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Effect of early life antibiotic use on serologic responses to oral rotavirus vaccine in the MAL-ED birth cohort study

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ABSTRACT

Background: Oral rotavirus vaccine efficacy is lower in low- and middle-income countries (LMICs) than in high-income countries. The degree to which antibiotic use impacts rotavirus vaccine immunogenicity in LMICs is unknown. Using data from a multisite prospective birth cohort study of malnutrition and enteric disease, MAL-ED, we examined the effect of early life antibiotic use on the immune response to rotavirus vaccine.

Methods: We assessed whether antibiotic use from birth up to 7 days following rotavirus vaccine series completion was associated with rotavirus seropositivity at 7 months of age in Brazil, Peru, and South Africa using a modified Poisson regression. We then used parametric g-computation to estimate the impact of hypothetical interventions that treated all children and alternatively prevented inappropriate antibiotic treatments on seropositivity.

Results: Of 537 children, 178 (33%) received at least one antibiotic course during the exposure window. Probability of seropositivity was 40% higher among children who had at least one course of antibiotics compared with those with no antibiotic exposure (PR: 1.40, 95% CI: 1.04, 1.89). There was no significant difference by the number of antibiotic courses received or total duration of antibiotics. Treating all children with antibiotics would be associated with a 19% (95% CI: 18%, 21%) absolute increase in seropositivity at 7 months. In contrast, removing inappropriate antibiotics would result in a 4% absolute reduction (95% CI: −5%, −2%) in seropositivity.

Conclusions: Early life antibiotic use was associated with increased seropositivity. However, a hypothetical intervention to remove inappropriate antibiotics would have little effect on overall seropositivity. Further investigation into the underlying mechanisms of antibiotic use on the infant gut microbiome and immune response are needed.

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1. Introduction

Although the burden of rotavirus has steadily declined over the past decade, rotavirus continues to be a leading cause of diarrhea among children younger than five years of age. According to the Global Burden of Diseases, Injuries, and Risk Factors Study, rotavirus was the leading cause of diarrhea-associated illness, accounting for 258 million diarrhea episodes, 1.5 million hospitalizations, and 128,500 deaths in 2016, with low- and middle-income countries (LMICs) experiencing disproportionate amounts of the world's rotavirus burden compared to high-income countries (HICs) [1–3]. The introduction and expanded use of oral rotavirus vaccines has contributed greatly to the reduction in rotavirus burden. However, rotavirus vaccine efficacy is greater in HICs compared to LMICs, while the disease burden is greater in the latter [4].

A proposed biological mechanism for the variability in oral rotavirus vaccine response is the composition of the gut microbiome, which is important for infant immune development and can be altered by antibiotic use [6–9]. Drug-related factors such as antibiotic class, timing of exposure, and route of administration have been shown to influence patterns of microbiota alteration, or dys-

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biosis, because of their different spectrum and bacterial targets [6,6,10]. The potential impact of antibiotics on early development of the microbiota and immunity is particularly relevant among children in LMICs because antibiotic use in these settings is highly common even in the first months of life [11]. More frequent antibiotic use can be explained by a wide range of factors, including a higher burden of disease, higher over-the-counter access [12,13], varying influence of caregiver and clinician preferences [14,15], and the limited availability of sensitive diagnostics to distinguish between viral and bacterial infections [16].

To date, studies of the effect of antibiotic use on vaccine response have focused on populations in HICs, despite the fact that the vaccine performs well in these settings [17,18]. Given the lack of studies in LMICs and the high use of antibiotics in early life, it is important to understand any potential unintended consequences of antibiotic administration that may affect the management of other communicable diseases in these settings. The objective of this analysis was to determine whether antibiotic use by children prior to completion of the rotavirus vaccine series was associated with vaccine response in three sites of the MAL-ED birth cohort study where rotavirus vaccines were part of the national immunization schedule [19]. We also estimated the expected effect of hypothetical mass antibiotic administration interventions as well as interventions to reduce inappropriate antibiotic use on rotavirus vaccine response in these LMIC settings.

2. Methods

2.1. Study population

The MAL-ED study design, descriptions of sites, surveillance, and microbiologic methods have been described previously in detail [19]. In summary, healthy infants from Dhaka (Bangladesh), Fortaleza (Brazil), Vellore (India), Bhaktapur (Nepal), Naushahro Feroze (Pakistan), Loreto (Peru), Venda (South Africa), and Haydom (United Republic of Tanzania) were enrolled within 17 days of birth between November 2009 and February 2012 and followed until 24 months of age – through February 2014. For this study, we included participants enrolled in 3 sites (Brazil, Peru, and South Africa) where the Rotarix® (GlaxoSmithKline, Rixensart, Belgium; RV1) vaccine was introduced nationally prior to the start of the study. The RV1 schedule was 6 and 14 weeks in South Africa compared to 8 and 16 weeks in Peru and Brazil. The original study was approved by local institutional review boards and ethical approval for our analysis was obtained through the University of North Carolina at Chapel Hill Institutional Review Board (IRB #: 20-1672).

2.2. Gastroenteritis and antibiotic use surveillance

Surveillance for illnesses and antibiotic use was conducted during twice weekly home visits by fieldworkers throughout the study period. Caregivers were asked a standardized questionnaire to assess symptoms of cough, fever, vomiting, diarrhea, and medication use. Stool samples were collected during diarrheal episodes (defined as $\geq$ 3 loose stools in a 24-hour period) and during routine monthly home visits [20]. All stool samples were preserved, transported, and then tested for rotavirus by enzyme immunoassay (EIA) using ProSpecT kits (Oxoid Ltd, Ely, United Kingdom) [21]. A subset of children with complete follow-up were assessed for rotavirus by reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR) using custom-designed TaqMan Array Cards (Thermo Fisher Scientific, Carlsbad, CA) and previously described methods [22].

