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Correlates of Rotavirus Vaccine Shedding and Seroconversion in a U.S. Cohort of Healthy Infants

Rachel M. Burke, PhD, MPH[*1], Daniel C. Payne, PhD[1], Monica McNeal, MS[2,3], Shannon C. Conrey, PhD, MS[3,4], Allison R. Burrell[2,3,4], Claire P. Mattison, MPH[1,5], Mary C. Casey-Moore, PhD [1], Slavica Mijatovic-Rustempasic, MSc[1], Rashi Gautam, PhD [1], Mathew D. Esona, PhD [1], Alexander W. Thorman, PhD[4], Michael D. Bowen, PhD [1], Umesh D. Parashar, MBBS, MPH[1], Jacqueline E. Tate, PhD[1], Ardythe L. Morrow, PhD, MSc[3,4], Mary A. Staat, MD, MPH[2,3]

1. Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA; 2. Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, USA; 3. Division of Infectious Diseases, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; 4. Department of Environmental and Public Health Sciences, University of Cincinnati College of Medicine, Cincinnati, OH, USA; 5. Cherokee Nation Assurance, Arlington, VA, USA

Background: Rotavirus is a leading cause of severe pediatric gastroenteritis; two highly effective vaccines are used in the US. We aimed to identify correlates of immune response to rotavirus vaccination in a US cohort.

Methods: PREVAIL is a birth cohort of 245 mother-child pairs enrolled 2017 – 2018 and followed for 2 years. Infant stool samples and symptom information were collected weekly. Shedding was defined as RT-PCR detection of rotavirus vaccine virus in stools collected 4–28 days after dose one. Seroconversion was defined as a threefold rise in IgA between the six-week and six-month blood draws. Correlates were analyzed using generalized estimating equations and logistic regression.

Results: Pre-vaccination IgG (OR=0.84, 95% CI [0.75–0.94] per 100-unit increase) was negatively associated with shedding. Shedding was also less likely among infants with a single-nucleotide polymorphism inactivating *FUT2* antigen secretion ("non-secretors") with non-

CORRESPONDING AUTHOR INFORMATION: Rachel M. Burke, PhD MPH, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Atlanta, GA 30329, USA, RBurke@cdc.gov

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secretor mothers, versus all other combinations (OR 0.37 [0.16–0.83]). Of 141 infants with data, 105 (74%) seroconverted; 78 (77%) had shed vaccine virus following dose one. Pre-vaccination IgG and secretor status were significantly associated with seroconversion. Neither shedding nor seroconversion significantly differed by vaccine product.

Discussion: In this US cohort, pre-vaccination IgG and maternal and infant secretor status were associated with rotavirus vaccine response.

KEY WORDS: Rotavirus, Rotavirus vaccine, Vaccine response

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the US Centers for Disease Control and Prevention (CDC).

BACKGROUND

Rotavirus is a leading cause of severe pediatric gastroenteritis worldwide [1-3]. In the United States, rotavirus activity has declined substantially since the introduction of rotavirus vaccines beginning in 2006 [4], and two live, oral rotavirus vaccines are licensed and approved by the Advisory Committee on Immunization Practices (ACIP) for use: Rotarix® (GlaxoSmithKline), a two-dose vaccine given at 2 and 4 months of age, and RotaTeq® (Merck), a three-dose vaccine given at 2, 4, and 6 months of age [5]. For US children born in 2018–19, full coverage with either vaccine reached 77.1% (95% confidence interval [CI]: 76.1 – 78.2%) by 8 months of age [6].

Both rotavirus vaccines have demonstrated good effectiveness against moderate to severe pediatric rotavirus gastroenteritis in the United States [7, 8] and in other high-income settings [9]. However, individual-level vaccine failures are possible in any setting [10, 11], suggesting that child and environmental factors may influence immunogenicity and vaccine efficacy. Previous research has pointed to child or maternal *FUT2* ("secretor") status, maternal IgG levels, and breastfeeding as potential influencers of infant immune response to rotavirus vaccination [12]. The *FUT2* gene is one of several genes that affect expression of histo-blood group antigens (HBGAs) on epithelial intestinal cells and saliva as well as the composition of oligosaccharides in breastmilk. Secretor-positive individuals, who express these HBGAs on epithelial cells, have been shown to be more susceptible to rotavirus infection. However, assessment of secretor status is a relatively recent trend in rotavirus vaccine studies, and limited real-world data are available on correlates of immune response to rotavirus vaccination among healthy U.S. infants.

Data from the PREVAIL (Pediatric Respiratory and Enteric Virus Acquisition and Immunogenesis Longitudinal) Cohort study [13], a longitudinal birth cohort in Cincinnati, Ohio, were analyzed to identify correlates of immune response to rotavirus vaccination, as measured by fecal shedding of the vaccine virus and seroresponse to the first two doses of rotavirus vaccine.

