


REVIEW



Update on rotavirus vaccine underperformance in low- to middle-income countries and next-generation vaccines

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ABSTRACT

In the decade since oral rotavirus vaccines (ORV) were recommended by the World Health Organization for universal inclusion in all national immunization programs, significant yet incomplete progress has been made toward reducing the burden of rotavirus in low- to middle-income countries (LMIC). ORVs continue to demonstrate effectiveness and impact in LMIC, yet numerous factors hinder optimal performance and evaluation of these vaccines. This review will provide an update on ORV performance in LMIC, the increasing body of literature regarding factors that affect ORV response, and the status of newer and next-generation rotavirus vaccines as of early 2020. Fully closing the gap in rotavirus prevention between LMIC and high-income countries will likely require a multifaceted approach accounting for biological and methodological challenges and evaluation and roll-out of newer and next-generation vaccines.

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Introduction

Rotaviruses are a leading cause of pediatric diarrheal disease and mortality worldwide, with rotavirus-associated deaths in children <5 years old estimated to be 128,500–215,000 yearly.^{1–5} Oral rotavirus vaccines (ORV) have demonstrated remarkable efficacy and effectiveness in high-income countries, but they suffer from reduced performance in low- to middle-income countries (LMIC). In this review, we will provide an LMIC-specific update on recent estimates of the performance of currently approved ORVs, review factors affecting ORV performance, and discuss the status of newer and next-generation vaccines. This review will not discuss cost-effectiveness or vaccine supply and delivery issues, which are nicely summarized elsewhere.⁶

Background

Rotaviruses are non-enveloped, double-stranded RNA viruses belonging to the *Reoviridae* family. Intact virions are composed of a complex, triple-layered icosahedral capsid and contain a genome of 11 segments encoding 12 viral proteins.⁷ Rotaviruses have traditionally been classified using a binary nomenclature based on the two outer capsid structural proteins: the surface glycoprotein VP7 (G) forms the shell of the outer capsid layer, out of which protrude multiple copies of VP4 (P), a protease-sensitive spike that is responsible for attachment to cellular receptors.⁸

Rotavirus infection causes acute gastroenteritis (AGE), characterized by vomiting, watery diarrhea, and often fever, leading to dehydration which may be fatal in severe cases without timely rehydration.⁹ The major targets of infection are the mature enterocytes of the small intestinal villi, although enteroendocrine cells may also be infected.^{10,11} Pathogenesis

involves both osmotic and secretory diarrhea via malabsorption by infected enterocytes, alteration of intracellular calcium homeostasis leading to disruption of cytoskeleton and tight junction integrity, an enterotoxin-like effect modulated by non-structural protein 4 (NSP4), and stimulation of the enteric nervous system.¹² Transmission is presumed to be mainly fecal-oral, although spread by contaminated water and fomites are also likely.^{10,13} In the pre-vaccine era, virtually all humans were infected during early childhood, with subsequent natural immunity developing after several rounds of childhood infection; for unclear reasons, children in lower-income settings appear to require a greater number of infections to gain less complete immunity.^{14,15}

In high-income countries, AGE due to rotavirus infection was historically one of the most significant causes of pediatric morbidity and health-care utilization.¹⁶ Effective disease control in the United States, for example, was not achieved until the late 2000s after ORV introduction,¹⁷ indicating that good hygiene and sanitation alone are insufficient to control transmission. Thus, improvements in community sanitation and public health infrastructures in LMIC are unlikely to substantially decrease rotavirus incidence, compounding the challenge of diarrheal disease control in regions where ORVs underperform. These settings shoulder a disproportionate burden of global rotavirus-associated mortality, with only four countries (India, Nigeria, Pakistan, Democratic Republic of Congo) suffering nearly half of all rotavirus deaths.⁵

Current oral rotavirus vaccines

Four ORVs are currently pre-qualified by the World Health Organization (WHO) for global use: RotaTeq (Merck & Co., Inc., Kenilworth, NJ), Rotarix (GlaxoSmithKline Biologicals,

Rixensart, Belgium), Rotavac (Bharat Biotech International Ltd., Hyderabad, India), and Rotasiil (Serum Institute of India Pvt. Ltd., Pune, India). All are oral, live-attenuated vaccines; selected characteristics of each are presented in Table 1. Both RotaTeq and Rotarix demonstrated excellent vaccine efficacy in clinical trials conducted in higher-income settings,^{19,20} but markedly reduced vaccine efficacy was observed in clinical trials in LMIC.^{21–24} Despite reduced efficacy, in high-incidence settings vaccination still provides a significant overall reduction in severe disease, prompting the WHO to universally recommend ORV inclusion in all national immunization programs in 2009.²⁵

Rotarix and RotaTeq

Following introduction of Rotarix and RotaTeq, real-world vaccine effectiveness studies continued to support the observation that these vaccines provide substantial benefit yet underperform in LMIC compared to high-income settings. In a comprehensive systematic review and meta-analysis using data published prior to December 2, 2016, Jonesteller et al. reported a median vaccine effectiveness for Rotarix of 57% in countries with high child mortality, which are typically LMIC, vs 84% in low child mortality countries; for RotaTeq, median vaccine effectiveness was 45% and 90% in high child mortality and low child mortality countries, respectively.²⁶ Since then, additional studies of Rotarix or RotaTeq effectiveness in LMIC report generally similar findings, with point estimates for adjusted effectiveness against rotavirus-associated AGE in children <5 ranging up to 68% (Table 2).^{27–35}

Despite decreased effectiveness in LMIC, substantial reductions in rotavirus-associated and all-cause AGE hospitalizations were observed in virtually all settings following national-

level introduction of Rotarix or RotaTeq. Recently, Burnett et al. analyzed manuscripts of vaccine impact published prior to December 31, 2019 and found that median reductions in rotavirus hospitalizations or emergency department (ED) visits and all-cause AGE hospitalization in children <5 years in high child-mortality countries were 50% (IQR, 41–65) and 26% (IQR, 20–50).³⁶ The largest study utilized the Global Rotavirus Surveillance Network (GRSN), which was established by WHO in 2008 with funding from Gavi, the Vaccine Alliance. Data from 305,789 cases in children <5 years admitted to the hospital with AGE from 2008 to 2016 were analyzed, representing 198 sites in 69 countries from all six major WHO regions (African Region, Region of the Americas, South-East Asia Region, European Region, Eastern Mediterranean Region, and Western Pacific Region). Overall, the study found a 39.6% (95% CI 35.4–43.8) relative reduction in the proportion of children admitted for AGE due to rotavirus post-vaccine introduction compared to pre-vaccine introduction.³⁷ In Latin America, data from sentinel sites in Bolivia, El Salvador, Guatemala, Honduras, Paraguay, and Venezuela showed a mean reduction of 16% (95% CI 10–22) in the proportion of acute diarrhea samples positive for rotavirus.³⁸ In Africa, percent reduction in rotavirus hospitalizations or emergency department visits was 46% among countries that had introduced ORV by 2013, and was 34% in countries that had introduced it later.³⁹

Decreases in rotavirus-associated or all-cause AGE mortality in children following vaccine introduction have also been reported. In Malawi, a 31% reduction (95% CI, 1–52) was observed in all-cause AGE mortality following Rotarix introduction,⁴⁰ and in Bolivia, a 53% reduction (95% CI, 47–56) in the proportion of deaths due to all-cause AGE was

Table 1. Overview of oral, live-attenuated rotavirus vaccines pre-qualified by the World Health Organization as of Jan 2020.

Vaccine	Manufacturer	Composition	Dosage	Formulation	Storage ^a	EPI schedule	WHO pre-qualification ^a
RotaTeq	Merck & Co., Inc.	Pentavalent human-bovine mono-reassortants: human G1, G2, G3, G4, P [8] on bovine rotavirus (WC3) backbone	2–2.8 x 10 ⁶ IU/serotype	Liquid	2–8 C, 24 mo	6, 10,	14 weeks
Oct 2008 Rotarix	GlaxoSmithKline Biologicals	Monovalent G1P[8] (strain RIX4414) human rotavirus	≥10 ⁶ CCID ₅₀	Liquid	2–8 C, 24 mo	6, 10 weeks	Mar 2009
Rotavac ^b	Bharat Biotech International Ltd.	Monovalent G9P[11] (strain 116E) natural human-bovine reassortant	≥10 ⁵ FFU	Liquid	–20 C, 60 mo After thaw: 2–8 C, 6 mo	6, 10,	14 weeks
Jan 2018 Rotasiil ^c	Serum Institute of India Pvt. Ltd.	Pentavalent human-bovine mono-reassortants (BRV-PV): human G1, G2, G3, G4, G9 on bovine rotavirus (UK) backbone	≥10 ^{5.6} FFU/serotype	Lyophilized	2–8 C, 30 mo	6, 10,	14 weeks
Sep 2018 Rotasiil Thermo	Same	Same	Same	Lyophilized	25 C, 30 mo	Same	Jan 2020

Abbreviations: CCID₅₀, cell culture infective dose 50; EPI, Expanded Programme on Immunizations; IU, international units; FFU, focus-forming units; WHO, World Health Organization

a. Ref¹⁸

b. Rotavac 5D: storage at 2–8 C but not yet pre-qualified by WHO

c. Rotasiil: liquid formulation requiring storage at 2–8 C not yet pre-qualified by WHO

Table 2. Summary of recent oral rotavirus vaccine effectiveness studies in LMIC.

Country	Vaccine	Setting	Age (years)	Adjusted vaccine effectiveness (95% CI)		Ref
				Any rotavirus gastroenteritis	Severe rotavirus gastroenteritis	
Bangladesh	Rotarix	Hospital, Outpatient	<2	41% (23–55)	43% (22–58)	27
Burkina Faso	RotaTeq	Hospital	<5	35% (–15–63)	39% (–18–68)	28
			6–11	58% (10–81)	NR	
			<5	68% (50–80)	NR	29
Lebanon	Rotarix, RotaTeq	Hospital	<5	62% (28–80)	75% (41–89)	30
Malawi	Rotarix	Hospital	<5	75% (45–89)	83% (54–94)	31
Philippines	Rotarix	Hospital	<5	62% (26–80)	67% (18–87)	32
Tanzania	Rotarix	Hospital	<2	49% (–30–80)	66% (–2–89)	33
Tanzania	Rotarix	Hospital	<5	75% (–8–94)	NR	
Thailand	Rotarix	Hospital	<18 mo	85% (27–97) ^a	NR	34
Zimbabwe	Rotarix	Hospital, ED	1–5	88% (76–94) ^b	NR	
			6–11 mo	–48% (–148–11)	–38% (–164–28)	35
				61% (21–81)	68% (13–88)	

Rotavirus detection by stool rotavirus enzyme immunoassay except as otherwise noted.

Abbreviations: NR, not reported

^aDetection by qPCR

^bDetection by polyacrylamide gel electrophoresis

observed.⁴¹ In the previously mentioned meta-analysis by Burnett et al., median reduction in AGE mortality was 37% (IQR, 24–41) for children <5 years in high child-mortality countries.³⁶ An estimated 134,714 (IQR, 112,321–154,654) hospitalizations and 20,986 (IQR, 18,924–22,822) deaths were prevented in 2016 in the 29 African countries that had introduced rotavirus vaccine by 2014; if all African countries had introduced vaccine, the numbers of hospitalizations and deaths prevented could have both been over twice these estimates.⁴² In Asia, ORVs introduction to all 43 Asian countries is estimated to decrease yearly rotavirus-associated hospitalizations by 710,000 (49% decline) and rotavirus-associated deaths by 35,000 (40% decline).⁴³

Indirect vaccine effects among unvaccinated individuals, such as reductions in rotavirus incidence among unvaccinated individuals (e.g. herd immunity) have also been noted in many high-income settings, but are infrequently observed in LMIC.⁴⁴ However, there may be additional indirect effects of vaccine introduction that may be unmeasured in typical impact studies. For example, in Bangladesh, which has a high burden of AGE hospitalizations and a chronic shortage of hospital beds, ORV use was estimated to free over 600 hospital beds per year, improving access to care for critical non-diarrheal pediatric illnesses.⁴⁵

Rotavac and Rotasiil

More recently, two ORVs manufactured in India, Rotavac, and Rotasiil, received WHO pre-qualification in 2018, after both demonstrated efficacy in clinical trials conducted in India (Rotavac, Rotasiil) and Niger (Rotasiil). A randomized, double-blind, placebo-controlled Rotavac trial performed from 2011 to 2012 in three sites in India demonstrated vaccine efficacy of 53.6% (95% CI, 35.0–66.9) against severe rotavirus gastroenteritis in the first year of life and 55.1% (39.9–66.4) until 2 years of age.^{46,47} A second formulation called Rotavac 5D has been developed and released, offering a ready-to-use liquid formulation stored at 2–8 C (versus Rotavac, which must be stored frozen for long-term storage), although it is not yet WHO pre-qualified.