Rotavirus diarrhea prior to vaccination completion was detected among diarrheal stools at an RT-qPCR [22,28] cycle threshold value < 35. If RT-qPCR data were unavailable, rotavirus was identified by EIA [21].

2.3. Data and definitions

Household demographics, maternal characteristics, and anthropometric measurements were collected at enrollment. Household socioeconomic status (SES) was assessed at 6, 12, 18, and 24 months using an index validated by the study team based on water and sanitation, eight household assets, maternal education, and monthly household income (WAMI) [23]. Anthropometric measurements and vaccination history were collected monthly.

The primary exposure was caregiver-reported oral or injected antibiotic treatment administered for any reason prior to or up to 7 days after the second dose of RV1, regardless of completion of the antibiotic course. Antibiotic name and class were verified by medication packaging or paperwork from a healthcare provider, and distinct antibiotic courses were separated by at least two antibiotic-free days [24]. We used a binary classification of antibiotic exposure, comparing at least one antibiotic course received to no courses received. Antibiotic exposures were categorized according to antibiotic class; if more than one antibiotic class was prescribed, then the exposure was attributed to more than one antibiotic class. We also measured the duration of antibiotic exposure as the total number of days during which antibiotics were received and the total number of antibiotic courses during the same exposure period.

We characterized inappropriate antibiotic use based on illness surveillance. If antibiotics were taken during any day of the illness, the illness was classified as treated with antibiotics; illnesses were separated by at least two symptom-free days. Illness definitions were based on the Integrated Management of Childhood Illness guidelines, as previously described [24,25]. Antibiotics taken for diarrhea without a caregiver report of at least one loose stool with visible blood (i.e., non-bloody diarrhea) were characterized as "inappropriate". Respiratory illness was defined as cough or shortness of breath. Acute lower respiratory tract illness was defined as cough or shortness of breath with a rapid respiratory rate determined by fieldworkers (defined by the average of two measurements per day that were: > 60 breaths per minute for infants < 2 months old; > 50 breaths per minute for ages 2 months to 1 year; and > 40 breaths per minute for age $\geq$ 1 year). Respiratory illnesses that did not meet the criteria for acute lower respiratory infections were considered upper respiratory infections (URIs). Antibiotics taken for URIs or vomiting only (i.e., vomiting not accompanied by diarrhea) were classified as "likely inappropriate". All other antibiotic use was considered appropriate.

We defined seropositivity as rotavirus-specific immunoglobulin A (IgA) antibody titer $\geq$ 20 U/mL, which has been established as a useful correlate of protection for rotavirus vaccines by Cheuvart et al. and Baker et al. [26,27]. IgA antibodies against rotavirus were measured by enzyme-linked immunosorbent assay (ELISA) of blood samples collected at 7 months of age from each child and stored at −20 °C.

2.4. Statistical analysis

Because the proportion of missing data for baseline covariates was 5% or less for all variables, we imputed the mean values of variables by site for individuals with missing data. We used modified Poisson regression with robust standard errors [29] to compare the prevalence of seropositivity at 7 months between those with and without antibiotic exposure up to 7 days after RV1 vaccination completion. We present comparisons in the form of crude (i.e., adjusted by site only) and adjusted prevalence ratios (PRs) and 95% confidence intervals (CIs). The following confounding vari-

ables were identified for inclusion in our analysis using a causal directed acyclic graph based on the substantive literature [30] (Supplementary Fig. 1): child sex, child age (in weeks) at vaccine completion, low birthweight (defined as birthweight < 2.5 kg), SES based on the WAMI Index [23], maternal education, maternal age, improved water and sanitation according to World Health Organization (WHO) definitions [31], number of diarrhea episodes prior to vaccine series completion, total number of days with diarrhea prior to vaccine series completion, presence of dehydration during diarrhea prior to vaccine series completion, underweight status (average weight-for-age Z score < −2 standard deviations (SD) from the WHO Child Growth Standards median [32]), and stunting status (average height-for-age Z score < −2 SD). We explored modeling continuous covariates as categorical, linear, quadratic, and restricted cubic splines, and optimal coding was determined by likelihood ratio tests at a significance level of 0.1 and Akaike's information criterion.

To estimate the impact of interventions to prevent antibiotic use, we used parametric g-computation to estimate counterfactual scenarios of antibiotic use [33,34]. We considered the following three interventions: treating all children with antibiotics, preventing all inappropriate antibiotic use within 7 days after vaccine completion, and additionally preventing likely inappropriate antibiotic courses within 7 days after vaccine completion (Table 1). We constructed 95% CIs by bootstrap with 1,000 replicates. We also estimated the number needed to treat (NNT), or the number of children who would need to receive the specified intervention prior to completing the vaccination series to observe a one-person change in RV1 seropositivity over 7 months.

In sensitivity analyses we re-estimated the PRs at 7 months under three different scenarios. First, we excluded children with natural rotavirus infections up to 7 days after RV1 vaccination completion from the analysis. In a second analysis, we recoded natural rotavirus infections as competing events (i.e., categorized children with natural rotavirus infections as seronegative at 7 months). Finally, given that a majority (80%) of antibiotic exposures occurred in Peru, we estimated the association between antibiotic use and seropositivity at that site only.