METHODS

Study population

The PREVAIL Cohort is a longitudinal study of 245 enrolled mother-child pairs residing in Cincinnati, Ohio. Methods have been previously described in detail [13], but those relevant to the present analysis are briefly outlined here. Mothers were recruited, consented, and enrolled 2017 – 2018 during the third trimester of pregnancy, and healthy, singleton infants were followed from birth. Infant stool samples and symptom information were collected weekly for the first 24 months of life, and mothers collected additional infant stool samples during episodes of acute gastroenteritis. Demographic information was collected at baseline, and mothers were periodically queried about infant care and feeding. Vaccination information was abstracted from state registries and provider records, and discrepancies were adjudicated. In this area, private insurance formularies cover RotaTeq, while public insurance covers Rotarix, although some deviations occur. Serum was collected from mothers at enrollment in the third trimester, and from infants at age six weeks, six months, and 12 months of age. Cord blood was collected at birth. Saliva was collected from mothers at enrollment and from infants at six weeks and 12 months of age, since some six-week samples were unsuccessfully genotyped. This study was reviewed and approved by the institutional review boards of CDC, Cincinnati Children's Hospital Medical Center, and the two enrolling birth hospitals.

Laboratory testing

Serum and cord blood samples were tested for anti-rotavirus IgG (mothers, infants, and cord blood) and IgA (infants only) using enzyme-linked immunosorbent assay (ELISA) at Cincinnati Children's Hospital Medical Center in the Laboratory for Specialized Clinical Studies [14].

All stool samples from the first year of life were tested for rotavirus according to the following procedures. Ten percent stool suspensions were prepared using phosphate-buffered saline followed by centrifugation at 3000 rpm for 10 minutes. Rotavirus RNA was extracted from suspension supernatants using the KingFisher™ Flex Purification System (Thermo Fisher Scientific, USA) with the MagMAX™-96 Viral RNA Isolation Kit (Thermo Fisher Scientific, USA), following the manufacturer's instructions. Rotavirus detection was performed on all RNA extracts using a real-time reverse transcription polymerase chain reaction (RT-PCR) assay [15, 16]. To differentiate between vaccine and wild-type (WT) strains, all rotavirus-positive samples were tested by RT-PCR assays designed to detect Rotarix® or RotaTeq® vaccine strain components [17]. The rotavirus-positive samples that tested negative for Rotarix® or RotaTeq® vaccine strains were genotyped using RT-PCR assays specific for rotavirus wild-type strains in singleplex format [17, 18]. Rotavirus-positive samples which did not genotype as vaccine or wild-type strains were re-extracted on MagNA Pure Compact Instrument (Roche Applied Science, Switzerland) with the MagNA Pure Compact Nucleic Acid Isolation Kit I and retested for vaccine-strain and wild-type genotype using the RT-PCR assays mentioned above. All

rotavirus-positive samples which did not yield a genotype by RT-PCR assays were amplified and sequenced [19].

To determine *FUT2* genotype, DNA was extracted from maternal and infant saliva samples. PCR testing defined secretor genotypes of the subjects based on a 428 G>A point mutation that results in a premature stop codon. Non-secretors were defined as individuals with the point mutation in both *FUT2* alleles (AA). Secretors were defined as individuals with GG or GA genotypes [20].

Statistical analysis

Analyses are limited to infants for whom at least one dose of rotavirus vaccine was reported. Descriptive analyses of the cohort and the prevalence of shedding by time post-vaccination are presented. Correlates of post-vaccination shedding were analyzed for the first dose only, given the possibility that the first dose would affect shedding probability in later doses. Covariates were selected *a priori* based on literature review. We included stools from all infants who received at least one dose of rotavirus vaccine and restricted the analysis of shedding correlates to stools collected between 4 and 28 days (inclusive) after the first dose and before the second dose. Stools collected 0 – 3 days post-vaccination were excluded from analyses of shedding correlates, due to the possibility of shedding representing "pass-through" rather than replication. Shedding was defined as detection of either Rotarix or RotaTeq vaccine virus in the stool. To maximize power, the individual stool was used as the unit of analysis; generalized estimating equations (GEE) with a binomial distribution and a logit link were used, with individual child as the clustering variable to account for within-child correlations. An exchangeable correlation matrix and robust standard errors were used, and models also controlled for days since vaccination as a linear variable.

Correlates of seroconversion were analyzed using logistic regression with the child as the unit of analysis. Seroconversion was defined as a threefold rise in IgA between the six-week and six-month blood draws, among children with blood drawn prior to (or day of) the first dose of rotavirus vaccine and at least 14 days after the second dose of rotavirus vaccine.

All models were assessed for collinearity and reduced as necessary until no collinearity remained.

In models of seroconversion and shedding, infant IgG at 6 weeks of age was imputed for 16 infants missing a 6-week blood draw using log-linear models with maternal and cord IgG as predictors of 6-week IgG. Breastfeeding status at time of vaccination was defined using queried dates of breastfeeding cessation and formula introduction. Exclusive breastfeeding was strictly defined as those infants who had received only breastmilk (never formula) since birth. Maternal and infant secretor status were tested separately in crude models but ultimately combined due to some suggestion of potential interaction; based on analysis, this was reduced into a single binary variable given small strata.

RESULTS

Characteristics of the Analysis Population

Of the 245 infants who met final study enrollment criteria, 225 (92%) received at least one dose of rotavirus vaccine; by the end of follow-up, 199 vaccinated infants (81%) had received a full course of rotavirus vaccination (2 doses of Rotarix or 3 doses of RotaTeq), while 26 (11%) remained only partially vaccinated against rotavirus. The first dose of rotavirus vaccine was typically administered according to ACIP guidelines (at mean age of ~2 months), and vaccine type of the first vaccination was nearly evenly distributed (RotaTeq 55%, Rotarix 45%) (Table 1). At the time of their first dose of rotavirus vaccine, 27% of infants were exclusively breastfed, while 47% of infants were exclusively formula fed. The majority of infants (80%) and mothers (79%) were secretors, and ~70% of mother-infant pairs had concordant secretor:secretor status, while ~10% had concordant non-secretor:non-secretor status.