Rotasiil is a lyophilized, heat-stable ORV that has been shown to be stable when stored up to 18 months at 37 C–40 C,⁴⁸ and was tested in two separate trials. A randomized, double-blind, placebo-controlled trial was performed among infants in Niger from 2014 to 2015; vaccine efficacy against severe rotavirus gastroenteritis was 66.7% (95% CI, 49.9–77.9).⁴⁹ A similar trial conducted at six sites in India from 2014 to 2015 reported vaccine efficacy against severe rotavirus gastroenteritis of 36% (95% CI, 11.7–53.6).⁵⁰ Rotasiil has demonstrated lot-to-lot consistency with similar immunogenicity to that of Rotarix, as assessed by serum rotavirus-specific IgA (RV-IgA) antibody concentration.⁵¹ A ready-to-use liquid formulation has been developed which demonstrated immunogenicity (RV-IgA) non-inferiority to lyophilized Rotasiil with lot-to-lot consistency, but must be maintained at 2–8 C.⁵² This formulation is not yet WHO pre-qualified. The discrepancy between the efficacy estimates for the Niger study and the India study are unclear. Both studies were designed and powered for a primary endpoint of vaccine efficacy after a pre-determined target number of cases was achieved, and different protocols for vaccine storage temperatures were used in each study but these seem unlikely to be contributing factors.^{49,50}

India has introduced Rotavac and Rotasiil at the national level, with roll-out having occurred in stages by state.⁵³ Outside of India, as of early 2020 Palestine⁵⁴ and Benin had introduced Rotavac and Democratic Republic of Congo had introduced Rotasiil, with a number of additional countries reportedly planning introduction or transition – Rotavac or Rotavac 5D: Egypt, Nigeria, Ghana, Sao Tome; Rotasiil, lyophilized: Mali, Uzbekistan; Rotasiil, liquid: Burkina Faso (Carl Kirkwood, personal communication).

Locally approved vaccines: Rotavin and Lanzhou lamb rotavirus (LLR) vaccine

Two countries, China and Vietnam, have developed vaccines approved and licensed for national use. In China, LLR (Lanzhou Institute of Biological Products, Lanzhou, China), a G10P[15] strain, is recommended once annually in children

from 2 months to 3 years of age. It has been available in China since 2000, but lack of inclusion in China's universal vaccine program and lack of formal phase 3 efficacy trials hamper understanding of the vaccine's true performance.⁵⁵ Single-dose effectiveness has been estimated from 34.9% (95% CI, 5.3–55.3) to 73.3% (95% CI, 61.2–81.6) in children <5 years, with improved performance observed with earlier vaccination.^{56–60} In Vietnam, Rotavin M-1, (POLYVAC, Hanoi, Vietnam), an attenuated G1[P8] strain of human rotavirus, has been licensed since 2012, based solely on immunogenicity data.⁶¹

Updated formulations of these vaccines are currently undergoing or have recently completed evaluation. A phase 3 trial to compare Rotavin-M1, which is stored frozen, with a newer liquid formulation simply called Rotavin, is reportedly in progress (ClinicalTrials.gov NCT0370336). Very recently, LLR3 (Lanzhou Institute of Biological Products, Lanzhou, China), a new G2, G3, G4 trivalent human-lamb mono-reassortant vaccine based on Lanzhou lamb rotavirus vaccine, was evaluated in a phase 3 clinical trial in China. Vaccine efficacy against any rotavirus AGE was 56.6% (95% CI, 47.5–60.1) and 70.3% (95% CI, 60.6–77.6) against severe rotavirus AGE through two epidemic seasons following vaccination.⁶² These estimates are similar to vaccine efficacies measured in phase 3 clinical trials of Rotarix in China,⁶³ and slightly lower than findings for RotaTeq.⁶⁴

Factors affecting oral vaccine performance

A number of factors have been proposed to contribute to poor ORV performance among children in LMIC, such as maternal antibodies, micronutrient deficiencies, gut dysbiosis, coinfections, environmental enteric dysfunction, and genetic factors.^{65,66} Most studies assessing factors related to ORV performance have utilized immunologic endpoints, typically RV-IgA seroconversion or concentration. RV-IgA is a suboptimal correlate of protection for ORVs in LMIC,^{67–71} so the effects of many factors on clinical protection remain incompletely understood. With this limitation in context, targeted interventions to improve ORV response have generally been unsuccessful (as further discussed below). Only two interventions, delay of the first ORV dose and separation of the first ORV dose from the first dose of oral polio vaccine have consistently demonstrated improvements in RV-IgA concentration or seroconversion.⁷² Unfortunately, these interventions would require significant adjustment to the WHO Expanded Programme on Immunizations (EPI) schedule, limiting feasibility.

Vaccine schedules and dosing

Delayed dosing

The EPI schedule recommends ORV vaccination at 6 and 10 weeks (Rotarix) or 6, 10, and 14 weeks of age (RotaTeq, Rotavac, Rotasiil). The rationale for delayed dosing is that it may mitigate the inhibitory effects of maternal antibodies, as further discussed below. In systematic reviews and meta-analyses, delayed dosing of Rotarix, defined as the first vaccine dose administered at or beyond 10 weeks of age, was associated

with improved RV-IgA seroconversion.^{72,73} Limited data also suggest improved clinical efficacy of delayed dosing. In Bangladesh, a delayed dosing schedule of 10 and 17 weeks demonstrated higher than expected efficacy against severe rotavirus diarrhea of 73.5% (95% CI, 45.8–87.0), but this was not directly compared to standard EPI scheduling.^{22,74} Pooled analyses from multiple Rotarix and RotaTeq studies suggested very modest improvements in protection from severe rotavirus gastroenteritis in children receiving a delayed first dose or increased interval between doses.⁷⁵ Whether delayed dosing can truly improve vaccine efficacy may require additional dedicated, adequately powered clinical trials.

Additional doses

A related issue is the potential benefit of additional vaccine doses. Assessments of an additional dose or doses of Rotarix at 14 weeks of life or later on ORV immunogenicity have yielded inconsistent conclusions. In Ghana, increased RV-IgA seroconversion and geometric mean concentration (GMC) was seen among infants who received an additional dose of Rotarix at 14 weeks compared to infants who received Rotarix at 6 and 10 weeks only.⁷⁶ However, a similar study in Pakistan was unable to demonstrate any effect, while a study in India found no differences with five doses of Rotarix compared to three.^{77,78} These studies suggest that the utility of this strategy may be location-specific. A study conducted at two sites in Africa (South Africa and Malawi) did demonstrate a slight improvement (not statistically significant) in year one vaccine efficacy with three doses compared to two doses among infants at the South Africa site, but not among infants in Malawi.⁷⁹ The initial WHO recommendation for a two-dose Rotarix regimen included review of these year one efficacy data from these two sites.²⁵ Subsequently, improved vaccine efficacy through 2 years of age was noted at both study sites in the three-dose group, as well as slightly increased RV-IgA seroconversion rates and GMC.^{23,79,80} However, these data alone were felt to be insufficient to recommend universal adoption of a three-dose regimen, although there was a call for additional research on the topic.⁸¹

Booster doses

Vaccine efficacy appears to be lower during the second year of life among children in LMIC,^{82,83} prompting investigations of a booster dose of ORV to counter waning immunity. A third (booster) dose of Rotarix administered with measles-mumps-rubella vaccine at 9 months was evaluated in Bangladesh. No effects on measles or rubella antibody titers were observed, and significant increases were seen in RV-IgA and RV-IgG seropositivity rates and geometric mean titer (GMT) in Rotarix booster recipients.⁸⁴ A similar study in Mali was conducted to administer a booster dose of RotaTeq with yellow fever, measles, and meningococcal A vaccines at 9 months. Seroresponses to measles and meningococcal A vaccine were similar in RotaTeq booster recipients compared to control, and RotaTeq recipients had significant increases in RV-IgA and RV-IgG concentration and seroresponse rates; the study was unable to demonstrate non-inferiority to yellow fever seroresponse (as defined by ≥ 4 -fold increase in titer), but responses to yellow fever vaccine were inexplicably quite low in both

groups, making the clinical significance of this result unclear. These findings suggest potential for extending the duration of vaccine protection, but the overall effect may be fairly modest: mathematical modeling suggests that a 9- or 12-month ORV booster might avert 4,000–19,600 deaths (3–16% reduction) among children aged 1–2 per year globally.⁸⁵

Size of vaccine inoculum

The potential effect of increasing vaccine inoculum has been widely discussed but few studies have directly addressed this topic in LMIC. For Rotarix, the effect of increasing vaccine inoculum, ranging from $1 \times 10^{4.1}$ to $1 \times 10^{6.4}$ focus-forming units (FFU)/dose, on seroconversion and RV-IgA GMC reached a plateau in studies from Europe, North America, Singapore, and Latin America.^{86–89} Early RotaTaq studies demonstrated a dose–response effect (range, 2.41×10^6 to 2.69×10^7 plaque-forming units/dose) of vaccine inoculum on G1-specific neutralizing antibody titers among children in Finland, although similar rates of RV-IgA seroconversion were seen.⁹⁰ Similarly, increased seroconversion was observed at higher dosages for both Rotavac (1×10^5 vs 1×10^4 FFU/dose) and Rotasil (range, 1×10^5 to $1 \times 10^{6.4}$ FFU/dose) in early studies in India.^{91,92} Our group recently performed a randomized-controlled trial comparing double the standard dose of Rotarix to standard dosing ($1 \times 10^{6.3}$ vs 1×10^6 FFU/dose) among infants in Dhaka, Bangladesh. No differences were observed in RV-IgA seroconversion in high- versus standard-dose recipients (46% and 42%, respectively) or in GMC (30.4 units/mL vs 22.8 units/mL, respectively).⁹³ The overall increase in vaccine inoculum was modest, which may help explain the apparent lack of benefit.

Maternal antibodies

Maternally-derived serum RV-IgG

The most plausible explanation for the beneficial effect of delayed ORV dosing noted above is the inhibitory effect of maternally derived, transplacentally acquired serum antibodies (RV-IgG). Higher levels of RV-IgG or neutralizing antibodies at the time of vaccination have consistently and convincingly been associated with decreased ORV immunogenicity.^{69,76,77,94–96} Delayed dosing may provide additional time for waning of maternal antibodies, diminishing their effect on infant ORV response. However, the apparent benefits of delayed dosing would need to be weighed against the logistical challenges associated with alterations to the EPI schedule, along with the potential increase in early episodes of rotavirus AGE in regions with significant incidence at very young ages.^{97,98}

Breast milk antibodies and antiviral factors

The role of breast milk on ORV response is less clear. Both breast milk antibodies and non-antibody breast milk components, such as lactoferrin, lactadherin, and human milk oligosaccharides (HMOs) diminish the infectivity of rotaviruses, including vaccine strains, *in vitro*.^{99–101} Formula-fed infants in Mexico achieved higher RV-IgA concentrations compared to breast-fed infants, even though RV-IgA geometric mean titer (GMT) in breast-fed infants was still quite robust.¹⁰² The

hypothesis that withholding breastfeeding at the time of vaccination could potentially improve infant vaccine response was tested in multiple LMICs. In clinical trials in India and South Africa, no differences in vaccine immunogenicity could be detected between infants in whom breastmilk was withheld around the time of vaccination compared to those breastfed at the time of vaccination,^{103,104} and in a similar study from Pakistan, RV-IgA seroconversion was paradoxically increased in the immediate breastfeeding arm.¹⁰⁵ Given the clear health benefits of breastfeeding and the lack of effect from short-term withholding of breastfeeding, further studies targeting breastfeeding are difficult to justify.