Table 1
Exposure contrasts for each hypothetical intervention.

Contrast	Referent	Index
Inappropriate antibiotics prevented	The observed exposure distribution among all children	The counterfactual exposure distribution after all inappropriate antibiotics are removed.
Likely inappropriate antibiotics also prevented	The observed exposure distribution among all children	The counterfactual exposure distribution after all inappropriate and likely inappropriate antibiotics are removed.
ass drug administration (MDA)	The observed exposure distribution among all children	The counterfactual exposure distribution had all children been treated with antibiotics.

All statistical analysis was performed in R statistical software, version 4.1.0 [35].

3. Results

We included 537 of 850 children (63%) enrolled in the Brazil, Peru, and South Africa sites who received both doses of RV1 and had blood samples collected at 7 months of age. Of the 313 children who were excluded from analyses, 135 (43%) infants dropped out prior to blood collection, 81 (26%) were missing a 7-month blood sample collection, 60 (19%) received only one RV1 dose, 16 (5%) were unvaccinated, 16 (5%) had blood samples collected but not assessed for rotavirus-specific IgA, and 5 (2%) had blood samples collected prior to completing the vaccine series. Most children (n = 237; 44%) were from Peru, compared to 35% from South Africa (n = 186) and 21% from Brazil (n = 114; Table 2).

The median time between birth and completion of the vaccination series was 19 weeks (interquartile range [IQR]: 17, 20).

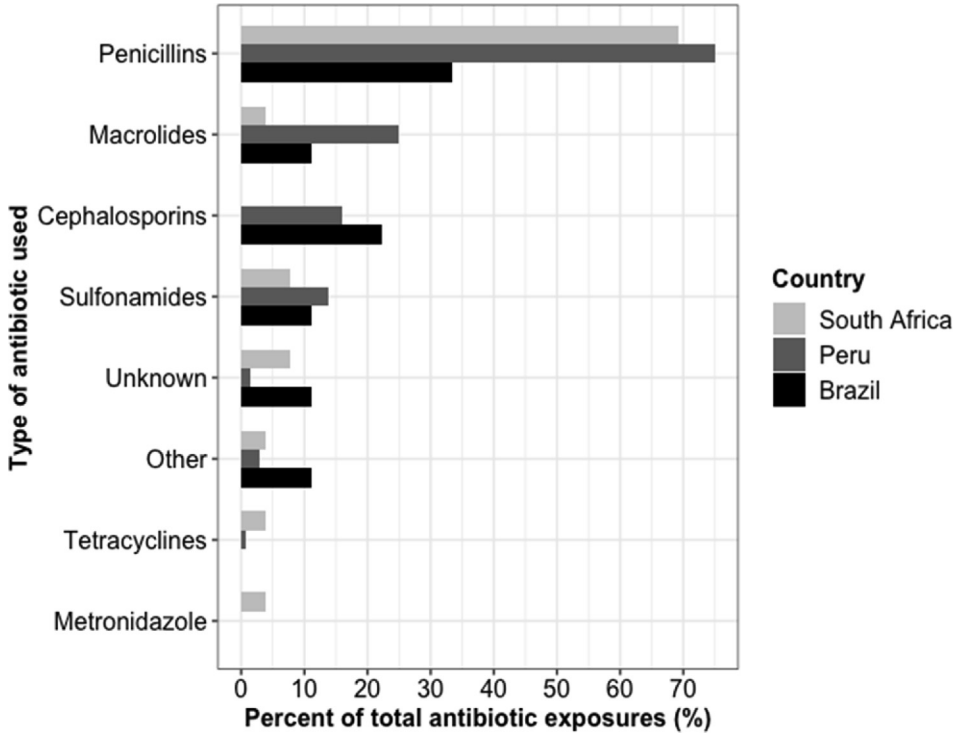

Fig. 1. Type of antibiotic used by site (n = 300 courses) up to 7 days after vaccine completion.

Table 2
Demographic characteristics of 537 children in the MAL-ED study with both RV1 vaccine doses.