Shedding

Overall, 4,232 stool samples from 204 infants were collected between 0 and 99 days following receipt of a rotavirus vaccine, with 1456 samples from 199 infants collected after dose 1 but before dose 2; 583/1456 were collected between 4 and 28 days post-vaccination. Shedding was highest after the first dose, with 81% (161/199) of infants having rotavirus vaccine virus detected in at least one stool sample; when stratifying by secretor status, shedding was somewhat less frequent among non-secretor infants or those with non-secretor mothers. Shedding was least frequent among non-secretor infants born to non-secretor mothers (Figure). Rotavirus vaccine virus was less frequently detected after the second (70/182 children shedding in ≥1 sample; 39%) dose of both vaccines and third (40/101 children; 40%) dose of RotaTeq. Rotavirus vaccine virus detections after dose 1 occurred most frequently among stools collected between 4 and 7 days following vaccination (84% of stools collected were positive for vaccine virus) and tapered off with increasing time since receipt (8 – 14 days post-vaccination: 58%; 15 – 21 days post-vaccination: 39%; 22 – 28 days post-vaccination: 33%).

In adjusted models, pre-vaccination IgG (OR 0.84, 95% CI: 0.75 – 0.94 per 100-unit increase) and days since vaccination (OR 0.89, 95% CI: 0.87 – 0.91 per day) were significantly negatively associated with shedding (Table 2). Shedding was significantly less likely among non-secretor infants with non-secretor mothers, as compared with all other combinations (OR 0.37, 95% CI: 0.16 – 0.83). This association was also statistically significant when comparing the non-secretor:non-secretor pairs to secretor:secretor pairs (OR 0.34, 95% CI: 0.15 – 0.79); other point estimates in the model remained similar (Supplemental Table 1).

When stratified by exclusive breastfeeding status, most findings were similar (Table 3). However, the relationship between secretor status and shedding appeared stronger among exclusively breastfed infants, as did the relationship between 6-week levels of infant IgG and shedding. Patterns were similar when stratifying by vaccine product (Supplemental Table 2) or

insurance type, with stronger relationships observed for secretor status and IgG among RotaTeq-vaccinated (or privately insured) infants, compared with Rotarix-vaccinated (or publicly insured) infants. However, exclusive breastfeeding was strongly correlated with both vaccine product and insurance type. At the time of vaccination, only 9 infants (with 28 stools in total) were vaccinated with Rotarix and exclusively breastfed while only 2 infants (with 4 stools in total) were vaccinated with Rotarix and privately insured. Point estimates comparing non-secretor:non-secretor dyads to all other combinations were similar to those comparing non-secretor:non-secretor dyads to secretor:secretor dyads across all models. Infant secretors were significantly more likely to shed, across strata of maternal secretor status, though the association did not reach the alpha = 0.05 significance level among children of secretor mothers (Supplemental Table 3).

Seroconversion

There were 141 infants who received at least two doses of rotavirus vaccine and had blood drawn before the first dose and at least 14 days after the second dose was received. Of these infants, 105 (75%) seroconverted, of whom at least 81 (77%) had also shed following the first dose of rotavirus vaccine (9 missing data). Among the 36 infants who did not seroconvert, 23 (64%) had shed after the first dose of vaccine ($p = 0.02$ for the association of shedding and seroconversion). In unadjusted analysis, shedding and seroconversion displayed similar patterns by secretor status (Figure). Although seroconversion was significantly higher among Rotarix vaccinees in a crude model, there was no association with vaccine product in the fully adjusted model (Table 4). After adjustment for covariates, only pre-vaccination IgG and maternal:infant secretor status were significantly associated with seroconversion after the second dose of rotavirus vaccine. In a separate model including both post-vaccination fecal shedding and pre-vaccination IgG, patterns were similar, though only pre-vaccine IgG was not significantly associated at the 0.05 significance level (Supplemental Table 4).

DISCUSSION

In this community-based cohort of healthy US infants, we observed a high prevalence of immune response to rotavirus vaccination as measured by post-vaccination shedding and seroconversion, regardless of which rotavirus vaccine (RotaTeq or Rotarix) was administered. While the high level of vaccine-induced immunogenicity across vaccine products is reassuring, notable differences in immunologic response were observed by extrinsic and intrinsic factors for both infant and mother. Fecal shedding was highest 4 – 7 days following the first dose of rotavirus vaccine and progressively declined over time and with subsequent doses. Infants having higher levels of pre-vaccination IgG at 6 weeks of age were significantly less likely to shed rotavirus vaccine virus or to seroconvert following their first dose of rotavirus vaccine. Non-secretor infants with non-secretor mothers were significantly less likely to shed or seroconvert, compared with all other combinations.