Histoblood group antigens (HBGA)

HBGA status, particularly secretor status, clearly affects susceptibility to rotavirus gastroenteritis. Secretor status is determined by the *FUT2* gene, which encodes an α -[1,2]-fucosyltransferase that controls the expression of various 2-fucosylated HBGAs on mucosal surfaces (such as the gut) and in exocrine secretions (including saliva and breast milk). In the gut, these HBGAs are proposed molecular receptors for viral attachment. Non-secretors, who do not express these fucosylated targets, have been found to be far less susceptible to AGE due to P[8] and P[4] rotaviruses, and individuals who lack Lewis (*FUT3*)-derived antigens are more susceptible to P[6] viruses.^{106–110} Therefore, a recent area of scrutiny has been the role of infant secretor status on ORV response, particularly for Rotarix, an attenuated P[8] strain. Studies from Ghana and Pakistan have reported decreased rates of RV-IgA seroconversion among infant secretors,^{111,112} while no such differences were found in studies from Bangladesh and Malawi.^{113,114} Additional findings from the Bangladesh study offer a potential explanation. In this study, infants born to maternal non-secretors had significantly increased risk of seroconversion compared to infants born to maternal secretors, and the greatest effect was observed in infants who were themselves secretor-positive but born to non-secretor mothers, with 73% seroconversion in secretor-positive infants born to genotype-confirmed non-secretor mothers, compared to 23% in those born to genotype-confirmed secretor mothers.¹¹⁴ A proposed mechanism for this effect is that *FUT2*-dependent HMOs present in breast milk may act as decoy receptors for vaccine-strain virus; this could explain why vaccinated infants without interference from breast milk ligands but who expressed 2-fucosylated HBGAs on the gut surface (i.e. secretor infants born to maternal non-secretors) had the highest rates of vaccine seroconversion.¹¹⁴ Inability to account for maternal secretor status is a possible explanation for the discordant results reported for infant secretor status across studies, but this requires further investigation.

Factors affecting the infant gut microenvironment

Oral polio vaccine interference

It has now been well demonstrated that concurrent administration of the first dose of oral polio vaccine (OPV) with the first dose of ORV decreases RV-IgA seroconversion and concentration.^{115–117} This phenomenon is likely due to

interference from intestinal OPV replication, which is most robust following the first dose of OPV and may out-compete ORV replication. With planned withdrawal of OPV as a part of the global polio eradication effort, this may become less of a factor if more countries can transition to inactivated polio vaccines (IPV). Unfortunately, this remains a challenge due to higher cost and supply issues of IPV and recent resurgences in outbreaks due to circulating vaccine-derived polioviruses, particularly due to Sabin strain 2.^{118–120} Genetically stable novel OPV vaccines (nOPV) that are less likely to revert to neurovirulence than Sabin strains are currently under development. nOPV against type 2 poliovirus is furthest in development, but since nOPV2 shedding in infants was comparable to historically observed rates of monovalent OPV2 shedding shortly after vaccination,¹²¹ nOPVs seem unlikely to differ in inhibitory effect on ORVs, although this remains to be determined.

Coinfections and microbiome

In Bangladesh, infection with non-polio enteroviruses at the time of ORV administration was associated with decreased RV-IgA seroconversion and concentration and increased vaccine failure.¹²² However, similar studies conducted in India and Zimbabwe found opposite effects, with enterovirus quantity positively associated with ORV RV-IgA seroconversion, although quantity of non-polio enteroviruses in these studies were inferred rather than directly measured due to lack of methods that specifically detect non-polio enteroviruses but not Sabin-strain polioviruses.^{115,117} It is possible that timing of vaccination could have affected these findings, as the Bangladesh study provided the first Rotarix dose at 10 weeks, concurrent with the second OPV dose, while infants in the Zimbabwe and India studies received their first doses of Rotarix and OPV concurrently at 6 weeks. No consistent pathogen-specific effects, including enterovirus, at the time of vaccination on ORV response have thus been observed.

The role of infant intestinal microbiome is an area of active interest. Investigators have reported significant differences in infant gut microbiota in Rotarix RV-IgA seroresponders and non-responders in cohorts from Pakistan and Ghana.^{123,124} In Pakistan, vaccine response correlated with higher relative abundance of bacteria belonging to *Clostridium* cluster XI and Proteobacteria, while in Ghana vaccine responders had an increased abundance of *Streptococcus bovis* and decreased abundance of Bacteroidetes compared to non-responders. This study was limited by very low RV-IgA response (15% seroconversion). In both studies, infants were also compared to a cohort of Dutch infants, with responders sharing greater similarity in overall microbiome composition with healthy Dutch infants, who were presumed to have good ORV response, compared to non-responders. In contrast, no consistent differences in microbiota composition or alpha or beta diversity could be detected in Rotarix seroconverters vs non-converters in India, although slightly more pre-vaccination bacterial taxa were observed in those who shed vaccine after the first dose.¹¹⁵ In Dutch adults who underwent microbiota modification via narrow- vs- wide-spectrum oral antibiotics followed by a single dose of Rotarix, those who received narrow-spectrum antibiotics (oral vancomycin) had an increased rate of RV-IgA boosting, defined as a two-fold increase, and

increased frequency of fecal vaccine shedding compared to controls, suggesting that alterations in microbiota could have detectable effects on ORV response.¹²⁵ Since all volunteers were adult males who had quite robust baseline RV-IgA levels, it remains unclear how these findings would translate to infants in LMIC.

An underexplored topic is maternal breast milk microbiome. Breast milk microbiome in mothers of neonates with symptomatic rotavirus infections due to neonatal G10P[11] rotavirus in India clustered differently from those with asymptomatic neonatal infection or uninfected neonates, with increased *Enterobacter/Klebsiella* relative abundance in breast milk of mothers of symptomatic infants.¹²⁶ Similar findings were observed for infant fecal microbiome. However, the relevance of these findings for ORV response also remain uncertain. The effects of infant and maternal microbiome on ORV responses will likely remain an area of intense interest, with further insights to come.

WASH

The impact of household-level water, sanitation, and hygiene (WASH) interventions on Rotarix response was evaluated in Zimbabwe in a cluster-randomized 2 × 2 factorial trial, which also assessed improved feeding interventions on child health outcomes. WASH interventions were associated with a modest increase in seroconversion among vaccinated infants in WASH groups versus non-WASH groups, with an absolute difference of 10.6% (95% CI 0.54–20.7).¹²⁷ Fewer infants in the WASH group were seropositive pre-vaccination, meaning this difference could have resulted from reductions in early RV exposure in the WASH group rather than a direct effect on vaccine response. However, exclusion of baseline-seropositive infants demonstrated a consistent effect, with an absolute difference of 9.8% (95% CI, –6–20.2). These results are tempered by the low rates of seroconversion observed, which was 35.4% in WASH infants who received both doses of vaccine. In this setting, WASH was not associated with significant reductions in rotavirus prevalence.¹²⁸ The ability of household-level WASH alone to improve ORV performance thus appears to be limited.

Probiotics and zinc supplementation

Zinc deficiency is common in children in LMIC and has long been recognized as important in the treatment of pediatric diarrhea, with recent evidence suggesting a specific role in protection from rotavirus diarrhea.²² Therefore, zinc supplementation has been proposed as a potential adjunct to aid in response to rotavirus vaccination. Similarly, probiotics have received interest as another possible intervention to induce a gut microbiota more favorable to oral rotavirus vaccine response. A study in India randomized infants into four groups: probiotic (10¹⁰ *Lactobacillus rhamnosus* GG) plus oral zinc (5 mg daily); probiotic only with zinc placebo; probiotic placebo with zinc only; or probiotic placebo and zinc placebo, all starting one week before initiating the Rotarix vaccine series until 6 weeks after the second dose.¹²⁹ Neither zinc nor probiotic was associated with significantly increased RV-IgA seroconversion, although a suggestion of benefit was noted for probiotic, with a 7.5% increase in seroconversion in

all infants who received probiotic compared to those who received none (97.5% CI −1.4–16.2).

Environmental enteric dysfunction (EED)

EED is a subclinical disorder of gut function and inflammation that affects impoverished populations in LMIC, presumably due to high enteropathogen exposure. It is associated with intestinal villus blunting, impaired barrier function, and malabsorption.¹³⁰ EED has long been proposed as an important variable in oral vaccine underperformance, but compelling evidence has proven difficult to produce, with large trials yielding conflicting results.¹³¹ The greatest barrier to this field has been lack of validated surrogate markers for EED: the current gold standard for diagnosis remains histopathologic examination of intestinal biopsy specimens, and an objective scoring index has only recently been developed.¹³² Numerous stool, serum, and urine biomarkers capturing specific aspects of EED-associated pathology (e.g. intestinal inflammation, permeability, malabsorption, translocation, etc.), have been evaluated in multiple settings with few consistent findings.^{133,134} Work to identify reliable noninvasive biomarkers as surrogates for biopsy-proven EED is currently ongoing, and may hopefully refine approaches toward EED detection and risk stratification in at-risk populations.¹³⁵

Factors impacting measurements of vaccine efficacy

At least part of the lower estimates of vaccine efficacy observed in LMIC may have to do with mathematical phenomena due to limitations in standard methods for measuring vaccine efficacy. For example, using data from a Rotarix trial performed in Bangladesh, researchers determined that traditional efficacy measurements that failed to account for immunity due to natural infections in the placebo group during periods of prior exposure underestimated overall efficacy by 7.1%, with a 13.5% increase specifically in year 2 efficacy when only evaluating rotavirus-naïve children.¹³⁶ They further developed a model to simulate variation in year 2 efficacy using data from other regions representing a spectrum of rotavirus incidence rates and vaccine efficacies. This model suggested that underestimation of year 2 vaccine efficacy was greatest in settings with calculated efficacy near 50%, which generally corresponds to efficacy estimates for many LMIC.

Similarly, lack of accounting for decreased natural susceptibility to rotavirus infections in individuals with non-secretor HBGA status was shown to decrease reported estimates of vaccine efficacy in Bangladesh.¹⁰⁷ In this study, efficacy against rotavirus-associated AGE of any severity among non-secretors was 31.7%, compared to 56.2% among secretors. The reduced efficacy observed among non-secretors appeared to be due to the natural protection afforded by non-secretor status in unvaccinated infants (which approached a 50% risk reduction compared to unvaccinated secretors): decreased susceptibility in non-secretors meant that vaccination in this group offered little additional protection. The difference in rotavirus incidence in vaccinated versus unvaccinated non-secretors was thus minimal, resulting in a reduced effect size. This led to a mathematical reduction in efficacy that did not reflect the biological mechanism for this outcome. Similar effects may

provide an incremental contribution to lower efficacy estimates in other trials, particularly in regions with a high population prevalence of non-secretors. In contrast, a study in Malawi found that non-secretors had a reduced risk of rotavirus vaccine failure, suggesting that secretor status does not impact vaccine performance estimates, but this analysis was performed using a case-control design and may not be directly comparable to efficacy estimates obtained from prospective clinical trials.¹¹³

Difficulties in attribution of diarrheal etiology have also come under increased scrutiny. Among infants in LMIC, coinfections with multiple enteropathogens are frequently observed.^{3,137} In the context of a vaccine trial, vaccinated infants with a rotavirus-positive diarrheal episode would be considered a case of vaccine failure. However, significant misattribution of diarrhea incidence to rotavirus could occur if these episodes were also frequently associated with coinfections with other etiologic agent of gastroenteritis, but for which testing was not performed. Post-vaccination, it is possible that rotavirus infections would be more likely to be asymptomatic, as is observed in subsequent episodes of natural infection,¹⁴ and therefore more frequently seen in the context of coinfection rather than symptomatic mono-infection. A high incidence of diarrhea due to other undetected pathogens could thus confound vaccine outcome measures in rotavirus efficacy trials. In Botswana, a recent study did not detect a difference in rotavirus vaccine effectiveness in patients with intestinal coinfections compared to those without.¹³⁸ In this study, co-pathogens were detected using either in-house multiplex PCR (nine targets) or a commercial gastrointestinal PCR panel (15 targets). A significant limitation of this study was a small sample size, which may have underpowered the study to reach more definitive conclusions, particularly as there did appear to be a possible effect of coinfections detected using the in-house panel: vaccine effectiveness was 48% in the coinfection group and 62% in the group with rotavirus infection alone. Similarly, detection of an even broader range of coinfections using Taqman Array Card (TAC) was performed in the Rotavac vaccine efficacy trial in India.¹³⁹ In this study, accounting for enteric coinfections led to an 11.3% increase in vaccine efficacy. Similar effects could help explain a portion of the lower vaccine efficacy estimates observed in LMIC, but further work will be required to resolve this issue.