Characteristic	No antibiotics up to 7 days after last vaccine dose (n = 359)	Antibiotics up to 7 days after last vaccine dose (n = 178)	Total (n = 537)
	N (%) or median [IQR]	N (%) or median [IQR]	N (%) or median [IQR]
Site			
Fortaleza, Brazil	105 (29.2)	9 (5.1)	114 (21.2)
Loreto, Peru	93 (25.9)	144 (80.9)	237 (44.1)
Venda, South Africa	161 (44.8)	25 (14.0)	186 (34.6)
Socioeconomic Characteristics			
Maternal age (in years)			
<20	71 (19.8)	46 (25.8)	117 (21.8)
20–25	82 (22.8)	43 (24.2)	125 (23.3)
26–30	80 (22.3)	43 (24.2)	123 (22.9)
31–35	60 (16.7)	23 (12.9)	83 (15.5)
36–40	28 (7.8)	7 (3.9)	35 (6.5)
>40	38 (10.6)	16 (9.0)	54 (10.1)
Maternal education			
No education	2 (0.6)	1 (0.6)	3 (0.6)
Primary education (1–8 years)	109 (30.4)	92 (51.7)	201 (37.4)
Secondary or higher (≥9 years)	248 (69.1)	85 (47.8)	333 (62.0)
WAMI Index*	0.75 [0.59, 0.86]	0.59 [0.48, 0.70]	0.70 [0.55, 0.81]
Crowding (≥2.5 people/ bedroom)	37 (10.3)	29 (16.3)	66 (12.3)
Monthly income in USD	195.3 [108.2, 351.7]	146.6 [108.1, 182.4]	168.9 [108.1, 307.8]
≥ 5 priority assets†	287 (79.9)	120 (67.4)	407 (75.8)
Child characteristics			
Male sex	188 (52.4)	99 (55.1)	286 (53.3)
Age (in weeks) at vaccine completion	19.0 [16.0, 20.0]	19.0 [19.0, 20.0]	19.0 [17.0, 20.0]
Low birthweight	25 (7.0)	11 (6.2)	36 (6.7)
Underweight in the first 3 months	11 (3.1)	6 (3.4)	17 (3.2)
Stunting in the first 3 months	54 (15.0)	34 (19.1)	88 (16.4)
Wasting in the first 3 months	1 (0.3)	0 (0)	1 (0.2)
Water, Sanitation & Hygiene			
Improved sanitation‡ *(2 missing)*	283 (79.3)	70 (39.3)	353 (65.0)
Improved drinking water§ *(3 missing)*	329 (92.4)	163 (91.6)	492 (92.0)
Antibiotics			
Age at first antibiotic exposure			
None	–	0 (0.0)	0 (0.0)
<6 months	–	176 (98.9)	176 (98.9)
6 months – 1 year	–	2 (1.1)	2 (1.1)
>1 year	–	0 (0.0)	0 (0.0)
Total antibiotic courses			
1	–	103 (57.9)	103 (57.9)
2+	–	75 (42.1)	75 (42.1)
Antibiotic class			
Penicillins	–	128 (71.9)	128 (71.9)
Cephalosporins	–	25 (14.0)	25 (14.0)
Sulfonamides	–	23 (12.9)	23 (12.9)
Macrolides	–	38 (21.3)	38 (21.3)
Tetracyclines	–	2 (1.1)	2 (1.1)
Metronidazole	–	1 (0.6)	1 (0.6)
Other	–	6 (3.4)	6 (3.4)
Unknown	–	5 (2.8)	5 (2.8)
Rotavirus			
Age at first rotavirus episode			
None	240 (66.9)	83 (46.6)	323 (60.1)
<6 months	25 (7.0)	23 (12.9)	48 (8.9)
6 months – 1 year	50 (13.9)	29 (16.3)	79 (14.7)
>1 year	44 (12.3)	43 (24.2)	87 (16.2)
Rotavirus infection up to 7 days after 2nd vaccine dose	24 (6.7)	21 (11.8)	45 (8.4)
Rotavirus-specific IgA titer (U/mL)	5.0 [1.0, 25.0]	6.0 [1.0, 33.8]	5.0 [1.0, 32.0]
Seropositive (rotavirus-specific IgA ≥ 20 U/mL)	102 (28.4)	59 (33.1)	161 (30.0)

* WAMI Index = improved Water and sanitation, eight selected Assets, Maternal education, and household Income.
† Assets measured were mattress, chair, table, TV, refrigerator, bank account, kitchen, < 2 people per room.
‡ Improved sanitation, based on WHO definitions, includes sanitation facilities that hygienically separate human excreta from human contact, such as those with sewer connections, septic system connections, pour-flush latrines, and pit latrines with a covered pit.
§ Improved drinking water, based on WHO definitions, is a source that adequately protects water from outside contamination and fecal matter by nature of its construction. Examples include piped household water, public standpipe, borehole, and protected spring.

Approximately one-third of children (n = 178, 33%) received at least one antibiotic course up to 7 days after their final vaccine dose. Among children with antibiotic exposures, 42% (n = 75) received two or more antibiotic courses. Of those, 60% (n = 45) received multiple antibiotic classes; 5 children received three different antibiotic classes. The most common antibiotic class pre-

scribed across all three sites was penicillins, accounting for 72% (n = 128) of caregiver-reported antibiotics (Fig. 1). Roughly one quarter (26%, n = 46) of children received antibiotics inappropriately, and an additional 17% (n = 30) of children had likely inappropriate antibiotic exposures. Nine percent (n = 17) of children had both inappropriate and likely inappropriate antibiotic exposures (Fig. 2).

Overall, the prevalence of seropositivity at 7 months was 30%. The crude association between antibiotic exposure up to 7 days following vaccine completion and 7-month RV1 seropositivity was small (Table 3). Seropositivity was higher among children exposed to antibiotics within 7 days of vaccine completion (n = 59, 33%) compared to those unexposed to antibiotics (n = 103, 28%). Despite the fact that a higher number of children receiving antibiotics were seropositive, the overall median IgA titer among children without antibiotic exposure (median: 5.0, IQR: 1.0, 15.0) was similar to those with antibiotic exposure (median: 6.0, IQR: 1.0, 33.8). After multivariable adjustment, the prevalence of seropositivity was 40% higher among children who had at least one course of antibiotics up to 7 days following vaccine completion compared with those who did not (Adjusted PR: 1.40, 95% CI: 1.04, 1.89). There was no significant difference in the association by the number of antibiotic courses received nor total duration of antibiotics. There were also no differences in prevalence of seropositivity based on antibiotic class.