The high prevalence of shedding in at least one sample following the first dose of rotavirus vaccine (81%) is comparable to that observed in other high-income settings where rotavirus was detected via RT-PCR [21-24]. This finding, taken together with the observation that shedding prevalence decreased for subsequent doses, suggests that most infants in our cohort mounted a robust mucosal immune response to rotavirus vaccine [25]. The high prevalence of shedding among day 4 – 7 samples also supports our choice to categorize days 0 – 3 post-vaccination as "pass-through" days rather than as shedding as a result of viral replication.

Pre-vaccination IgG, representing placentally transferred maternal antibodies, was negatively associated with rotavirus vaccine response for both the shedding and seroconversion outcomes. Other studies in low- and middle-income countries have found anti-rotavirus antibodies can inhibit immunologic take of live, attenuated rotavirus vaccines, but this finding has not been suggested in a US sample until now [24, 26-31]. Increased transfer of maternal IgG with increasing gestational age may also explain our finding of reduced shedding among infants born at term or post-term.

Though exclusive breastfeeding at time of vaccination was associated with seroconversion in unadjusted models, no significant associations remained with shedding or seroconversion after adjustment for covariates. This finding supports previous research showing that withholding of breastfeeding was not an effective means of improving immune response to rotavirus vaccination [12]. Breastfeeding is still recommended as the best way to nourish infants.

We found moderate but inconsistent associations with sociodemographic factors. The heterogeneous performance of rotavirus vaccines across country income / child mortality strata has been well demonstrated in both clinical trials and observational studies [9, 32], but few data have been generated regarding any differences in performance across socioeconomic strata within a country. Unfortunately, given the high degree of correlation among race, education, insurance status, vaccine product, and breastfeeding practices in our study population, it is difficult to interpret our results, which may be affected by residual confounding or small strata. Future research in a larger population may facilitate more informative comparisons.

In our cohort, secretor infants were more likely to shed vaccine virus compared with non-secretor infants, although the relationship between secretor status and seroconversion was less pronounced. Secretor status has been shown to influence wild-type and vaccine-type rotavirus susceptibility (as measured via shedding) in a genotype-dependent manner, with secretor-positive infants usually found to be more susceptible to P[8] rotaviruses, although findings are inconsistent [33-39]. Secretor-positive infants have also been found to seroconvert more frequently following Rotarix or RotaTeq vaccination (both based on P[8] strains), when compared with secretor-negative infants [34, 40-42].

The impact of maternal secretor status is more complicated, since maternal and infant secretor status are correlated, and maternal secretor status also affects breastmilk composition. Secretor

mothers produce fucosylated oligosaccharides in breastmilk; these fucosylated oligosaccharides are structurally like glycoproteins found on the surfaces of infant epithelial cells and thus may act as decoy receptors for pathogens such as rotavirus [43]. We might hypothesize that breastfed infants of secretor mothers would be less likely to shed vaccine virus (and potentially less likely to seroconvert), compared with breastfed infants of non-secretor mothers. When we stratified by maternal secretor status, exclusive breastfeeding was negatively associated with shedding among infants with secretor mothers (though the effect did not reach significance), but null for infants with non-secretor mothers. Given the small sample sizes, this was not definitive, and it was not possible to stratify seroconversion models by breastfeeding status. To our knowledge, only one previous study has also assessed maternal and infant secretor status in combination. Williams et al. analyzed secretor status in a subset of Bangladeshi infants enrolled in a Rotarix vaccine trial [44] where they found maternal secretor status to be more influential than infant secretor status, in contrast with our findings. These discrepancies may be the result of differences in the populations (such as breastfeeding practices, economic status, or rotavirus exposure), small sample sizes among some strata, or other unmeasured factors. For instance, although maternal secretor status was not associated with infant pre-vaccination anti-rotavirus IgG in the Bangladeshi cohort, we found infant IgG to be significantly higher among those born to secretor-positive mothers in our cohort, even when adjusting for infant secretor status (data not shown), suggesting the influence of previous maternal rotavirus exposure on maternally derived IgG.

This study is one of few to comprehensively assess immune response to rotavirus vaccination among a cohort of healthy infants in a high-income country setting. Collection of weekly stool samples allowed a detailed description of shedding over time, including correlations with both maternal and infant factors. However, we note the following limitations. First, small sample sizes in some strata resulted in lack of precision for some estimates, particularly stratified analyses. Additionally, correlations between socioeconomic factors and which vaccine product was administered (due to formulary differences between public and private insurers) limited our ability to interpret differences by vaccine product or socioeconomic status. Further, for analyses of seroconversion, the second serum measurement was taken at 6 months of age, which is before the RotaTeq vaccine series would be complete; a post-RotaTeq-completion serum sample was not available. We did not, in this analysis, directly assess neutralizing antibody levels in maternal breastmilk, which may have helped provide context to our findings. Despite these limitations, our major findings were consistent across several sensitivity analyses, lending confidence to our results. While this analysis focused on the possible role of *FUT2* secretor status in infant immune response to rotavirus vaccine, *FUT3*, another gene that affects rotavirus susceptibility, was not assessed here [43, 45]. Although our cohort was similar demographically to the broader Cincinnati population, our findings may not be generalizable to the US population.