Clearly, the underperformance of current ORVs in LMIC is multifactorial. A small but important component of the reduced efficacy observed in these regions may have to do with limitations in approaches for measuring vaccine performance. However, a spectrum of biological factors is also clearly involved, meaning any single intervention is unlikely to fully achieve levels of ORV performance comparable to high-income settings. Such interventions (e.g. delayed or extra dosing, booster doses, increased vaccine inoculum, staggered OPV administration, micronutrient or probiotic supplementation) have had limited success in improving oral vaccine responses, as detailed above. Even if they demonstrated larger effects, none are easily implemented as they would all require large-scale restructuring of current WHO vaccine schedules or add significant cost to the global rotavirus vaccine effort, at a time when access to current vaccines is already suboptimal. In 2019,

an estimated 85 million children still did not have access to vaccine.¹⁴⁰ Therefore, next-generation vaccines will likely be needed to fully bridge this gap. A number of newer vaccines are currently undergoing clinical evaluation or development, including oral, live-attenuated vaccines and parenteral non-replicating rotavirus vaccines.

Newer and next-generation rotavirus vaccines

An overview of newer and next-generation vaccines is provided in Table 3.

Newer oral vaccines

RV3-BB

A promising newer ORV is RV3-BB (PT BioFarma, Bandung, Indonesia). This vaccine consists of a neonatal strain of G3P [6] human rotavirus (RV3). As a naturally attenuated neonatal strain, it was found to cause wild-type asymptomatic infection very early in life and is infectious and immunogenic in spite of high levels of maternally derived serum or breast milk antibodies, providing a rational alternative path for improving oral rotavirus vaccine responses.¹⁴¹ RV3-BB demonstrated efficacy in a double-blind, placebo-controlled Phase 2b trial in Indonesia using both infant and neonatal dosing.¹⁴² In this trial, participants were randomized to neonatal dosing at 0–5 days, 8 weeks, and 14 weeks of age, infant dosing at 8, 14, and 18 weeks of age, or placebo. Vaccine efficacy against severe rotavirus AGE through 18 months was 75% (95% CI, 44–91) in the neonatal-schedule group and 51% (95% CI, 7–76) in the infant-schedule group. Efficacy was even higher for the first year of life, estimated at 94% (95% CI, 56–99) in the neonatal-schedule group and 77% (95% CI, 31–92) in the infant-schedule group. A phase 2 dose-ranging study to confirm appropriate dosing in African infants has been performed in Malawi, although results are not yet available (ClinicalTrials.gov NCT03483116). A phase

3 trial in Indonesia using RV3-BB produced under Halal manufacture (PT BioFarma) is scheduled to commence in mid-2020; pending trial results, developers plan to submit for local licensure and national roll-out in Indonesia, followed by WHO pre-qualification (Julie Bines, personal communication).

Other unique characteristics of RV3-BB make it an intriguing addition to the ORV repertoire. For example, high rates of vaccine take were observed irrespective of infant HBGA status, and immune response appeared to be less susceptible to interference from OPV, with vaccinated infants who received concurrent OPV demonstrating similar rates of antibody seroconversion and GMT compared to vaccinated infants who received IPV.^{143,144} Interestingly, Rotavac is also derived from a neonatal rotavirus strain (G9P[11]) but showed efficacy levels similar to those of other ORVs in LMIC. Whether strain-specific differences or adoption of a neonatal dosing regimen means RV3-BB's promising findings from Indonesia can be replicated in other locations remains to be seen.

Non-replicating rotavirus vaccines (NRRVs)

Given the ongoing challenges with oral vaccine underperformance, the potential of parenteral NRRVs has received intense interest. The potential advantages of such vaccines include: circumventing the so-called “tropical barrier” presented by multiple factors (e.g. EED) that prevent successful take of live-attenuated vaccines in the gut, hopefully leading to improved efficacy; reduced cold-chain footprint and cost; opportunity via sequential scheduling strategies (e.g., “prime-boost”) to augment (rather than replace) current ORV programs; and development of combination vaccines to facilitate administration and improve access. A meeting of NRRV vaccine developers was organized by PATH and held in Geneva, Switzerland in June 2019; additional information regarding the following vaccine candidates are available in

Table 3. Overview of newer and next-generation vaccines under evaluation or in development.

Vaccine type	Manufacturer/Developer	Composition	Status
Oral, live-attenuated			
RV3-BB	PT BioFarma	Monovalent G3P[6] (strain RV3) neonatal human rotavirus	Phase 2/3
Non-replicating, parenteral			
P2-VP8*	PATH SK Bioscience	Trivalent subunit vaccine: tetanus toxoid P2 fused to P[4] (strain DS1), P[6] (strain 1076), and P[8] (strain Wa) VP8*	Phase 3
CDC-9	CDC Serum Institute of India Pvt. Ltd	Monovalent inactivated human rotavirus strain CDC-9	Preclinical
116E	Zhifei Lvzhu Biopharmaceutical Co., Ltd. Bharat Biotech International Ltd.	Monovalent inactivated human rotavirus strain 116E	Preclinical
VLP VP2/6/7	Mitsubishi/Medicago	Virus-like particle	Phase 1
VLP VP2/4/6/7	Baylor College of Medicine	Virus-like particle	Preclinical
VP6 + norovirus	Tampere University, Finland	Nanoparticle: VP6 nanotubes or microspheres + norovirus viral-like protein admixture	Preclinical
VP6 + norovirus	Cincinnati Children's Hospital Medical Center	Sub-viral particles (norovirus S or P) expressing rotavirus VP8* particle	Preclinical
VP8* mRNA	CureVac	VP8* mRNA	Preclinical

Newer formulations of already licensed vaccines are not included.

the meeting proceedings¹⁴⁵. An overview of NRRV candidates currently in development is provided in Table 3.

Subunit vaccines

Furthest along in development is a trivalent P2-VP8* subunit vaccine developed by PATH. This vaccine consists of a truncated segment of VP8* that contains all known VP8*-specific neutralizing epitopes fused to the P2 tetanus toxoid T cell epitope.¹⁴⁶ An initial monovalent formulation consisting of VP8* derived from G1P[8] Wa strain rotavirus was well tolerated and immunogenic in a phase 1 trial among South African infants.¹⁴⁷ All children received Rotarix following completion of study vaccination, which provided a unique opportunity to assess mucosal immunity: if recipients of study vaccine inhibited shedding of Rotarix (a homotypic P [8] virus) following oral challenge, this would suggest that the antibodies induced by the parenteral vaccine provided sterilizing immunity in the gut mucosa. Indeed, those who received study vaccine had reduced frequencies of stool Rotarix shedding following the first Rotarix dose compared to placebo recipients, with the overall percent reduction in shedding at any measured time point in vaccinated infants compared to placebo recipients ranging from 49% to 66%, depending on the investigational vaccine dose received. However, serum neutralizing responses to heterotypic rotavirus strains were poor overall. Based on these results, a trivalent formulation that added DS1 strain P[4] VP8* and 1076 strain P[6] VP8* underwent a subsequent phase 1/2 trial in the same location. In this study, the trivalent formulation was well tolerated and robust IgG and neutralizing seroresponses to P[4], P[6], and P[8] rotaviruses were observed, although serum IgA responses to each individual antigen were modest (20–34%) across all three dosages evaluated.¹⁴⁸ Similar to the previous trial, Rotarix was given to all infants after study vaccination, and post-Rotarix shedding data were collected in a subset of infants: compared to placebo, significant reductions in shedding were again observed in infants who received parenteral vaccine, but only at the highest dose (90 µg) administered, which is quite high for a typical infant vaccine injection.¹⁴⁸

Since functional antibodies in the gut are primarily IgA, it remains to be seen whether strong serum IgG and neutralizing antibodies induced by this vaccine can mediate sufficient mucosal immunity to prevent symptomatic AGE due to rotavirus. Observational data demonstrating the importance of maternally derived serum RV-IgG in infants (see above) gives reason for optimism, but the extent to which VP8*-specific antibodies alone can mediate this effect is unknown. A commercial partner, SK Bioscience (Seoul, South Korea) has been identified for this vaccine and a multinational phase 3 efficacy trial evaluating three 90 µg doses of trivalent P2-VP8* at sites in Africa (Ghana, Malawi, Zambia) and India is currently in progress (ClinicalTrials.gov NCT04010488).

Inactivated vaccines

A monovalent, heat-inactivated whole virus vaccine consisting of G1P[8] strain CDC-9 human rotavirus developed by the Centers for Disease Control and Prevention is currently in development. Unlike other rotavirus strains, CDC-9 is

reported to grow to high titer and demonstrate very high stability in the infectious, triple-layered particle form.^{149,150} Since the mechanisms of immunity and the effectors necessary or sufficient for protection from rotavirus AGE remain incompletely understood, an inactivated, intact virion is an attractive choice for a non-replicating vaccine, since this would promote presentation of all surface neutralizing epitopes. This vaccine has undergone extensive pre-clinical evaluation, demonstrating strong induction of homotypic and heterotypic serum neutralizing antibody responses and reductions in fecal virus shedding upon oral challenge in vaccinated animals compared to placebo in multiple animal models,^{150–153} including when delivered intradermally using a novel-coated skin microneedle patch.^{152,154} Two commercial partners, Serum Institute of India Pvt. Ltd. (Pune, India) and Zhifei Lvzhu Biopharmaceutical Co., Ltd. (Beijing, China) have reported initiation of Good Manufacturing Process (GMP) production and regulatory approvals processes in preparation for phase 1 clinical trials¹⁴⁶.

Bharat Biotech is reportedly also developing an inactivated version of Rotavac for parenteral administration. No data regarding its progress or performance are publicly available to date¹⁴⁶.

Virus-like particle (VLP) and nanoparticle vaccines

Double- or triple-layered VLPs containing various combinations of all major capsid layer proteins (VP2, VP6, VP7, and/or VP4) expressed in recombinant baculovirus-infected insect cell culture have been developed as potential NRRV candidates by Baylor College of Medicine (Houston, TX).^{153,155} These candidates have also undergone extensive preclinical testing in multiple animal models, including the demonstration of broad heterotypic neutralizing antibody induction in mice immunized with a G1 VP7-containing construct.^{156–158} Immunization with this approach is now used to stimulate generation of protective antibodies in a commercial bovine colostrum formulation that was approved by the United States Department of Agriculture in 2017 for passive vaccination to prevent calf scours (First Defense Tri-Shield, Immucell, Portland, ME).¹⁵⁹ Despite successful use in veterinary medicine and the growing list of successful human VLP-based vaccines, insect cell-produced VLP vaccine candidates for rotavirus have yet to advance past the preclinical phase.

More recently, Medicago Inc. (Quebec City, Canada), a subsidiary of Mitsubishi Tanabe Pharma Corporation (Osaka, Japan), has applied its proprietary technology using plant-based VLP production toward development of a rotavirus VLP vaccine. A randomized, placebo-controlled, descending age dose-escalation phase 1 trial to evaluate the safety and immunogenicity of their candidate vaccine MT-5625 in adults, toddlers, and infants has been completed in Australia and South Africa (ClinicalTrials.gov NCT03507738), but results have not yet been released.