We estimated that seropositivity at 7 months would have been 19 percentage points higher had all participants received antibiotics compared to the observed exposure distribution (prevalence difference [PD]: 0.19, 95% CI: 0.18, 0.21; Table 4). Exposing six children to antibiotics before vaccination completion would be expected to result in one additional child who was RV1 seropositive at 7 months (NNT: 6, 95% CI: 5, 6). The prevention of inappropriate antibiotics, or antibiotic treatment for non-bloody diarrhea, would result in a 2 percentage point reduction in seropositivity from the observed seroprevalence (PD: −0.02, 95% CI: −0.03, −0.01). Preventing likely inappropriate antibiotics, or antibiotics taken for URI and vomiting only, in addition to antibiotics taken for non-bloody diarrhea would result in a 4 percentage point reduction in 7-month seropositivity compared to seroprevalence under the observed antibiotic exposure distribution (PD: −0.04, 95% CI: −0.05, −0.02).

3.1. Sensitivity analyses

Excluding the 45 (8%) children with natural rotavirus infections up to 7 days after the second vaccine dose from the analysis, the prevalence of seropositivity was 41% higher among children who had at least one course of antibiotics compared with those who did not (Adjusted PR: 1.41, 95% CI: 1.02, 1.97; Supplementary Table 1). Though similar to the estimate in the main analysis, this estimate was slightly less precise. Considering natural rotavirus infection as a competing risk further increased the strength of the association between antibiotic use and prevalence of seropositivity among children who had at least one course of antibiotics (Adjusted PR: 1.52, 95% CI: 1.09, 2.13). Within Peru only, where 80% of antibiotic exposures occurred, the prevalence of seropositivity was 28% higher among children who had received at least one course of antibiotics (Adjusted PR: 1.28, 95% CI: 0.99, 2.89).

3.2. Discussion

Our study is one of the first to find that antibiotic treatment early in life among children living in LMICs may be associated with increased RV1 immunogenicity. Results did not differ based on the duration of antibiotic treatment or antibiotic class. Based on these results, further exploration of the relationship between antibiotic use, the gut microbiome, and rotavirus vaccine response is warranted.

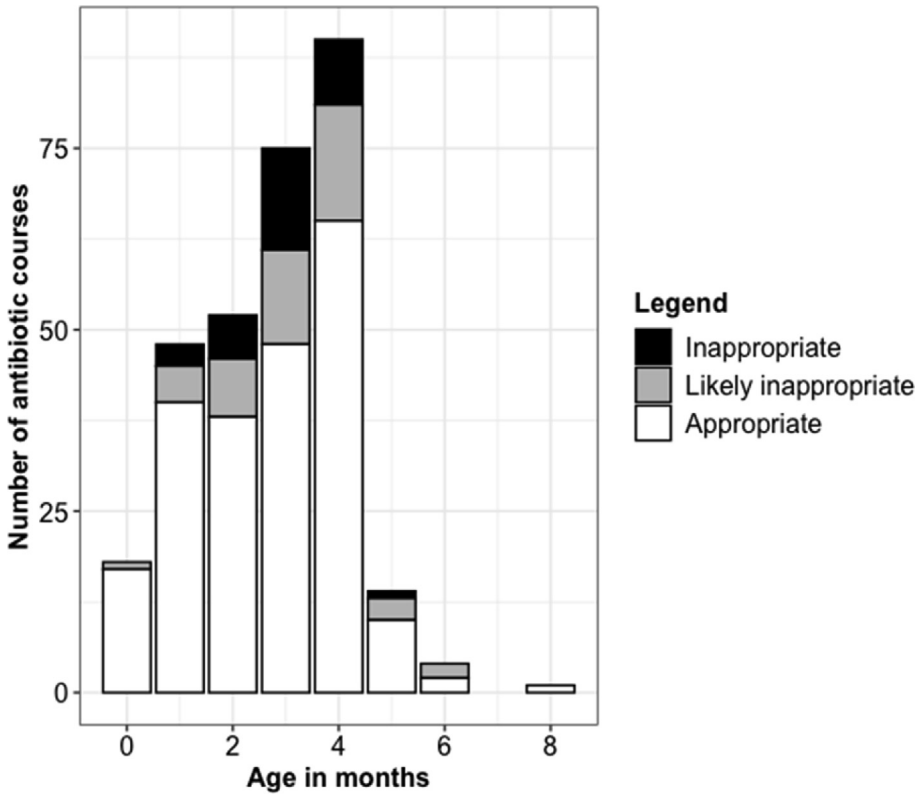

Fig. 2. Appropriateness of antibiotic courses received up to 7 days after vaccine completion by age among 178 children who received at least one course of antibiotics.

Table 3
Association between antibiotic exposure up to 7 days after vaccination and RV1 seropositivity at 7 months among 537 children in the MAL-ED study.

