweight' (WAZ) if their z-score was less than -2 standard deviations below the mean per the WHO Child Growth Standards (66).

Statistical Analysis

All statistical analysis were performed using SAS 9.4 (Carey, NC). Chi-square tests of proportions and ANOVA tests were conducted to look for differences in participant characteristics across the four groups presented in Table 1. P-values of < 0.05 were considered statistically significant. We used multivariable

linear regression (MLR) to investigate the association between infant vaccination status and fecal viral shedding quantity during episodes of RVGE. Generalized estimating equations with robust standard error were used to account for clustering of RVGE episodes in the same child. Only children who experienced ≥ 1 episodes of RVGE between 10 weeks and 1 year of age were included in the final analytic dataset. Because we restricted the study population based on post-randomization factors, we adjusted for potential confounding variables including child age, WAZ, HAZ, exclusive breastfeeding at the time of episode, and time from symptom onset to stool collection.

In addition, we stratified the model to investigate potential effect modification by disease severity (modified Vesikari score) and child age. Age was dichotomized to examine the impact of vaccination on fecal shedding among children 10 weeks to 6 months of age compared with older children 6 to 12 months of age. Finally, an additional interaction assessment was conducted to examine potential differences in shedding between unvaccinated and vaccination children after receipt of 1 dose (administered at week 10) vs 2 doses (administered at week 17) of RV1. We stratified the effect estimates for two time intervals: 10 weeks to 19 weeks and 19 weeks to 1 year. We chose 19 weeks as the cutoff to allow a two-week period for children to mount an immune response to the second dose of RV1.

Results

Description of Study Population

700 children were enrolled in PROVIDE within 7 days of birth, and 350 were randomized to each RV1 vaccine arm. Of these, 107 (15.3%) children dropped out of the study within the first year, 58 in the vaccine arm and 49 in the control arm (64). 174 (24.9%) children experienced at least one episode of RVGE between the ages of 10 weeks and one year of life. 17.8% (n=62) of vaccinated children experienced at least one breakthrough episode of RVGE, compared with 32.0% (n=112) of unvaccinated children experiencing at least one RVGE episode (Table 1).

Child characteristics by randomization arm and RVGE are shown in Table 1. We found no significant differences in characteristics across the four groups. Approximately half of all children were male. Median age at enrollment was 5 days, except among unvaccinated children who did not acquire RVGE during the study period, whose median age at enrollment was 6 days. 45.9% (n=28) of vaccinated children with breakthrough RVGE were exclusively breastfed at 18 weeks. 39.6% (n=44) of unvaccinated children who experienced at least one episode of RVGE were exclusively breastfed at 18 weeks.

Description of Quantity of Fecal Shedding

There were 184 total episodes of RVGE detected by ELISA during the study period. Quantity of fecal viral shedding ranged from 3.71 to 11.00 log copies per gram of stool, measured by qPCR. Median viral load was 7.99 (IQR 7.01, 8.76) log copies per gram of stool for episodes of RVGE occurring in children between 10 weeks and 6 months of age and 8.27 (IQR 7.64, 9.08) log copies per gram of stool for episodes occurring in older children 6 months to 1 year of age (Table 2). Median viral load was 8.24 (IQR 7.42, 9.45) log copies per gram of stool among severe episodes of RVGE, 7.94 (IQR 7.24, 8.77) log copies per gram of stool among moderate episodes of RVGE, and 8.53 (IQR 7.68, 9.20) log copies per gram of stool among mild episodes of RVGE (Table 2). In the 14 episodes where the child was exclusively breastfed at the time of the episode, median quantity of fecal shedding was 8.72 (IQR 7.55, 9.30) log copies per gram of stool as compared to 8.20 (IRQ 7.55, 8.92) log copies per gram of stool for episodes occurring in children who were not exclusively breastfed prior to the episode (Table 2).