Several nanoparticle-based vaccines are also in the pre-clinical phase of development. A group at Tampere University, Finland, has developed a combined rotavirus/norovirus vaccine candidate containing self-assembled rotavirus VP6 nanoparticles expressed in recombinant

baculovirus-infected insect cells, including in both nanotube and microsphere configurations.^{160,161} Interestingly, VP6 appeared to have the additional benefit of acting as an adjuvant for norovirus immunogenicity in mice.¹⁶¹ Another proposed combination rotavirus/norovirus construct under development at Cincinnati Children's Hospital Medical Center (Cincinnati, OH) uses rotavirus VP8* expressed on the surface of recombinant norovirus P or S subviral particles. Noroviruses contain a single outer capsid structural protein, VP1, which contains two domains, the shell (S) and protrusion (P) domains, each of which can be independently expressed to form self-assembling sub-viral particles as a platform for foreign antigen presentation.¹⁶² Both P and S formulations were immunogenic in mice and the S-particle construct reduced fecal viral shedding in vaccinated mice following oral challenge with a VP8*-homologous strain.^{162,163}

mRNA vaccines

Finally, the Bill and Melinda Gates Foundation has partnered with CureVac (Tübingen, Germany) to leverage their mRNA-based technologies toward development of a rotavirus VP8* mRNA vaccine¹⁴⁶. This project is also in the preclinical stage of development and public data are not yet available. The overall mRNA approach has been evaluated as a plausible approach for vaccine development.¹⁶⁴

Future vaccine prospects

The recent development of a plasmid-based reverse genetics system for rotavirus is an important breakthrough with significant potential to accelerate rotavirus research. Hopefully, this new technology will augment existing platforms (as detailed above) and assist in development of new vaccine candidates.¹⁶⁵

Additional challenges

In addition to the issues related to vaccine development, clinical evaluation in the field remains challenging. Next-generation vaccines will likely be targeted specifically to LMIC, where large-scale field trials remain more difficult to perform, often due to relative limitations in research infrastructure, supplies procurement, and transportation, posing added challenges to overall research capacity. New studies may need to carefully consider and account for the growing list of biological and mathematical phenomena that may confound accurate measures of true vaccine efficacy. Furthermore, the availability and use of multiple WHO pre-qualified ORVs means that placebo-controlled efficacy trials are now difficult to justify ethically. Future studies may need to rely on active comparator arms, as was the approach adopted for the current phase 3 trivalent P2-VP8* efficacy study (NCT04010448).

Under such conditions, one potential approach would be using bridging studies comparing immunogenicity outcomes. The currently accepted standard for ORV immunogenicity, RV-IgA, may be a plausible approach for new oral vaccines, despite being a sub-optimal correlate of protection in LMIC.^{69,166} RV-IgA is likely a non-mechanistic correlate

of protection, as immunized infants without a post-immunization RV-IgA response still appear to have greater protection from rotavirus diarrhea than non-immunized infants, and similarly those with high RV-IgA may still develop disease.^{69,166} Nevertheless, current evidence suggests that it may be "reasonably likely to predict clinical benefit," although it has not been validated as a true surrogate endpoint.^{67,166} However, RV-IgA may not be as applicable for assessment of NRRVs, as immune responses induced by parenteral vaccination may differ from those induced by live-attenuated oral vaccines. Until a true rotavirus immune correlate of protection that can be induced by both parenteral and oral vaccines can be confirmed, trials of NRRVs are likely to ultimately require clinical endpoints. Non-inferiority studies may be a reasonable option, but opportunities to demonstrate improved efficacy compared to current vaccines may be significantly hindered by the substantially larger sample sizes required.

Conclusions

Current ORVs have had substantial impact on rotavirus morbidity and mortality throughout LMIC, but continue to underperform in these settings relative to high-income countries. Coupled with ongoing challenges related to ORV cost, supply, and access, the full global potential of rotavirus vaccines is still not being realized. Overcoming this challenge will require a multifaceted approach, taking into consideration the multiple factors that impact ORV performance and the likely need for newer and next-generation vaccines. Ongoing exploration to identify better immune correlates of protection following vaccination for both ORVs and NRRVs are needed. Indeed, the landscape for rotavirus vaccines may substantially change in coming years, and hopefully the second decade following universal WHO rotavirus vaccine pre-qualification will see increasing progress toward reducing rotavirus vaccine underperformance and rotavirus-associated morbidity and mortality among children in LMIC.

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References

- Troeger C, Khalil IA, Rao PC, Cao S, Blacker BF, Ahmed T, Armah G, Bines JE, Brewer TG, Colombara DV, et al. Rotavirus vaccination and the global burden of rotavirus diarrhoea among children younger than 5 years. *JAMA Pediatr.* 2018;172:958–65. doi:10.1001/jamapediatrics.2018.1960.
- Liu J, Platts-Mills JA, Juma J, Kabir F, Nkeze J, Okoi C, Operario DJ, Uddin J, Ahmed S, Alonso PL, 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(10051):1291–301. doi:10.1016/S0140-6736(16)31529-X.
- Platts-Mills JA, Liu J, Rogawski ET, Kabir F, Lertsethtakarn P, Sigua M, Khan SS, Prahara J, Murei A, Nshama R, et al. Use of quantitative molecular diagnostic methods to assess the aetiology, burden, and clinical characteristics of diarrhoea in children in low-resource settings: a reanalysis of the MAL-ED cohort study. *Lancet Glob Health.* 2018;6(12):e1309–e18. doi:10.1016/S2214-109X(18)30349-8.
- Clark A, Black R, Tate J, Roose A, Kotloff K, Lam D, Blackwelder W, Parashar U, Lanata C, Kang G, et al. Estimating global, regional and national rotavirus deaths in children aged <5 years: current approaches, new analyses and proposed improvements. *PLoS One.* 2017;12:e0183392.
- Tate JE, Burton AH, Boschi-Pinto C, Parashar UD. World health organization-coordinated global rotavirus surveillance N. Global, regional, and national estimates of rotavirus mortality in children <5 years of age, 2000–2013. *Clin Infect Dis.* 2016;62:S96–S105.
- Steele AD, Victor JC, Carey ME, Tate JE, Atherly DE, Pecinka C, Diaz Z, Parashar UD, Kirkwood CD. Experiences with rotavirus vaccines: can we improve rotavirus vaccine impact in developing countries? *Hum Vaccin Immunother.* 2019;15(6):1215–27. doi:10.1080/21645515.2018.1553593.
- Estes MK. Principles of Mucosal Immunology. In: Smith PD, MacDonald TT, Blumberg RS, editors. *Viral Infections: rotavirus infection.* New York, NY: Garland Science; 2013. p. 377–80.
- Greenberg HB, Estes MK. Rotaviruses: from pathogenesis to vaccination. *Gastroenterology.* 2009;136(6):1939–51. doi:10.1053/j.gastro.2009.02.076.
- Dennehy PH. Rotavirus vaccines: an overview. *Clin Microbiol Rev.* 2008;21(1):198–208. doi:10.1128/CMR.00029-07.
- Crawford SE, Ramani S, Tate JE, Parashar UD, Svensson L, Hagbom M, Franco MA, Greenberg HB, O’Ryan M, Kang G, et al. Rotavirus infection. *Nat Rev Dis Primers.* 2017;3:17083.
- Hagbom M, Istrate C, Engblom D, Karlsson T, Rodriguez-Diaz J, Buesa J, Taylor JA, Laitto VM, Magnusson KE, Ahlman H, et al. Rotavirus stimulates release of serotonin (5-HT) from human enterochromaffin cells and activates brain structures involved in nausea and vomiting. *PLoS Pathog.* 2011;7:e1002115. doi:10.1371/journal.ppat.1002115.
- Angel J, Franco MA, Greenberg HB. Rotavirus vaccines: recent developments and future considerations. *Nat Rev Microbiol.* 2007;5:529–39.
- Dennehy PH. Transmission of rotavirus and other enteric pathogens in the home. *Pediatr Infect Dis J.* 2000;19(Supplement):S103–5. doi:10.1097/00006454-200010001-00003.
- Velazquez FR, Matson DO, Calva JJ, Guerrero L, Morrow AL, Carter-Campbell S, Glass RI, Estes MK, Pickering LK, Ruiz-Palacios GM. Rotavirus infection in infants as protection against subsequent infections. *N Engl J Med.* 1996;335(14):1022–28. doi:10.1056/NEJM199610033351404.
- Gladstone BP, Ramani S, Mukhopadhyaya I, Muliylil J, Sarkar R, Rehman AM, Jaffar S, Gomara MI, Gray JJ, Brown DW, et al. Protective effect of natural rotavirus infection in an Indian birth cohort. *N Engl J Med.* 2011;365(4):337–46. doi:10.1056/NEJMoa1006261.
- Ramig RF. Pathogenesis of intestinal and systemic rotavirus infection. *J Virol.* 2004;78(19):10213–20. doi:10.1128/JVI.78.19.10213-10220.2004.
- Payne DC, Parashar U. Chapter 13: rotavirus. Roush SW, Baldy LB, Kirkconnell Hall MA, editors. *Manual for the surveillance of vaccine-preventable diseases: centers for disease control and prevention.* Last reviewed. May 18 2018.
- WHO Prequalified Vaccines. 2020 September 9 [accessed 2020 September 10]. <https://extranet.who.int/pqvdata/>.
- Ruiz-Palacios GM, Perez-Schael I, Velazquez FR, Abate H, Breuer T, Clemens SC, Chevart B, Espinoza F, Gillard P, Innis BL, et al. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. *N Engl J Med.* 2006;354(1):11–22. doi:10.1056/NEJMoa052434.
- Vesikari T, Matson DO, Dennehy P, Van Damme P, Santosham M, Rodriguez Z, Dallas MJ, Heyse JF, Goveia MG, Black SB, et al. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. *N Engl J Med.* 2006;354:23–33. doi:10.1056/NEJMoa052664.
- Armah GE, Sow SO, Breiman RF, Dallas MJ, Tapia MD, Feikin DR, Binka FN, Steele AD, Laserson KF, Anshah NA, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in sub-Saharan Africa: a randomised, double-blind, placebo-controlled trial. *Lancet.* 2010;376(9741):606–14. doi:10.1016/S0140-6736(10)60889-6.
- Colgate ER, Haque R, Dickson DM, Carmolli MP, Mychaleckyj JC, Nayak U, Qadri F, Alam M, Walsh MC, Diehl SA, et al. Delayed dosing of oral rotavirus vaccine demonstrates decreased risk of rotavirus gastroenteritis associated with serum zinc: a randomized controlled trial. *Clin Infect Dis.* 2016;63(5):634–41. doi:10.1093/cid/ciw346.
- Madhi SA, Cunliffe NA, Steele D, Witte D, Kirsten M, Louw C, Ngwira B, Victor JC, Gillard PH, Chevart BB, et al. Effect of human rotavirus vaccine on severe diarrhoea in African infants. *N Engl J Med.* 2010;362(4):289–98. doi:10.1056/NEJMoa0904797.
- Zaman K, Dang DA, Victor JC, Shin S, Yunus M, Dallas MJ, Podder G, Vu DT, Le TP, Luby SP, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in Asia: a randomised, double-blind, placebo-controlled trial. *Lancet.* 2010;376:615–23. doi:10.1016/S0140-6736(10)60755-6.
- WHO. Rotavirus vaccines: an update. *Wkly Epidemiol Rec.* 2009; 84: 533–40.
- Jonesteller CL, Burnett E, Yen C, Tate JE, Parashar UD. Effectiveness of rotavirus vaccination: a systematic review of the first decade of global postlicensure data, 2006–2016. *Clin Infect Dis.* 2017;65:840–50. doi:10.1093/cid/cix369.
- Zaman K, Sack DA, Neuzil KM, Yunus M, Moulton LH, Sugimoto JD, Fleming JA, Hossain I, Arifeen SE, Azim T, et al. Effectiveness of a live oral human rotavirus vaccine after programmatic introduction in Bangladesh: A cluster-randomized trial. *PLoS Med.* 2017;14(4):e1002282. doi:10.1371/journal.pmed.1002282.
- Bonkougou IJO, Aliabadi N, Leshem E, Kam M, Nezien D, Drabo MK, Nikiema M, Ouedraogo B, Medah I, Konate S, et al. Impact and effectiveness of pentavalent rotavirus vaccine in children <5 years of age in Burkina Faso. *Vaccine.* 2018;36:7170–78.
- Ali Z, Harastani H, Hammadi M, Reslan L, Ghanem S, Hajar F, Sabra A, Haidar A, Inati A, Rajab M, et al. Rotavirus genotypes and vaccine effectiveness from a sentinel, hospital-based, surveillance study for three consecutive rotavirus seasons in Lebanon. *PLoS One.* 2016;11(8):e0161345. doi:10.1371/journal.pone.0161345.
- Bennett A, Pollock L, Jere KC, Pitzer VE, Parashar U, Tate JE, Heyderman RS, Mwansambo C, French N, Nakagomi O, et al. Direct and possible indirect effects of vaccination on rotavirus hospitalisations among children in Malawi four years after programmatic introduction. *Vaccine.* 2018;36(47):7142–48. doi:10.1016/j.vaccine.2018.04.030.
- Lopez AL, Daag JV, Esparagoza J, Bonifacio J, Fox K, Nyambat B, Parashar UD, Ducusin MJ, Tate JE. Effectiveness of monovalent rotavirus vaccine in the Philippines. *Sci Rep.* 2018;8(1):14291. doi:10.1038/s41598-018-32595-9.