‘		Number of children	Prevalence of seropositivity	Prevalence ratio (95% CI)	
				Crude*	Adjusted†
Antibiotics up to 7 days after vaccination	No	359	0.28	1.	1.
	Yes	178	0.33	1.22 (0.90, 1.65)	1.40 (1.04, 1.89)
Number of antibiotic courses	0	359	0.28	1.	1.
	1	103	0.34	1.20 (0.86, 1.69)	1.40 (1.01, 1.95)
	2	75	0.32	1.25 (0.82, 1.90)	1.39 (0.88, 2.19)
Total days of antibiotics	0	359	0.28	1.	1.
	1–7 days	97	0.34	1.24 (0.88, 1.76)	1.46 (1.04, 2.05)
	> 7 days	81	0.32	1.18 (0.80, 1.76)	1.31 (0.86, 1.68)
Penicillins	No antibiotics	359	0.28	1.	1.
	Any penicillins‡	128	0.36	1.18 (0.84, 1.65)	1.29 (0.92, 1.80)
	Non-penicillin antibiotics	50	0.32	1.31 (0.86, 2.00)	1.73 (1.10, 2.72)
Macrolides	No antibiotics	359	0.28	1.	1.
	Any macrolides	38	0.32	1.17 (0.69, 1.99)	1.47 (0.83, 2.62)
	Non-macrolide antibiotics	140	0.34	1.23 (0.90, 1.68)	1.39 (1.02, 1.90)
Cephalosporins	No antibiotics	359	0.28	1.	1.
	Any cephalosporins	25	0.33	1.18 (0.63, 2.20)	1.42 (0.76, 2.65)
	Non-cephalosporin antibiotics	153	0.32	1.22 (0.89, 1.67)	1.40 (1.02, 1.91)
Sulfonamides	No antibiotics	359	0.28	1.	1.
	Any sulfonamides	23	0.33	1.28 (0.70, 2.36)	1.60 (0.78, 3.29)
	Non-sulfonamide antibiotics	155	0.35	1.21 (0.88, 1.65)	1.38 (1.01, 1.89)

* Crude prevalence ratio adjusted for site
† Prevalence ratio adjusted for covariates described in the Methods section
‡ ‘Any’ of a drug class combines that class only and multiple classes including that class

Table 4
Estimated population-level impact of potential interventions to vary antibiotic exposure on 7-month RV1 seropositivity among 537 children in the MAL-ED birth cohort.

Contrast	# Exposed to antibiotics	Prevalence of seropositivity	PD* (95% CI)	NNT (95% CI)
Removal of inappropriate antibiotics				
Observed	178	0.49	0	
Inappropriate antibiotics removed	132	0.46	−0.02 (−0.03, −0.01)	45 (31, 85)
Likely inappropriate antibiotics also removed	102	0.45	−0.04 (−0.05, −0.02)	29 (22, 42)
MDA				
Observed	178	0.49	0	
All exposed	537	0.68	0.19 (0.18, 0.21)	6 (5, 6)

Abbreviations: MDA, mass drug administration; NNT, number needed to treat; PD, prevalence differenec
* PD adjusted for covariates described in the Methods section

Although we hypothesized that antibiotics might disrupt the gut microbiome and blunt the immune response to the rotavirus vaccine, we did not observe negative impacts of antibiotics on seropositivity following rotavirus vaccination. In fact, antibiotics may have improved the response to the live oral vaccine. Our findings of an improved immune response to the rotavirus vaccine after antibiotic exposure are similar to what was found in a mouse model by Uchiyama et al. [36]. In contrast, a recent analysis of data from a randomized controlled study of healthy infants in the United States receiving RotaTeq® (Merck and Co, Westpoint, Pennsylvania) and RV1 did not identify any effect of antibiotic use on serologic response to oral rotavirus vaccine [18]. No other clinical studies have demonstrated a positive effect of antibiotic use on oral rotavirus immunogenicity [17]. However, there are other potential impacts of early life antibiotic use on the gut microbiome and child health that were not measured in our study.

These findings suggest that antibiotic receipt does modulate the enteric immune response, though the mechanisms of action are not fully understood. A proposed explanation for our results, based on a recent study by Gozalbo-Rovira et al., is the presence of histo-blood group antigen (HBGA)-like “decoys” on the surface of intestinal bacteria [37]. The bacterial HBGAs could serve as decoy receptors preventing rotavirus or the attenuated vaccine strain from binding to HBGAs on the intestinal epithelial cells; if the intestinal bacteria are not present, the virus is unable to bind and replicate [36,38].

The varied diversity and composition of the gut microbiome between children in LMICs and HICs has been suggested as a potential explanation for observed differences in oral rotavirus vaccine performance [39–41]. In high pathogen exposure settings, antibiotic use may decrease dysbiosis of the intestinal microbiota, and by “resetting” the microbiota, improve the enteric immune response. Antibiotics appear to play a role in the complex interplay between the gut microbiome, vaccine, virus, and host’s immune system, and mechanistic studies are needed to further understand this relationship.

We also observed that more children with antibiotic exposures (53%) experienced rotavirus gastroenteritis compared to those with no antibiotic exposure (23%). Notably, a greater proportion of infants with antibiotic exposures experienced rotavirus gastroenteritis later, after one year of age. This could indicate that despite being associated with increased RV1 seropositivity in our study, antibiotic receipt decreases the duration of vaccine-induced immunity. Alternatively, it is possible that children who received antibiotics had more exposure to enteric pathogens than children who did not receive antibiotics, resulting in more diarrheal episodes later in life.

Estimates of the potential impact of interventions to reduce antibiotic use among children prior to vaccine completion are relevant to public health policy. The intervention exposing all children to antibiotics is similar to proposed mass drug administration (MDA) interventions in LMICs [42–44]. For exam-

ple, a randomized controlled trial in India determined that a three-day course of azithromycin prior to receipt of oral poliovirus vaccine (OPV) found no effect on subsequent OPV immunogenicity [42]. Unlike traditional MDA interventions, antibiotic exposure in our study was mixed in terms of timing, antibiotic class, and duration, all of which can affect seropositivity. While the estimates of interventions to remove inappropriate and likely inappropriate antibiotics were small in magnitude, they correspond to antimicrobial stewardship interventions. In settings with a higher incidence of inappropriate antibiotic use, the magnitude of impact could be higher.