Time from symptom onset to stool collection ranged from 0-7 days. In stools collected within 1 day of symptom onset, median viral load was 8.23 (IQR 7.59, 9.08) log copies per gram of stool. The median was 8.13 (IQR 7.51, 8.96) log copies per gram of stool in specimens collected 2-3 days from symptom onset and 8.47 (IQR 7.19, 8.86) log copies per gram of stool in specimens collect four or more days from symptom onset (Table 2).

Effect of Vaccination

We analyzed 184 episodes of RVGE detected in children between 10 weeks and 1 year of life that had complete data for all 6 explanatory variables in the multivariable linear regression model. Children in the vaccine arm had significantly lower levels of fecal viral shedding compared to children in the control arm after controlling for age, WAZ, HAZ, exclusive breastfeeding at time of episode, and time in days since symptom onset. The average amount of fecal viral shedding during episodes of RVGE was 0.59 (95% CI: 0.19, 0.99) log copies per gram of stool lower among vaccinated as compared to unvaccinated children (Table 3).

Interaction Assessment

There was no evidence of statistically significant interaction by age ($p = 0.58$), after controlling for WAZ, HAZ, exclusive breastfeeding at time of episode, and time in days since symptom onset. Among those 6 to 12 months of age, the average amount of viral shedding was 0.50 (95% CI: -0.003, 1.01) log copies per gram of stool lower among vaccinated children than among unvaccinated children. The effect was slightly stronger for younger children. Among those 10 weeks to 6 months of age, vaccinated children shed, on average, 0.73 (95% CI: 0.07, 1.40) log copies per gram of stool less than unvaccinated children (Table 3). Regardless of age group, vaccinated children had lower levels of shedding than unvaccinated children.

We also tested for interaction between vaccination and disease severity (modified Vesikari score), using mild disease as the reference group. There were 149 episodes of RVGE with complete data for modified Vesikari score. Of these, 44 were classified as mild, 57 as moderate, and 48 as severe. We found no evidence of statistically significant interaction between vaccination and disease severity on quantity of fecal shedding (Table 3). The largest effect estimate was found among children with moderate disease compared to children with episodes of other severities. Among children with moderate RVGE, the average amount of fecal viral shedding was 0.85 (95% CI: 0.18, 1.52) log copies per gram of stool lower among vaccinated as compared to unvaccinated children (Table 3). Mean fecal shedding among children

with mild or severe disease was also lower among vaccinated vs. unvaccinated children, but these estimates were imprecise (Table 3).

In our interaction assessment by time (after 1 dose vs 2 doses of RV1), there were 22 episodes of RVGE among children ages 10 and 19 weeks of life. The other 162 episodes occurred in children between the ages of 19 weeks and 1 year of life. Our results show no evidence of interaction by these time intervals (Table 3). However, due to the small number of episodes occurring between 10 and 19 weeks of life, these results are imprecise.

Discussion

In a birth cohort of Bangladeshi children 10 weeks to 1 year of age, lower quantities of virus were detected in stools from vaccinated children with symptomatic rotavirus infection than in stools from symptomatic unvaccinated children. This relationship did not differ by child age or disease severity. Our analysis suggested that even one dose of RV1 may be sufficient to reduce viral shedding during episodes of RVGE among vaccinated as compared to unvaccinated children, although these results should be interpreted with caution given the small number of episodes among children ages 10 to 19 weeks.

These results suggest that RV1 vaccination reduces shedding burden among breakthrough cases of RVGE, in addition to preventing RVGE cases entirely. Assuming quantity of rotavirus detected by qPCR is a reasonable proxy for the quantity of infectious virus shed, our results also suggest that breakthrough cases among vaccinated children may have lower transmission potential than cases among unvaccinated children, though further study is needed. Furthermore, these results strengthen the evidence for the potential health benefits of vaccination in settings where vaccine efficacy is reduced and in highly endemic settings where routine rotavirus vaccination has not yet been introduced.

Since previous studies have reported higher levels of shedding among children with severe disease compared to children with mild or moderate disease, we were interested in stratifying our analysis on disease severity (53,56). However, we found no evidence of interaction between vaccination and disease

severity. Our results suggest that vaccination works equally as well at reducing shedding between vaccinated compared to unvaccinated children among cases of severe vs. moderate vs. mild RVGE.