32. Jani B, Hokororo A, McHomvu J, Cortese MM, Kamugisha C, Mujuni D, Kallova D, Parashar UD, Mwenda JM, Lyimo D, et al. Detection of rotavirus before and after monovalent rotavirus vaccine introduction and vaccine effectiveness among children in mainland Tanzania. *Vaccine*. 2018;36(47):7149–56. doi:10.1016/j.vaccine.2018.01.071.
33. Platts-Mills JA, Amour C, Gratz J, Nshama R, Walongo T, Mujaga B, Maro A, McMurry TL, Liu J, Mduma E, et al. Impact of rotavirus vaccine introduction and postintroduction etiology of diarrhea requiring hospital admission in Haydom, Tanzania, a rural African setting. *Clin Infect Dis*. 2017;65(7):1144–51. doi:10.1093/cid/cix494.
34. Tharmaphornpilas P, Jiamsiri S, Boonchaiya S, Rochanathimoke O, Thinyoung W, Tuntiwitayapun S, Guntapong R, Riewpaiboon A, Rasdjarmrearnsook AO, Glass RI. Evaluating the first introduction of rotavirus vaccine in Thailand: moving from evidence to policy. *Vaccine*. 2017;35:796–801. doi:10.1016/j.vaccine.2016.12.043.
35. Mujuru HA, Burnett E, Nathoo KJ, Ticklay I, Gonah NA, Mukaratirwa A, Berejena C, Manangazira P, Rupfute M, Weldegebriel GG, et al. Monovalent rotavirus vaccine effectiveness against rotavirus hospitalizations among children in Zimbabwe. *Clin Infect Dis*. 2019;69(8):1339–44. doi:10.1093/cid/ciy1096.
36. Burnett E, Parashar UD, Tate JE. Global impact of rotavirus vaccination on diarrhea hospitalizations and deaths among children <5 years old: 2006–2019. *J Infect Dis*. 2020. doi:10.1093/infdis/jiaa081.
37. Aliabadi N, Antoni S, Mwenda JM, Weldegebriel G, Biey JNM, Cheikh D, Fahmy K, Tebeb N, Ashmony HA, Ahmed H, et al. Global impact of rotavirus vaccine introduction on rotavirus hospitalisations among children under 5 years of age, 2008–16: findings from the global rotavirus surveillance Network. *Lancet Glob Health*. 2019;7:e893–e903. doi:10.1016/S2214-109X(19)30207-4.
38. Shioda K, de Oliveira LH, Sanwogou J, Rey-Benito G, Nunez Azzad D, Castillo RE, Gamarra Ramirez ML, Von Horoch MR, Weinberger DM, Pitzer VE. Identifying signatures of the impact of rotavirus vaccines on hospitalizations using sentinel surveillance data from Latin American countries. *Vaccine*. 2020;38(2):323–29. doi:10.1016/j.vaccine.2019.10.010.
39. Weldegebriel G, Mwenda JM, Chakauya J, Daniel F, Masresha B, Parashar UD, Tate JE. Impact of rotavirus vaccine on rotavirus diarrhoea in countries of East and Southern Africa. *Vaccine*. 2018;36(47):7124–30. doi:10.1016/j.vaccine.2017.10.050.
40. Bar-Zeev N, King C, Phiri T, Beard J, Mvula H, Crampin AC, Heinsbroek E, Lewycka S, Tate JE, Parashar UD, et al. Impact of monovalent rotavirus vaccine on diarrhoea-associated post-neonatal infant mortality in rural communities in Malawi: a population-based birth cohort study. *Lancet Glob Health*. 2018;6(9):e1036–e44. doi:10.1016/S2214-109X(18)30314-0.
41. Inchauste L, Patzi M, Halvorsen K, Solano S, Montesano R, Iniguez V. Impact of rotavirus vaccination on child mortality, morbidity, and rotavirus-related hospitalizations in Bolivia. *Int J Infect Dis*. 2017;61:79–88. doi:10.1016/j.ijid.2017.06.006.
42. Shah MP, Tate JE, Mwenda JM, Steele AD, Parashar UD. Estimated reductions in hospitalizations and deaths from childhood diarrhea following implementation of rotavirus vaccination in Africa. *Expert Rev Vaccines*. 2017;16(10):987–95. doi:10.1080/14760584.2017.1371595.
43. Burnett E, Tate JE, Kirkwood CD, Nelson EAS, Santosham M, Steele AD, Parashar UD. Estimated impact of rotavirus vaccine on hospitalizations and deaths from rotavirus diarrhea among children <5 in Asia. *Expert Rev Vaccines*. 2018;17:453–60.
44. Rosettie KL, Vos T, Mokdad AH, Flaxman AD, Khalil I, Troeger C, Weaver MR. Indirect rotavirus vaccine effectiveness for the prevention of rotavirus hospitalization: a systematic review and meta-analysis. *Am J Trop Med Hyg*. 2018;98(4):1197–201. doi:10.4269/ajtmh.17-0705.
45. Saha S, Santosham M, Hussain M, Black RE, Saha SK. Rotavirus vaccine will improve child survival by more than just preventing diarrhea: evidence from Bangladesh. *Am J Trop Med Hyg*. 2018;98(2):360–63. doi:10.4269/ajtmh.17-0586.
46. Bhandari N, Rongsen-Chandola T, Bavdekar A, John J, Antony K, Taneja S, Goyal N, Kawade A, Kang G, Rathore SS, et al. Efficacy of a monovalent human-bovine (116E) rotavirus vaccine in Indian infants: a randomised, double-blind, placebo-controlled trial. *Lancet*. 2014;383(9935):2136–43. doi:10.1016/S0140-6736(13)62630-6.
47. Bhandari N, Rongsen-Chandola T, Bavdekar A, John J, Antony K, Taneja S, Goyal N, Kawade A, Kang G, Rathore SS, et al. Efficacy of a monovalent human-bovine (116E) rotavirus vaccine in Indian children in the second year of life. *Vaccine*. 2014;32(Suppl 1):A110–6. doi:10.1016/j.vaccine.2014.04.079.
48. Naik SP, Zade JK, Sabale RN, Pisal SS, Menon R, Bankar SG, Gairola S, Dhare RM. Stability of heat stable, live attenuated Rotavirus vaccine (ROTASII®). *Vaccine*. 2017;35(22):2962–69. doi:10.1016/j.vaccine.2017.04.025.
49. Isanaka S, Guindo O, Langendorf C, Matar Seck A, Plikaytis BD, Sayinzoga-Makombe N, McNeal MM, Meyer N, Adehossi E, Djibo A, et al. Efficacy of a low-cost, heat-stable oral rotavirus vaccine in Niger. *N Engl J Med*. 2017;376(12):1121–30. doi:10.1056/NEJMoa1609462.
50. Kulkarni PS, Desai S, Tewari T, Kawade A, Goyal N, Garg BS, Kumar D, Kanungo S, Kamat V, Kang G, et al. A randomized Phase III clinical trial to assess the efficacy of a bovine-human reassortant pentavalent rotavirus vaccine in Indian infants. *Vaccine*. 2017;35(45):6228–37. doi:10.1016/j.vaccine.2017.09.014.
51. Rathi N, Desai S, Kawade A, Venkatramanan P, Kundu R, Lalwani SK, Dubey AP, Venkateswara Rao J, Narayanappa D, Ghildiyal R, et al. A Phase III open-label, randomized, active controlled clinical study to assess safety, immunogenicity and lot-to-lot consistency of a bovine-human reassortant pentavalent rotavirus vaccine in Indian infants. *Vaccine*. 2018;36(52):7943–49. doi:10.1016/j.vaccine.2018.11.006.
52. Kawade A, Babji S, Kamath V, Raut A, Kumar CM, Kundu R, Venkatramanan P, Lalwani SK, Bavdekar A, Juvekar S, et al. Immunogenicity and lot-to-lot consistency of a ready to use liquid bovine-human reassortant pentavalent rotavirus vaccine (ROTASII - Liquid) in Indian infants. *Vaccine*. 2019;37(19):2554–60. doi:10.1016/j.vaccine.2019.03.067.
53. Malik A, Haldar P, Ray A, Shet A, Kapuria B, Bhadana S, Santosham M, Ghosh RS, Steinglass R, Kumar R. Introducing rotavirus vaccine in the universal immunization programme in India: from evidence to policy to implementation. *Vaccine*. 2019;37(39):5817–24. doi:10.1016/j.vaccine.2019.07.104.
54. Debellut F, Jaber S, Bouzya Y, Sabbah J, Barham M, Abu-Awwad F, Hjaija D, Ramlawi A, Pecenka C, Clark A, et al. Introduction of rotavirus vaccination in Palestine: an evaluation of the costs, impact, and cost-effectiveness of ROTARIX and ROTAVAC. *PLoS One*. 2020;15(2):e0228506. doi:10.1371/journal.pone.0228506.
55. Kirkwood CD, Steele AD. Rotavirus vaccines in China: improvement still required. *JAMA Netw Open*. 2018;1(4):e181579. doi:10.1001/jamanetworkopen.2018.1579.
56. Fu C, Wang M, Liang J, He T, Wang D, Xu J. Effectiveness of Lanzhou lamb rotavirus vaccine against rotavirus gastroenteritis requiring hospitalization: a matched case-control study. *Vaccine*. 2007;25(52):8756–61. doi:10.1016/j.vaccine.2007.10.036.
57. Li J, Zhang Y, Yang Y, Liang Z, Tian Y, Liu B, Gao Z, Jia L, Chen L, Wang Q. Effectiveness of Lanzhou lamb rotavirus vaccine in preventing gastroenteritis among children younger than 5 years of age. *Vaccine*. 2019;37:3611–16. doi:10.1016/j.vaccine.2019.03.069.
58. Fu C, He Q, Xu J, Xie H, Ding P, Hu W, Dong Z, Liu X, Wang M. Effectiveness of the Lanzhou lamb rotavirus vaccine against gastroenteritis among children. *Vaccine*. 2012;31(1):154–58. doi:10.1016/j.vaccine.2012.10.078.
59. Fu C, Tate JE, Jiang B. Effectiveness of Lanzhou lamb rotavirus vaccine against hospitalized gastroenteritis: further analysis and update. *Hum Vaccin*. 2010;6:953. doi:10.4161/hv.6.11.12847.
60. Zhen SS, Li Y, Wang SM, Zhang XJ, Hao ZY, Chen Y, Wang D, Zhang YH, Zhang ZY, Ma JC, et al. Effectiveness of the live attenuated rotavirus vaccine produced by a domestic manufacturer