In summary, immune response to rotavirus vaccination was high among this cohort of US-based infants, with a high frequency of post-vaccination shedding across both RotaTeq and Rotarix vaccines and a post-dose-2 seroconversion rate of nearly 75%. Pre-vaccination IgG and maternal

and infant secretor status were associated with vaccine response, suggesting complicated inter-relationships among innate infant susceptibility to rotavirus mediated through transplacental maternal antibodies and possibly breastmilk (including the roles of neutralizing IgA as well as "decoy" fucosylated oligosaccharides). Additional research should consider the role of both infant and maternal secretor status, as well as include a comprehensive evaluation of breastfeeding practices.

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Author Contributions: RMB, DCP, ALM, and MAS conceptualized this study. RMB and SCC performed data curation. RMB performed formal analysis, software programming, and data visualization. ALM and MAS acquired financial support and oversaw the research and investigation process. MM, MCCM, SMR, RG, MDE, and MDB performed laboratory analyses and interpreted results. DCP, ALM, MAS, UDP, and JET provided oversight. RMB drafted the manuscript. All authors critically reviewed and edited the manuscript.

References

1. Collaborators GBDDD. Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Infect Dis **2017**; 17(9): 909-48.
2. Troeger C, Khalil IA, Rao PC, et al. Rotavirus Vaccination and the Global Burden of Rotavirus Diarrhea Among Children Younger Than 5 Years. JAMA Pediatr **2018**; 172(10): 958-65.
3. Collaborators GBDDD. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect Dis **2018**; 18(11): 1211-28.
4. Hallowell BD, Parashar UD, Curns A, DeGroote NP, Tate JE. Trends in the Laboratory Detection of Rotavirus Before and After Implementation of Routine Rotavirus Vaccination - United States, 2000-2018. MMWR Morb Mortal Wkly Rep **2019**; 68(24): 539-43.
5. Cortese MM, Parashar UD, CDC. Prevention of rotavirus gastroenteritis among infants and children: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep **2009**; 58(RR-2): 1-25.
6. Hill HA, Chen M, Elam-Evans LD, Yankey D, Singleton JA. Vaccination Coverage by Age 24 Months Among Children Born During 2018-2019 - National Immunization Survey-Child, United States, 2019-2021. MMWR Morb Mortal Wkly Rep **2023**; 72(2): 33-8.

7. Payne DC, Selvarangan R, Azimi PH, et al. Long-term Consistency in Rotavirus Vaccine Protection: RV5 and RV1 Vaccine Effectiveness in US Children, 2012-2013. Clin Infect Dis **2015**; 61(12): 1792-9.
8. Pindyck T, Tate JE, Parashar UD. A decade of experience with rotavirus vaccination in the United States - vaccine uptake, effectiveness, and impact. Expert Rev Vaccines **2018**; 17(7): 593-606.
9. Burnett E, Parashar UD, Tate JE. Real-world effectiveness of rotavirus vaccines, 2006-19: a literature review and meta-analysis. Lancet Glob Health **2020**; 8(9): e1195-e202.
10. Burke RM, Tate JE, Han GS, et al. Rotavirus Vaccination Coverage During a Rotavirus Outbreak Resulting in a Fatality at a Subacute Care Facility. J Pediatric Infect Dis Soc **2020**; 9(3): 287-92.
11. Burke RM, Groom HC, Naleway AL, et al. Rotavirus Vaccine Is Effective Against Rotavirus Gastroenteritis Resulting in Outpatient Care: Results From the Medically Attended Acute Gastroenteritis (MAAGE) Study. Clin Infect Dis **2021**; 72(11): 2000-5.
12. Velasquez DE, Parashar U, Jiang B. Decreased performance of live attenuated, oral rotavirus vaccines in low-income settings: causes and contributing factors. Expert Rev Vaccines **2018**; 17(2): 145-61.
13. Morrow AL, Staat MA, DeFranco EA, et al. Pediatric Respiratory and Enteric Virus Acquisition and Immunogenesis in US Mothers and Children Aged 0-2: PREVAIL Cohort Study. JMIR Res Protoc **2021**; 10(2): e22222.
14. Bernstein DI, Smith VE, Sherwood JR, et al. Safety and immunogenicity of live, attenuated human rotavirus vaccine 89-12. Vaccine **1998**; 16(4): 381-7.
15. Katz EM, Gautam R, Bowen MD. Evaluation of an Alternative Recombinant Thermostable Thermus thermophilus (rTth)-Based Real-Time Reverse Transcription-PCR Kit for Detection of Rotavirus A. J Clin Microbiol **2017**; 55(5): 1585-7.
16. Mijatovic-Rustempasic S, Tam KI, Kerin TK, et al. Sensitive and specific quantitative detection of rotavirus A by one-step real-time reverse transcription-PCR assay without antecedent double-stranded-RNA denaturation. J Clin Microbiol **2013**; 51(9): 3047-54.
17. Gautam R, Esona MD, Mijatovic-Rustempasic S, Ian Tam K, Gentsch JR, Bowen MD. Real-time RT-PCR assays to differentiate wild-type group A rotavirus strains from Rotarix((R)) and RotaTeq((R)) vaccine strains in stool samples. Hum Vaccin Immunother **2014**; 10(3): 767-77.
18. Gautam R, Mijatovic-Rustempasic S, Esona MD, Tam KI, Quaye O, Bowen MD. One-step multiplex real-time RT-PCR assay for detecting and genotyping wild-type group A rotavirus strains and vaccine strains (Rotarix(R) and RotaTeq(R)) in stool samples. PeerJ **2016**; 4: e1560.
19. Mijatovic-Rustempasic S, Esona MD, Williams AL, Bowen MD. Sensitive and specific nested PCR assay for detection of rotavirus A in samples with a low viral load. J Virol Methods **2016**; 236: 41-6.
20. Thorman AW, Adkins G, Conrey SC, et al. Gut Microbiome Composition and Metabolic Capacity Differ by FUT2 Secretor Status in Exclusively Breastfed Infants. Nutrients **2023**; 15(2).
21. Hsieh YC, Wu FT, Hsiung CA, Wu HS, Chang KY, Huang YC. Comparison of virus shedding after lived attenuated and pentavalent reassortant rotavirus vaccine. Vaccine **2014**; 32(10): 1199-204.
22. Ye S, Whiley DM, Ware RS, Kirkwood CD, Lambert SB, Grimwood K. Multivalent Rotavirus Vaccine and Wild-type Rotavirus Strain Shedding in Australian Infants: A Birth Cohort Study. Clin Infect Dis **2018**; 66(9): 1411-8.