Our study has several key strengths. First, the data come from the PROVIDE trial, which employs a rigorous randomized control design to assign participants to a vaccination arm, thereby balancing both measured and unmeasured confounders between the vaccination and control groups (63). Second, few rotavirus studies have incorporated PCR testing into the analysis. This study used qPCR to measure quantity of rotavirus per gram of stool, something that is not possible with the less sensitive ELISA. Third, multivariable regression methods allowed us to control for post-randomization factors that may confound the relationship between vaccination and fecal shedding, including age, nutritional status, breastfeeding, and time from symptom onset. For example, studies from India and Malawi showed that viral load among symptomatic cases declines with time from symptom onset (53,54). Our analysis controlled for differences in time of stool collection relative to the start of symptoms to account for these inherent differences in shedding quantity over time. Finally, to our knowledge, this study is the first to examine rotavirus fecal shedding among vaccinated compared with unvaccinated children. While previous studies from India and Malawi have characterized general shedding patterns, these studies had little to no variability in vaccination status among participants (53,54,56,67).

Our analysis is subject to the following limitations. Since stool specimens were only collected from children presenting with diarrhea, we were unable to examine shedding characteristics among asymptomatic cases of rotavirus infection. It is important to study how efficiently asymptomatic cases shed virus to understand the full web of transmission dynamics. Second, only diarrheal episodes that tested positive by ELISA were considered as cases of RVGE. Since ELISA has limited sensitivity, infections with low-level viral shedding were likely missed. Given that our study found lower average quantities of viral shedding among vaccinated compared to unvaccinated children, breakthrough cases may be more subject to this misclassification. Third, although PCR tests are highly sensitive in detecting rotavirus DNA, one disadvantage is that they are unable to distinguish between live and dead virus

particles. Since only live virus particles cause illness, our ability to make inferences about the transmission potential among vaccinated compared to unvaccinated children is limited. Next, only one stool sample from each episode of diarrhea was collected, and these samples were collected at varying times from symptom onset. Ideally, we would have taken serial stool samples at predetermined time intervals for each RVGE episode. This would have allowed for better control over time as a confounding factor and would have allowed us to analyze shedding patterns throughout the course of illness. Lastly, our data come from a densely-populated, urban setting in Bangladesh. Results may not be generalizable to child populations in high income, semi-urban, or rural settings.

Conclusions & Future Research

In addition to preventing disease, rotavirus vaccination reduces the quantity of virus shed in stool during episodes of RVGE. While viral load is an important determinant of secondary rotavirus transmission, additional studies are needed to determine its impact on community-level transmission dynamics. Shedding differences among older children should also be investigated to examine if the negative association between vaccination and viral load is sustained beyond the first year of life. Additionally, quantifying the impact of vaccination on shedding among asymptomatic cases would expand our understanding of the role of vaccination in preventing the spread of rotavirus. Our work demonstrates that rotavirus vaccination is a critical tool reducing the burden of disease in communities with ongoing transmission, even in settings with reduced vaccine efficacy.

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Tables

Table 1. Child characteristics by vaccination arm and rotavirus detection by ELISA