- in China studied using a population-based case-control design. *Emerg Microbes Infect.* 2015;4:e64. doi:10.1038/emi.2015.64.
61. Dang DA, Nguyen VT, Vu DT, Nguyen TH, Nguyen DM, Yuhuan W, Baoming J, Nguyen DH, Le TL, Rotavin MVTG. A dose-escalation safety and immunogenicity study of a new live attenuated human rotavirus vaccine (Rotavin-M1) in Vietnamese children. *Vaccine.* 2012;30(Suppl 1):A114–21. doi:10.1016/j.vaccine.2011.07.118.
 62. Xia S, Du J, Su J, Liu Y, Huang L, Yu Q, Xie Z, Gao J, Xu B, Gao X, et al. Efficacy, immunogenicity and safety of a trivalent live human-lamb reassortant rotavirus vaccine (LLR3) in healthy Chinese infants: A randomized, double-blind, placebo-controlled trial. *Vaccine.* 2020;38(46):7393–400. doi:10.1016/j.vaccine.2020.04.038.
 63. Li RC, Huang T, Li Y, Luo D, Tao J, Fu B, Si G, Nong Y, Mo Z, Liao X, et al. Human rotavirus vaccine (RIX4414) efficacy in the first two years of life: a randomized, placebo-controlled trial in China. *Hum Vaccin Immunother.* 2014;10:11–18. doi:10.4161/hv.26319.
 64. Mo Z, Mo Y, Li M, Tao J, Yang X, Kong J, Wei D, Fu B, Liao X, Chu J, et al. Efficacy and safety of a pentavalent live human-bovine reassortant rotavirus vaccine (RV5) in healthy Chinese infants: A randomized, double-blind, placebo-controlled trial. *Vaccine.* 2017;35(43):5897–904. doi:10.1016/j.vaccine.2017.08.081.
 65. Parker EP, Ramani S, Lopman BA, Church JA, Iturriza-Gomara M, Prendergast AJ, Grassly NC. Causes of impaired oral vaccine efficacy in developing countries. *Future Microbiol.* 2018;13:97–118. doi:10.2217/fmb-2017-0128.
 66. Desselberger U. Differences of rotavirus vaccine effectiveness by country: likely causes and contributing factors. *Pathogens.* 2017;6(4):65. doi:10.3390/pathogens6040065.
 67. Angel J, Steele AD, Franco MA. Correlates of protection for rotavirus vaccines: possible alternative trial endpoints, opportunities, and challenges. *Hum Vaccin Immunother.* 2014;10(12):3659–71. doi:10.4161/hv.34361.
 68. Chevart B, Neuzil KM, Steele AD, Cunliffe N, Madhi SA, Karkada N, Han HH, Vinals C. Association of serum anti-rotavirus immunoglobulin A antibody seropositivity and protection against severe rotavirus gastroenteritis: analysis of clinical trials of human rotavirus vaccine. *Hum Vaccin Immunother.* 2014;10(2):505–11. doi:10.4161/hv.27097.
 69. Lee B, Carmolli M, Dickson DM, Colgate ER, Diehl SA, Uddin MI, Islam S, Hossain M, Rafique TA, Bhuiyan TR, 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. doi:10.1093/cid/ciy076.
 70. Liu GF, Hille D, Kaplan SS, Goveia MG. Postdose 3 G1 serum neutralizing antibody as correlate of protection for pentavalent rotavirus vaccine. *Hum Vaccin Immunother.* 2017;13(10):2357–63. doi:10.1080/21645515.2017.1356522.
 71. Angel J, Franco MA, Greenberg HB. Rotavirus immune responses and correlates of protection. *Curr Opin Virol.* 2012;2(4):419–25. doi:10.1016/j.coviro.2012.05.003.
 72. Church JA, Parker EP, Kirkpatrick BD, Grassly NC, Prendergast AJ. Interventions to improve oral vaccine performance: a systematic review and meta-analysis. *Lancet Infect Dis.* 2019;19(2):203–14. doi:10.1016/S1473-3099(18)30602-9.
 73. Gruber JF, Gruber LM, Weber RP, Becker-Dreps S, Jonsson Funk M. Rotavirus vaccine schedules and vaccine response among infants in low- and middle-income countries: a systematic review. *Open Forum Infect Dis.* 2017;4(2):ofx066. doi:10.1093/ofid/ofx066.
 74. Kirkpatrick BD, Colgate ER, Mychaleckyj JC, Haque R, Dickson DM, Carmolli MP, Nayak U, Taniuchi M, Naylor C, Qadri F, et al. The “performance of rotavirus and oral polio vaccines in developing countries” (PROVIDE) study: description of methods of an interventional study designed to explore complex biologic problems. *Am J Trop Med Hyg.* 2015;92:744–51.
 75. Gruber JF, Becker-Dreps S, Hudgens MG, Brookhart MA, Thomas JC, Jonsson Funk M. Timing of rotavirus vaccine doses and severe rotavirus gastroenteritis among vaccinated infants in low- and middle-income countries. *Epidemiology.* 2018;29(6):867–75. doi:10.1097/EDE.0000000000000909.
 76. Armah G, Lewis KD, Cortese MM, Parashar UD, Anshah A, Gazley L, Victor JC, McNeal MM, Binka F, Steele AD, et al. Controlled trial of the impact of alternative dosing schedules on the immune response to human rotavirus vaccine in rural Ghanaian infants. *J Infect Dis.* 2016;213:1678–85. doi:10.1093/infdis/jiw023.
 77. Ali SA, Kazi AM, Cortese MM, Fleming JA, Parashar UD, Jiang B, McNeal MM, Steele D, Bhutta Z, Zaidi A. 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):1772–79. doi:10.1093/infdis/jiu335.
 78. Kompithra RZ, Paul A, Manoharan D, Babji S, Sarkar R, Mathew LG, Kang G. Immunogenicity of a three dose and five dose oral human rotavirus vaccine (RIX4414) schedule in south Indian infants. *Vaccine.* 2014;32(Suppl 1):A129–33. doi:10.1016/j.vaccine.2014.03.002.
 79. Cunliffe NA, Witte D, Ngwira BM, Todd S, Bostock NJ, Turner AM, Chimpeni P, Victor JC, Steele AD, Bouckennooghe A, et al. Efficacy of human rotavirus vaccine against severe gastroenteritis in Malawian children in the first two years of life: a randomized, double-blind, placebo controlled trial. *Vaccine.* 2012;30(Suppl 1):A36–43. doi:10.1016/j.vaccine.2011.09.120.
 80. Madhi SA, Kirsten M, Louw C, Bos P, Aspinall S, Bouckennooghe A, Neuzil KM, Steele AD. Efficacy and immunogenicity of two or three dose rotavirus-vaccine regimen in South African children over two consecutive rotavirus-seasons: a randomized, double-blind, placebo-controlled trial. *Vaccine.* 2012;30(Suppl 1):A44–51. doi:10.1016/j.vaccine.2011.08.080.
 81. WHO. Rotavirus vaccines. WHO position paper - January 2013. *Wkly Epidemiol Rec.* 2013; 88: 49–64.
 82. Patel M, Glass RI, Jiang B, Santosham M, Lopman B, Parashar U. A systematic review of anti-rotavirus serum IgA antibody titer as a potential correlate of rotavirus vaccine efficacy. *J Infect Dis.* 2013;208(2):284–94. doi:10.1093/infdis/jit166.
 83. Clark A, van Zandvoort K, Flasche S, Sanderson C, Bines J, Tate J, Parashar U, Jit M. Efficacy of live oral rotavirus vaccines by duration of follow-up: a meta-regression of randomised controlled trials. *Lancet Infect Dis.* 2019;19(7):717–27. doi:10.1016/S1473-3099(19)30126-4.
 84. Zaman K, Fleming JA, Victor JC, Yunus M, Bari TI, Azim T, Rahman M, Mowla SM, Bellini WJ, McNeal M, et al. Noninterference of rotavirus vaccine with measles-rubella vaccine at 9 months of age and improvements in antirotavirus immunity: a randomized trial. *J Infect Dis.* 2016;213(11):1686–93. doi:10.1093/infdis/jiw024.
 85. Burnett E, Lopman BA, Parashar UD. Potential for a booster dose of rotavirus vaccine to further reduce diarrhea mortality. *Vaccine.* 2017;35(51):7198–203. doi:10.1016/j.vaccine.2017.10.027.
 86. Dennehy PH, Brady RC, Halperin SA, Ward RL, Alvey JC, Fischer FH Jr., Innis BL, Rathfon H, Schuind A, De Vos B, et al. Comparative evaluation of safety and immunogenicity of two dosages of an oral live attenuated human rotavirus vaccine. *Pediatr Infect Dis J.* 2005;24(6):481–88. doi:10.1097/01.inf.0000164763.55558.71.
 87. Phua KB, Quak SH, Lee BW, Emmanuel SC, Goh P, Han HH, De Vos B, Bock HL. Evaluation of RIX4414, a live, attenuated rotavirus vaccine, in a randomized, double-blind, placebo-controlled phase 2 trial involving 2464 Singaporean infants. *J Infect Dis.* 2005;192(Suppl 1):S6–S16. doi:10.1086/431511.
 88. Salinas B, Perez Schael I, Linhares AC, Ruiz Palacios GM, Guerrero ML, Yarzabal JP, Cervantes Y, Costa Clemens S, Damaso S, Hardt K, et al. Evaluation of safety, immunogenicity and efficacy of an attenuated rotavirus vaccine, RIX4414: A randomized, placebo-controlled trial in Latin American infants. *Pediatr Infect Dis J.* 2005;24:807–16. doi:10.1097/01.inf.0000178294.13954.a1.