A strength of the study was the twice weekly frequency of antibiotic surveillance to capture antibiotic usage. The study was limited by our inability to definitively distinguish between appropriate and inappropriate antibiotic treatment. Only self-reported information regarding the indication for antibiotics was available, and other unknown symptoms or co-infections may have warranted antibiotic treatment. Also, antibiotic use did not occur equally among the three sites in the analysis. Nearly 80% of antibiotic use occurred in Peru, where a sensitivity analysis showed no significant difference in seropositivity between those with and without antibiotic exposure. However, reduced power and precision due to a smaller analytic sample likely contributes to this finding. Another limitation is the potential for misclassification due to natural rotavirus infections that occurred during the 7-month exposure window. We addressed this by exploring the reclassification of seropositivity in sensitivity analyses. Also, a 7-month time point rather than the standard 28 days post-vaccination time point was used to measure seropositivity due to data availability. Finally, we were unable to account for asymptomatic rotavirus infections not captured during routine monthly stool collection.

Despite these limitations, these data should reassure parents and healthcare providers that if antibiotics are needed around the time of rotavirus vaccination, serologic responses will not be adversely affected. However, by providing evidence that antibiotic use may positively impact vaccine response, these findings support further investigation into the effects of antibiotic use on the infant gut microbiome and immune response. The possibility that antibiotics may boost rotavirus vaccination performance should be further explored and weighed against the growing threat of antibiotic resistance and the potential long-term consequences of antibiotic use.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: DTS reports financial support was provided by the Robert Wood Johnson Foundation. SBD, PT, and ETRM report financial support from the National Institute of Allergy and Infectious Diseases. All other authors declare no competing interests.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2022.03.023.

References

[1] Troeger C, Khalil IA, Rao PC, Cao S, Blacker BF, Ahmed T, et al. Rotavirus Vaccination and the Global Burden of Rotavirus Diarrhea among Children Younger Than 5 Years. JAMA Pediatr 2018;172(10):958.
[2] Troeger C, Forouzanfar M, Rao PC, Khalil I, Brown A, Reiner RC, et al. 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.
[3] Tate JE, Burton AH, Boschi-Pinto C, et al. Global, Regional, and National Estimates of Rotavirus Mortality in Children <5 Years of Age, 2000–2013. Clin Infect Dis 2016;62:S96–S105.
[4] Soares-Weiser K, Bergman H, Henschke N, et al. Vaccines for preventing rotavirus diarrhoea: vaccines in use. Cochrane Database Syst Rev 2019; 3 (2019):CD008521.
[5] Ianiro G, Tilg H, Gasbarrini A. Antibiotics as deep modulators of gut microbiota: Between good and evil. Gut 2016;65(11):1906–15.
[6] Neuman H, Forsythe P, Uzan A, et al. Antibiotics in early life: dysbiosis and the damage done. FEMS Microbiol Rev 2018;42:489–99.
[7] Ciabattini A, Olivieri R, Lazzeri E, et al. Role of the microbiota in the modulation of vaccine immune responses. Front Microbiol 2019;3:1305.
[8] Butel M-J, Waligora-Dupriet A-J, Wydau-Dematteis S. The developing gut microbiota and its consequences for health. J Dev Orig Health Dis 2018;9 (6):590–7.
[9] Vangay P, Ward T, Gerber J, Knights D. Antibiotics, Pediatric Dysbiosis, and Disease. Cell Host Microbe 2015;17(5):553–64.
[10] Sulis G, Adam P, Nafade V, Gore G, Daniels B, Daftary A, et al. Antibiotic prescription practices in primary care in low- and middle-income countries: A systematic review and meta-analysis. PLOS Med 2020;17(6):e1003139.
[11] Ayukekbong JA, Ntemgwa M, Atabe AN. The threat of antimicrobial resistance in developing countries: Causes and control strategies. Antimicrob Resist Infect Control 2017;6:1–8.
[12] Bebell LM, Muiru AN. Antibiotic use and emerging resistance—how can resource- limited countries turn the tide? Glob Heart 2014;9(3):347.
[13] Zhang J, Cameron D, Quak SH, Kadim M, Mohan N, Ryoo E, et al. Rates and determinants of antibiotics and probiotics prescription to children in Asia-Pacific countries. Benef Microbes 2020;11(4):329–38.
[14] Kandeel A, El-Shoubary W, Hicks L, Fattah M, Dooling K, Lohiniva A, et al. Patient attitudes and beliefs and provider practices regarding antibiotic use for acute respiratory tract infections in Minya. Egypt. *Antibiotics* 2014;3 (4):632–44.
[15] Pavlinac PB, Denno DM, John-Stewart GC, Onchiri FM, Naulikha JM, Odundo EA, et al. Failure of syndrome-based diarrhea management guidelines to detect shigella infections in kenyan children. J Pediatric Infect Dis Soc 2016;5 (4):366–74.
[16] Harris VC, Haak BW, Handley SA, Jiang B, Velasquez DE, Hykes BL, et al. Effect of Antibiotic-Mediated Microbiome Modulation on Rotavirus Vaccine Immunogenicity:. A Human Randomized-Control Proof-of-Concept Trial. *Cell Host Microbe*. 2018;24(2):197–207.e4.
[17] Anderson EJ, Lopman B, Yi J, Libster R, Creech CB, El-Khorazaty J, et al. Effect of Concomitant Antibiotic and Vaccine Administration on Serologic Responses to Rotavirus Vaccine. J Pediatric Infect Dis Soc 2020;9(4):479–82.
[18] Miller M, Acosta AM, Chavez CB, et al. The MAL-ED study: A multinational and multidisciplinary approach to understand the relationship between enteric pathogens, malnutrition, gut physiology, physical growth, cognitive development, and immune responses in infants and children up to 2 years of. Clin Infect Dis 2014;59:S193–206.
[19] Richard SA, Barrett LJ, Guerrant RL, Checkley W, Miller MA. Disease surveillance methods used in the 8-site MAL-ED cohort study. Clin Infect Dis 2014;59:S220–4.
[20] Houpt E, Gratz J, Kosek M, Zaidi AKM, Qureshi S, Kang G, et al. Microbiologic methods utilized in the MAL-ED cohort study. Clin Infect Dis 2014;59: S225–32.
[21] Liu J, Gratz J, Amour C, Nshama R, Walongo T, Maro A, et al. Optimization of quantitative PCR methods for enteropathogen detection. PLoS ONE 2016;11 (6):e0158199.
[22] Psaki SR, Seidman JC, Miller M, Gottlieb M, Bhutta ZA, Ahmed T, et al. Measuring socioeconomic status in multicountry studies: Results from the eight-country MAL-ED study. Popul Health Metr 2014;12(1).
[23] Rogawski ET, Platts-Mills JA, Seidman JC, John S, Mahfuz M, Ulak M, et al. Use of antibiotics in children younger than two years in eight countries: A prospective cohort study. Bull World Health Organ 2017;95(1):49–61.
[24] WHO. Handbook: IMCI Integrated Management of Childhood Illness. Geneva: World Health Organization; 2005.