23. Markkula J, Hemming-Harlo M, Vesikari T. Shedding of oral pentavalent bovine-human reassortant rotavirus vaccine indicates high uptake rate of vaccine and prominence of G-type G1. Vaccine **2020**; 38(6): 1378-83.
24. Parker EPK, Bronowski C, Sindhu KNC, et al. Impact of maternal antibodies and microbiota development on the immunogenicity of oral rotavirus vaccine in African, Indian, and European infants. Nat Commun **2021**; 12(1): 7288.
25. Lee B, Kader MA, Colgate ER, et al. Oral rotavirus vaccine shedding as a marker of mucosal immunity. Sci Rep **2021**; 11(1): 21760.
26. Lee B, Carmolli M, Dickson DM, et al. Rotavirus-Specific Immunoglobulin A Responses Are Impaired and Serve as a Suboptimal Correlate of Protection Among Infants in Bangladesh. Clin Infect Dis **2018**; 67(2): 186-92.
27. Becker-Dreps S, Vilchez S, Velasquez D, et al. Rotavirus-specific IgG antibodies from mothers' serum may inhibit infant immune responses to the pentavalent rotavirus vaccine. Pediatr Infect Dis J **2015**; 34(1): 115-6.
28. Moon SS, Groome MJ, Velasquez DE, et al. Prevaccination Rotavirus Serum IgG and IgA Are Associated With Lower Immunogenicity of Live, Oral Human Rotavirus Vaccine in South African Infants. Clin Infect Dis **2016**; 62(2): 157-65.
29. Isanaka S, Garba S, Plikaytis B, et al. Immunogenicity of an oral rotavirus vaccine administered with prenatal nutritional support in Niger: A cluster randomized clinical trial. PLoS Med **2021**; 18(8): e1003720.
30. Ali SA, Kazi AM, Cortese MM, et al. Impact of different dosing schedules on the immunogenicity of the human rotavirus vaccine in infants in Pakistan: a randomized trial. J Infect Dis **2014**; 210(11): 1772-9.
31. Payne DC, McNeal M, Staat MA, et al. Persistence of Maternal Anti-Rotavirus Immunoglobulin G in the Post-Rotavirus Vaccine Era. J Infect Dis **2021**; 224(1): 133-6.
32. Lamberti LM, Ashraf S, Walker CL, Black RE. A Systematic Review of the Effect of Rotavirus Vaccination on Diarrhea Outcomes Among Children Younger Than 5 Years. Pediatr Infect Dis J **2016**; 35(9): 992-8.
33. Payne DC, Currier RL, Staat MA, et al. Epidemiologic Association Between FUT2 Secretor Status and Severe Rotavirus Gastroenteritis in Children in the United States. JAMA Pediatr **2015**; 169(11): 1040-5.
34. Pollock L, Bennett A, Jere KC, et al. Nonsecretor Histo-blood Group Antigen Phenotype Is Associated With Reduced Risk of Clinical Rotavirus Vaccine Failure in Malawian Infants. Clin Infect Dis **2019**; 69(8): 1313-9.
35. Yang TA, Hou JY, Huang YC, Chen CJ. Genetic Susceptibility to Rotavirus Gastroenteritis and Vaccine Effectiveness in Taiwanese Children. Sci Rep **2017**; 7(1): 6412.
36. Loureiro Tonini MA, Pires Goncalves Barreira DM, Bueno de Freitas Santolin L, et al. FUT2, Secretor Status and FUT3 Polymorphisms of Children with Acute Diarrhea Infected with Rotavirus and Norovirus in Brazil. Viruses **2020**; 12(10).
37. Lee B, Dickson DM, deCamp AC, et al. Histo-Blood Group Antigen Phenotype Determines Susceptibility to Genotype-Specific Rotavirus Infections and Impacts Measures of Rotavirus Vaccine Efficacy. J Infect Dis **2018**; 217(9): 1399-407.
38. Bucardo F, Reyes Y, Ronnelid Y, et al. Histo-blood group antigens and rotavirus vaccine shedding in Nicaraguan infants. Sci Rep **2019**; 9(1): 10764.