Characteristic	RV1, breakthrough (n=62)		RV1, no breakthrough (n=288)		No RV1, RV detected (n=112)		No RV1, RV not detected (n=238)	
	mdn	*IQR*	*mdn*	*IQR*	*mdn*	*IQR*	*mdn*	*IQR*
Child attributes								
Sex, Male (n,%)	n=40	64.5%	n=142	49.3%	n=61	54.5%	n=125	52.5%
Median age at enrollment (days)	5	4, 6	5	3, 6	5	3.5, 6	6	4, 7
Weight at enrollment (kg)	2.8	2.5, 2.9	2.7	2.5, 3.0	2.7	2.5, 3.0	2.8	2.5, 3.1
Length at enrollment (cm)	48.5	47.2, 49.9	48.5	47.3, 49.6	48.7	47.5, 50.0	48.9	47.5, 50.1
Has $\geq$ 1 siblings <5 years (n,%)	19	30.6%	77	26.7%	33	29.5%	59	24.8%
Weight-for-age z score at 10 wk[a]	-1.09	-1.82, -0.54	-0.87	-1.60, -0.34	-0.96	-1.54, -0.42	-0.84	-1.50, -0.16
Height-for age z score at 10 wk[a]	-1.11	-1.53, -0.48	-0.97	-1.63, -0.45	-0.98	-1.49, -0.44	-0.84	-1.62, -0.34
Weight-for-height z score at 10 wk[a]	-0.14	-1.22, 0.55	-0.08	-0.62, 0.63	-0.03	-0.81, 0.62	0.07	-0.65, 0.69
Exclusively breastfed at 18 wk[b] (n,%)	28	45.9%	126	51.0%	44	39.6%	96	50.5%

[a] n = 62 RV1 & breakthrough, n = 256 RVI & no breakthrough, n = 112 No RV1 & RV detected, n = 214 No RV1 & RV not detected

[b] n = 61 RV1 & breakthrough, n = 247 RVI & no breakthrough, n = 111 No RV1 & RV detected, n = 190 No RV1 & RV not detected

Table 2. Quantity of fecal shedding (by qPCR) by episode and child characteristics among cases of RVGE (positive by ELISA)

Characteristic	Shedding Quantity			
	Number of episodes (n)	Median	IQR	Range
Episode Attributes				
Severity (Vesikari score)				
Mild (< 7)	44	8.53	7.68, 9.20	5.25 – 10.53
Moderate (7-10)	57	7.94	7.24, 8.77	3.71 – 10.11
Severe (≥11)	48	8.24	7.42, 9.45	4.35 – 11.00
Time from symptom onset				
0 – 1 days	93	8.23	7.59, 9.08	4.35 – 10.75
2 – 3 days	67	8.13	7.51, 8.96	3.71 – 11.00
≥ 4 days	24	8.47	7.19, 8.86	5.34 – 10.53
Child Attributes				
Child age at episode				
10 wk to 6 months	63	7.99	7.01, 8.76	4.60 – 10.53
6 months to 1 yr	121	8.27	7.64, 9.08	3.71 – 11.00
Underweight (WAZ < -2)				
Yes	32	8.44	7.37, 9.28	4.35 – 10.53
No	152	8.20	7.56, 8.89	3.71 – 11.00
Stunted (HAZ < -2)				
Yes	26	8.37	7.47, 9.95	4.35 – 10.53
No	158	8.20	7.55, 8.90	3.71 – 11.00
Exclusive breastfeeding at episode				
Yes	14	8.72	7.55, 9.30	5.59 – 10.04
No	170	8.20	7.55, 8.92	3.71 – 11.00

Table 3. Association of vaccination with quantify of fecal viral shedding by qPCR

Analysis	n	Effect Estimate (95% CI)	pvalue for heterogeneity
Overall Effect[a]	184	-0.59 (-0.99, -0.19)	-
Interaction with Age[b]			
< 6 months	184	-0.73 (-1.40, -0.07)	REF
≥ 6 months	184	-0.50 (-1.01, 0.003)	0.58
Interaction with Severity[a]			
Mild	44	-0.48 (-1.19, 0.24)	REF
Moderate	57	-0.85 (-1.52, -0.18)	0.46
Severe	48	-0.66 (-1.67, 0.35)	0.77
Interaction with Time[a]			
10wk – 19wk	22	-1.40 (-2.48, -0.31)	REF
19wk – 1 yr	162	-0.51 (-0.96, -0.06)	0.13

[a] Controlling for age, WAZ, HAZ, exclusive breastfeeding at time of episode, and time (days) since symptom onset

[b] Controlling for WAZ, HAZ, exclusive breastfeeding at time of episode, and time (days) since symptom onset