[25] Cheuvart B, Neuzil KM, Steele AD, Cunliffe N, Madhi SA, Karkada N, et al. Association of serum anti-rotavirus immunoglobulin A antibody seropositivity and protection against severe rotavirus gastroenteritis. Hum Vaccin Immunother 2014;10(2):505–11.
[26] Baker JM, Tate JE, Leon J, Haber MJ, Pitzer VE, Lopman BA. Postvaccination serum antirotavirus immunoglobulin A as a correlate of protection against rotavirus gastroenteritis across settings. J Infect Dis 2020;222:309–18.
[27] Liu J, Platts-Mills JA, Juma J, Kabir F, Nkeze J, Okoi C, et al. Use of quantitative molecular diagnostic methods to identify causes of diarrhoea in children: a reanalysis of the GEMS case- control study. Lancet 2016;388:1291–301.
[28] Zou G. A Modified Poisson Regression Approach to Prospective Studies with Binary Data. Am J Epidemiol 2004;159(7):702–6.
[29] Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. Epidemiology 1999;10(1):37–48.
[30] WHO, United Nations Children's Fund. Progress on Drinking Water, Sanitation and Hygiene: 2017 Update and SDG Baseline. Geneva: World Health Organization; 2017.
[31] WHO. WHO child growth standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development. Geneva: World Health Organization; 2006.
[32] Ahern J, Hubbard A, Galea S. Estimating the effects of potential public health interventions on population disease burden: A step-by-step illustration of causal inference methods. Am J Epidemiol 2009;169(9):1140–7.
[33] Westreich D. From exposures to population interventions: Pregnancy and response to HIV therapy. Am J Epidemiol 2014;179(7):797–806.
[34] R Core Team. R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing; 2021. , https://www.R-project.org/.
[35] Uchiyama R, Chassaing B, Zhang B, Gewirtz AT. Antibiotic treatment suppresses rotavirus infection and enhances specific humoral immunity. J Infect Dis 2014;210(2):171–82.
[36] Gozalbo-Rovira R, Rubio-del-Campo A, Santiso-Bellón C, Vila-Vicent S, Buesa J, Delgado S, et al. Interaction of intestinal bacteria with human rotavirus during infection in children. Int J Mol Sci 2021;22(3):1010.
[37] Lundin A, Bok CM, Aronsson L, Björkholm B, Gustafsson J-Å, Pott S, et al. Gut flora, Toll-like receptors and nuclear receptors: a tripartite communication that tunes innate immunity in large intestine. Cell Microbiol 2008;10(5):1093–103.
[38] Vlasova AN, Takanashi S, Miyazaki A, Rajashekara G, Saif LJ. How the gut microbiome regulates host immune responses to viral vaccines. Curr Opin Virol 2019;37:16–25.
[39] Parker EPK, Grassly NC. Enhancing Rotavirus Vaccination: A Microbial Fix? Cell Host Microbe 2018;24(2):195–6.
[40] Fix J, Chandrashekhar K, Perez J, Bucardo F, Hudgens MG, Yuan L, et al. Association between gut microbiome composition and rotavirus vaccine response among Nicaraguan infants. Am J Trop Med Hyg 2020;102(1):213–9.
[41] Grassly NC, Praharaj I, Babji S, Kaliappan SP, Giri S, Venugopal S, et al. The effect of azithromycin on the immunogenicity of oral poliovirus vaccine: a double-blind randomised placebo-controlled trial in seronegative Indian infants. Lancet Infect Dis 2016;16(8):905–14.
[42] Hart JD, Kalua K, Keenan JD, Lietman TM, Bailey RL. Effect of Mass Treatment with Azithromycin on Causes of Death in Children in Malawi: Secondary Analysis from the MORDOR Trial. Am J Trop Med Hyg 2020;103(3):1319–28.
[43] Keenan JD, Bailey RL, West SK, Arzika AM, Hart J, Weaver J, et al. Azithromycin to Reduce Childhood Mortality in Sub-Saharan Africa. N Engl J Med 2018;378(17):1583–92.