39. Magwira CA, Kgosana LP, Esona MD, Seheri ML. Low fecal rotavirus vaccine virus shedding is significantly associated with non-secretor histo-blood group antigen phenotype among infants in northern Pretoria, South Africa. Vaccine **2020**; 38(52): 8260-3.
40. Kazi AM, Cortese MM, Yu Y, et al. Secretor and Salivary ABO Blood Group Antigen Status Predict Rotavirus Vaccine Take in Infants. J Infect Dis **2017**; 215(5): 786-9.
41. Armah GE, Cortese MM, Dennis FE, et al. Rotavirus Vaccine Take in Infants Is Associated With Secretor Status. J Infect Dis **2019**; 219(5): 746-9.
42. Bucardo F, Nordgren J, Reyes Y, Gonzalez F, Sharma S, Svensson L. The Lewis A phenotype is a restriction factor for Rotateq and Rotarix vaccine-take in Nicaraguan children. Sci Rep **2018**; 8(1): 1502.
43. Orczyk-Pawilowicz M, Lis-Kuberka J. The Impact of Dietary Fucosylated Oligosaccharides and Glycoproteins of Human Milk on Infant Well-Being. Nutrients **2020**; 12(4).
44. Williams FB, Kader A, Colgate ER, et al. Maternal Secretor Status Affects Oral Rotavirus Vaccine Response in Breastfed Infants in Bangladesh. J Infect Dis **2021**; 224(7): 1147-51.
45. Rodriguez-Diaz J, Garcia-Mantrana I, Vila-Vicent S, et al. Relevance of secretor status genotype and microbiota composition in susceptibility to rotavirus and norovirus infections in humans. Sci Rep **2017**; 7: 45559.

TABLES

Table 1: Demographics of rotavirus-vaccinated infants and their mothers enrolled in the PREVAIL (Pediatric Respiratory and Enteric Virus Acquisition and Immunogenesis Longitudinal) Cohort—Cincinnati, Ohio, 2017 – 2018 (N = 225)

	N(%) or mean (SD)
Demographics	
Maternal Education	
More than High School	125 (55.6%)
High School or Less	100 (44.4%)
Maternal Race	
Non-White	108 (48.0%)
White	117 (52.0%)
Feeding Practices at 1st Vaccination	
Exclusive Breastfeeding	60 (26.7%)
Mixed Breast- and Formula-feeding	60 (26.7%)
Formula-feeding only	105 (46.7%)
Birth Characteristics	
Delivery Type	
Caesarian	88 (39.1%)
Vaginal	137 (60.9%)
Gestational Weeks at Delivery	
36 - 37 Gestational weeks	45 (20.2%)
38 - 39 Gestational weeks	140 (62.8%)
40 - 41 Gestational weeks	38 (17.0%)
Birthweight (kg)	3.2 ± 0.5
Secretor Status	

	N(%) or mean (SD)
Infant and Mother Both Secretors	137 (69.5%)
Infant Secretor, Mother Non-Secretor	19 (9.6%)
Infant Non-Secretor, Mother Secretor	21 (10.7%)
Infant and Mother Both Non-Secretors	20 (10.2%)
Previous Rotavirus Exposure	
Rotavirus detection before 1st recorded dose of vaccine	16 (7.1%)
IgG titer at 6 weeks of age (GMT)	127 ± 3
Vaccination Characteristics	
Final Vaccination Status	
Fully vaccinated	199 (88.4%)
Partially vaccinated	26 (11.6%)
Product for 1st Dose	
Rotarix	101 (45.3%)
RotaTeq	122 (54.7%)
Age at 1st dose of rotavirus vaccine (days)	67 ± 17
Outcomes (among those with data)	
Shedding	
Shed in at least one sample taken 4 – 28 days post-dose 1	147/188 (78.2%)
Shedding among samples taken 4 – 28 days post-dose 1	300/583 (51.5%)
Seroconversion	105/141 (74.5%)

Table 2: Crude and adjusted odds ratios for vaccine virus shedding 4 – 28 days following the first dose of rotavirus vaccination among infants enrolled in the PREVAIL (Pediatric Respiratory and Enteric Virus Acquisition and Immunogenesis Longitudinal) Cohort—Cincinnati, Ohio, 2017 – 2018. (N = 567 stools from 181 children)

	Crude			Adjusted		
	OR*	**95% CI**	**P value****	**OR***	**95% CI**	**P value****
Demographics						
Maternal Education: High School or less (vs. more)	2.00	(1.26, 3.18)	0.003	2.20	(0.99, 4.85)	0.052
Maternal White Race (vs. all others)	0.74	(0.47, 1.14)	0.17	1.89	(0.88, 4.06)	0.10
Feeding Practices						
Exclusively Breastfeeding (vs. not exclusively breastfeeding***)	0.64	(0.41, 1.00)	0.052	0.68	(0.39, 1.19)	0.18
Birth Characteristics						
Vaginal Delivery (vs. Caesarian)	0.95	(0.61, 1.47)	0.83	0.92	(0.54, 1.57)	0.75
40 – 41 Gestational weeks (vs. 36 – 37 weeks)**	0.42	(0.21, 0.84)	0.023	0.37	(0.15, 0.92)	0.019
38 – 39 Gestational weeks (vs. 36 – 37 weeks)**	0.87	(0.50, 1.50)	-	0.95	(0.44, 2.04)	-
Birthweight (500g increase)	0.89	(0.70, 1.11)	0.30	1.08	(0.79, 1.48)	0.63
Secretor Status						
Both Non-Secretors (vs. all other combinations)	0.50	(0.25, 1.03)	0.059	0.37	(0.16, 0.83)	0.016
Previous Rotavirus Exposure						
Previous Infant Rotavirus Infection	1.52	(0.67, 3.49)	0.32	1.28	(0.44, 3.74)	0.65
IgG at 6 weeks of age (100-unit increase) ****	0.87	(0.80, 0.96)	0.004	0.84	(0.75, 0.94)	0.002
Vaccination Characteristics						
Product (RotaTeq vs. Rotarix)	0.59	(0.37, 0.93)	0.023	0.81	(0.36, 1.81)	0.60
Days Since Vaccination (1-day increase)	0.90	(0.88, 0.92)	< 0.0001	0.89	(0.87, 0.91)	< 0.0001

*GEE logistic regression, clustered by infant. **Wald Chi Square, Robust SE. Generalized Wald presented for gestational weeks.

***Mixed or formula feeding. ****Imputed from maternal and cord blood IgG for 16 infants missing a week-6 blood draw.

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Table 3: Adjusted odds ratios for rotavirus vaccine virus shedding 4 – 28 days following the first dose of vaccination, stratified by exclusive breastfeeding at time of first vaccination, among infants enrolled in the PREVAIL (Pediatric Respiratory and Enteric Virus Acquisition and Immunogenesis Longitudinal) Cohort—Cincinnati, Ohio, 2017 – 2018.

	Exclusively breastfed infants (N = 179 stools, 57 infants)			**Non-exclusively breastfed infants (N = 392 stools, 127 infants)**		
	OR*	**95% CI**	**P value****	**OR***	**95% CI**	**P value****
Demographics						
Maternal White Race (vs. all others)	0.67	(0.23, 1.98)	0.47	0.89	(0.48, 1.64)	0.71
Secretor Status						
Both Non-Secretors (vs. all other combinations)	0.31	(0.10, 0.98)	0.046	0.51	(0.19, 1.36)	0.18
Previous Rotavirus Exposure						
Week-6 IgG (100-unit increase)	0.75	(0.63, 0.91)	0.003	0.90	(0.79, 1.04)	0.16
Vaccination Characteristics						
Days Since Vaccination (1-day increase)	0.88	(0.83, 0.93)	< 0.0001	0.90	(0.88, 0.93)	< 0.0001

*GEE logistic regression, clustered by infant. **Wald Chi Square, Robust SE.

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Table 4: Crude and adjusted odds ratios for rotavirus seroconversion at 6 months of age, following 2 doses of rotavirus vaccine, among infants enrolled in the PREVAIL (Pediatric Respiratory and Enteric Virus Acquisition and Immunogenesis Longitudinal) Cohort—Cincinnati, Ohio, 2017 – 2018. (N = 134)

	Crude			Adjusted		
	OR*	**95% CI**	**P value****	**OR***	**95% CI**	**P value****
Demographics						
Maternal Education: High School or less (vs. more)	2.87	(1.21, 7.67)	0.023	1.27	(0.28, 5.88)	0.76
Maternal White Race (vs. all others)	0.25	(0.09, 0.58)	0.003	0.31	(0.04, 1.75)	0.21
Feeding Practices						
Exclusively Breastfed at Dose 1 (vs. not exclusively breastfed***)	0.35	(0.16, 0.76)	0.008	0.47	(0.18, 1.22)	0.12
Birth Characteristics						
40 - 41 Gestational weeks (vs. 36 – 37 weeks)	0.25	(0.07, 0.83)	0.029	0.38	(0.09, 1.47)	0.35
38 - 39 Gestational weeks (vs. 36 – 37 weeks)	0.70	(0.21, 1.95)	0.52	0.99	(0.28, 3.14)	-
Secretor Status						
Both Non-Secretors (vs. all other combinations)	0.44	(0.14, 1.45)	0.16	0.24	(0.06, 0.90)	0.033
Previous Rotavirus Exposure						
6-week IgG (100-unit increase)	0.87	(0.76, 1.00)	0.043	0.84	(0.72, 0.97)	0.021
Vaccination Characteristics						
Product (RotaTeq vs. Rotarix)	0.30	(0.11, 0.71)	0.01	2.21	(0.33, 17.53)	0.43
Infant Age at Vaccination (1-week increase)	0.85	(0.61, 1.19)	0.35	0.80	(0.51, 1.24)	0.31

*Logistic regression. **Wald Chi Square. Likelihood ratio test presented for gestational age. ***Mixed or formula fed.

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FIGURE

Figure Legend: Percent of infants (N=141) enrolled in the PREVAIL (Pediatric Respiratory and Enteric Virus Acquisition and Immunogenesis Longitudinal) Cohort (Cincinnati, Ohio, 2017 – 2018) exhibiting vaccine virus shedding 4 – 28 days following the first dose (dark bars) or seroconversion (3-fold rise in IgA) following the second dose (light bars) of rotavirus vaccine, stratified by infant secretor status (A), maternal secretor status (B), or infant-maternal secretor status (C), among those with seroconversion data. Non-secretors were defined as individuals with a 428 G>A point mutation in both *FUT2* alleles (AA). Secretors were defined as individuals with GG or GA genotypes.