89. Vesikari T, Karvonen A, Korhonen T, Espo M, Lebacqz E, Forster J, Zepp F, Delem A, De Vos B. Safety and immunogenicity of RIX4414 live attenuated human rotavirus vaccine in adults, toddlers and previously uninfected infants. *Vaccine*. 2004;22(21--22):2836–42. doi:10.1016/j.vaccine.2004.01.044.
90. Vesikari T, Clark HF, Offit PA, Dallas MJ, DiStefano DJ, Gouveia MG, Ward RL, Schodol F, Karvonen A, Drummond JE, et al. Effects of the potency and composition of the multivalent human-bovine (WC3) reassortant rotavirus vaccine on efficacy, safety and immunogenicity in healthy infants. *Vaccine*. 2006;24(22):4821–29. doi:10.1016/j.vaccine.2006.03.025.
91. Dhingra MS, Kundu R, Gupta M, Kanungo S, Ganguly N, Singh MP, Bhattacharya MK, Ghosh R, Kumar R, Sur D, et al. Evaluation of safety and immunogenicity of a live attenuated tetravalent (G1-G4) Bovine-Human Reassortant Rotavirus vaccine (BRV-TV) in healthy Indian adults and infants. *Vaccine*. 2014;32(Suppl 1):A117–23. doi:10.1016/j.vaccine.2014.03.069.
92. Zade JK, Kulkarni PS, Desai SA, Sabale RN, Naik SP, Dhare RM. Bovine rotavirus pentavalent vaccine development in India. *Vaccine*. 2014;32(Suppl 1):A124–8. doi:10.1016/j.vaccine.2014.03.003.
93. Lee B, Dickson DM, Alam M, Afreen S, Kader A, Afrin F, Ferdousi T, Damon CF, Gullickson SK, McNeal MM, et al. The effect of increased inoculum on oral rotavirus vaccine take among infants in Dhaka, Bangladesh: A double-blind, parallel group, randomized, controlled trial. *Vaccine*. 2020;38(1):90–99. doi:10.1016/j.vaccine.2019.09.088.
94. Appaihgari MB, Glass R, Singh S, Taneja S, Rongsen-Chandola T, Bhandari N, Mishra S, Vrtati S. Transplacental rotavirus IgG interferes with immune response to live oral rotavirus vaccine ORV-116E in Indian infants. *Vaccine*. 2014;32(6):651–56. doi:10.1016/j.vaccine.2013.12.017.
95. Becker-Dreps S, Vilchez S, Velasquez D, Moon SS, Hudgens MG, Zambrana LE, Jiang B. Rotavirus-specific IgG antibodies from mothers' serum may inhibit infant immune responses to the pentavalent rotavirus vaccine. *Pediatr Infect Dis J*. 2015;34:115–16. doi:10.1097/INF.0000000000000481.
96. Moon SS, Groome MJ, Velasquez DE, Parashar UD, Jones S, Koen A, van Niekerk N, Jiang B, Madhi SA. Prevacination rotavirus serum IgG and IgA are associated with lower immunogenicity of live, oral human rotavirus vaccine in South African infants. *Clin Infect Dis*. 2016;62:157–65. doi:10.1093/cid/civ828.
97. Steele AD, Madhi SA, Cunliffe NA, Vesikari T, Phua KB, Lim FS, Nelson EA, Lau YL, Huang LM, Karkada N, et al. Incidence of rotavirus gastroenteritis by age in African, Asian and European children: relevance for timing of rotavirus vaccination. *Hum Vaccin Immunother*. 2016;12:2406–12. doi:10.1080/21645515.2016.1179412.
98. Hasso-Agopsowicz M, Ladva CN, Lopman B, Sanderson C, Cohen AL, Tate JE, Riveros X, Henao-Restrepo AM, Clark A; Global Rotavirus Surveillance N, et al. Global review of the age distribution of rotavirus disease in children aged <5 years before the introduction of rotavirus vaccination. *Clin Infect Dis*. 2019;69:1071–78.
99. Moon SS, Tate JE, Ray P, Dennehy PH, Archary D, Coutsooudis A, Bland R, Newell ML, Glass RI, Parashar U, et al. Differential profiles and inhibitory effect on rotavirus vaccines of nonantibody components in breast milk from mothers in developing and developed countries. *Pediatr Infect Dis J*. 2013;32:863–70.
100. Moon SS, Wang Y, Shane AL, Nguyen T, Ray P, Dennehy P, Baek LJ, Parashar U, Glass RI, Jiang B. Inhibitory effect of breast milk on infectivity of live oral rotavirus vaccines. *Pediatr Infect Dis J*. 2010;29:919–23. doi:10.1097/INF.0b013e3181e232ea.
101. Laucirica DR, Triantis V, Schoemaker R, Estes MK, Ramani S. Milk oligosaccharides inhibit human rotavirus infectivity in MA104 cells. *J Nutr*. 2017;147:1709–14.
102. Bautista-Marquez A, Velasquez DE, Esparza-Aguilar M, Luna-Cruz M, Ruiz-Moran T, Sugata K, Jiang B, Parashar U, Patel M, Richardson V. Breastfeeding linked to the reduction of both rotavirus shedding and IgA levels after Rotarix(R) immunization in Mexican infants. *Vaccine*. 2016;34:5284–89. doi:10.1016/j.vaccine.2016.09.006.
103. Groome MJ, Moon SS, Velasquez D, Jones S, Koen A, van Niekerk N, Jiang B, Parashar UD, Madhi SA. Effect of breastfeeding on immunogenicity of oral live-attenuated human rotavirus vaccine: a randomized trial in HIV-uninfected infants in Soweto, South Africa. *Bull World Health Organ*. 2014;92:238–45. doi:10.2471/BLT.13.128066.
104. Rongsen-Chandola T, Strand TA, Goyal N, Flem E, Rathore SS, Arya A, Winje BA, Lazarus R, Shanmugasundaram E, Babji S, et al. Effect of withholding breastfeeding on the immune response to a live oral rotavirus vaccine in North Indian infants. *Vaccine*. 2014;32(Suppl 1):A134–9. doi:10.1016/j.vaccine.2014.04.078.
105. Ali A, Kazi AM, Cortese MM, Fleming JA, Moon S, Parashar UD, Jiang B, McNeal MM, Steele D, Bhutta Z, et al. Impact of withholding breastfeeding at the time of vaccination on the immunogenicity of oral rotavirus vaccine—a randomized trial. *PLoS One*. 2015;10:e0127622. doi:10.1371/journal.pone.0127622.
106. Kambhampati A, Payne DC, Costantini V, Lopman BA. Host genetic susceptibility to enteric viruses: a systematic review and metaanalysis. *Clin Infect Dis*. 2016;62(1):11–18. doi:10.1093/cid/civ873.
107. Lee B, Dickson DM, deCamp AC, Ross Colgate E, Diehl SA, Uddin MI, Sharmin S, Islam S, Bhuiyan TR, Alam M, 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;217:1399–407. doi:10.1093/infdis/jiy054.
108. Sun X, Guo N, Li J, Yan X, He Z, Li D, Jin M, Xie G, Pang L, Zhang Q, et al. Rotavirus infection and histo-blood group antigens in the children hospitalized with diarrhoea in China. *Clin Microbiol Infect*. 2016;22(8):740 e1–3. doi:10.1016/j.cmi.2016.06.007.
109. Ayouni S, Sdiri-Loulizi K, de Rougemont A, Estienney M, Ambert-Balay K, Aho S, Hamami S, Aouni M, Neji-Guediche M, Pothier P, et al. Rotavirus P[8] Infections in persons with secretor and non-secretor phenotypes, Tunisia. *Emerg Infect Dis*. 2015;21(11):2055–58. doi:10.3201/eid2111.141901.
110. Nordgren J, Sharma S, Bucardo F, Nasir W, Gunaydin G, Ouermi D, Nitiema LW, Becker-Dreps S, Simpre J, Hammarstrom L, et al. Both Lewis and secretor status mediate susceptibility to rotavirus infections in a rotavirus genotype-dependent manner. *Clin Infect Dis*. 2014;59:1567–73. doi:10.1093/cid/ciu633.
111. Armah GE, Cortese MM, Dennis FE, Yu Y, Morrow AL, McNeal MM, Lewis KDC, Awuni DA, Armachie J, Parashar UD. Rotavirus vaccine take in infants is associated with secretor status. *J Infect Dis*. 2019;219(5):746–49. doi:10.1093/infdis/jiy573.
112. Kazi AM, Cortese MM, Yu Y, Lopman B, Morrow AL, Fleming JA, McNeal MM, Steele AD, Parashar UD, Zaidi AKM, et al. Secretor and salivary ABO blood group antigen status predict rotavirus vaccine take in infants. *J Infect Dis*. 2017;215(5):786–89. doi:10.1093/infdis/jix028.
113. Pollock L, Bennett A, Jere KC, Dube Q, Mandolo J, Bar-Zeev N, Heyderman RS, Cunliffe NA, Iturriza-Gomara M. Nonsecretor histo-blood group antigen phenotype is associated with reduced risk of clinical rotavirus vaccine failure in Malawian infants. *Clin Infect Dis*. 2019;69:1313–19. doi:10.1093/cid/ciy1067.
114. Williams FB, Kader A, Colgate ER, Dickson DM, Carmolli M, Uddin MI, Sharmin S, Islam S, Bhuiyan TR, Alam M, et al. Maternal secretor status affects oral rotavirus vaccine response in breastfed infants in Bangladesh. *J Infect Dis*. 2020. doi:10.1093/infdis/jiaa101.
115. Parker EPK, Praharaj I, Zekavati A, Lazarus RP, Giri S, Operario DJ, Liu J, Houpt E, Iturriza-Gomara M, Kampmann B, et al. Influence of the intestinal microbiota on the immunogenicity of oral rotavirus vaccine given to infants in south India. *Vaccine*. 2018;36(2):264–72. doi:10.1016/j.vaccine.2017.11.031.
116. Baker JM, Tate JE, Leon J, Haber MJ, Lopman BA. Antirotavirus IgA seroconversion rates in children who receive concomitant oral poliovirus vaccine: A secondary, pooled analysis of Phase II and III

- trial data from 33 countries. *PLoS Med.* 2019;16:e1003005. doi:10.1371/journal.pmed.1003005.
117. Church JA, Rogawski McQuade ET, Mutasa K, Taniuchi M, Rukobo S, Govha M, Lee B, Carmolli MP, Chasekwa B, Ntozini R, et al. Enteropathogens and rotavirus vaccine immunogenicity in a cluster randomized trial of improved water, sanitation and hygiene in rural Zimbabwe. *Pediatr Infect Dis J.* 2019;38(12):1242–48. doi:10.1097/INF.0000000000002485.
 118. Jorba J, Diop OM, Iber J, Henderson E, Zhao K, Quddus A, Sutter R, Vertefeuille JF, Wenger J, Wassilak SGF, et al. Update on vaccine-derived poliovirus outbreaks - worldwide, January 2018-June 2019. *MMWR Morb Mortal Wkly Rep.* 2019;68:1024–28. doi:10.15585/mmwr.mm6845a4.
 119. Polio endgame strategy 2019-2023: eradication, integration, certification and containment. Geneva, Switzerland: World Health Organization; 2019. (WHO/Polio/19.04). Licence: CC BY-NC-SA 3.0 IGO
 120. Strategy for the response to type 2 circulating vaccine-derived poliovirus 2020-2021: an addendum to the polio endgame strategy 2019-2023. Geneva, Switzerland: World Health Organization; 2020. (WHO/Polio/20.02). License CC BY-NC-SA 3.0 IGO
 121. Clinical summary for novel oral polio vaccine type 2 (nOPV2). Global polio eradication initiative; 2020 May [accessed 2020 June 01]. <http://polioeradication.org/nopv2/>.
 122. Taniuchi M, Platts-Mills JA, Begum S, Uddin MJ, Sobuz SU, Liu J, Kirkpatrick BD, Colgate ER, Carmolli MP, Dickson DM, et al. Impact of enterovirus and other enteric pathogens on oral polio and rotavirus vaccine performance in Bangladeshi infants. *Vaccine.* 2016;34(27):3068–75. doi:10.1016/j.vaccine.2016.04.080.
 123. Harris V, Ali A, Fuentes S, Korpela K, Kazi M, Tate J, Parashar U, Wiersinga WJ, Giaquinto C, de Weerth C, et al. Rotavirus vaccine response correlates with the infant gut microbiota composition in Pakistan. *Gut Microbes.* 2018;9(2):93–101. doi:10.1080/19490976.2017.1376162.
 124. Harris VC, Armah G, Fuentes S, Korpela KE, Parashar U, Victor JC, Tate J, de Weerth C, Giaquinto C, Wiersinga WJ, et al. Significant Correlation Between the Infant Gut Microbiome and Rotavirus Vaccine Response in Rural Ghana. *J Infect Dis.* 2017;215(1):34–41. doi:10.1093/infdis/jiw518.
 125. Harris VC, Haak BW, Handley SA, Jiang B, Velasquez DE, Hykes BL Jr., Droit L, Berbers GAM, Kemper EM, van Leeuwen EMM, 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):197–207e4. doi:10.1016/j.chom.2018.07.005.
 126. Ramani S, Stewart CJ, Laucirica DR, Ajami NJ, Robertson B, Autran CA, Shinge D, Rani S, Anandan S, Hu L, et al. Human milk oligosaccharides, milk microbiome and infant gut microbiome modulate neonatal rotavirus infection. *Nat Commun.* 2018;9(1):5010. doi:10.1038/s41467-018-07476-4.
 127. Church JA, Rukobo S, Govha M, Lee B, Carmolli MP, Chasekwa B, Ntozini R, Mutasa K, McNeal MM, Majo FD, et al. The Impact of Improved Water, Sanitation, and Hygiene on Oral Rotavirus Vaccine Immunogenicity in Zimbabwean Infants: substudy of a Cluster-randomized Trial. *Clin Infect Dis.* 2019;69(12):2074–81. doi:10.1093/cid/ciz140.
 128. Rogawski McQuade ET, Platts-Mills JA, Gratz J, Zhang J, Moulton LH, Mutasa K, Majo FD, Tavengwa N, Ntozini R, Prendergast AJ, et al. Impact of water quality, sanitation, hand-washing, and nutritional interventions on enteric infections in rural Zimbabwe: the Sanitation Hygiene Infant Nutrition Efficacy (SHINE) trial. *J Infect Dis.* 2020;221(8):1379–86. doi:10.1093/infdis/jiz179.
 129. Lazarus RP, John J, Shanmugasundaram E, Rajan AK, Thiagarajan S, Giri S, Babji S, Sarkar R, Kaliappan PS, Venugopal S, et al. The effect of probiotics and zinc supplementation on the immune response to oral rotavirus vaccine: A randomized, factorial design, placebo-controlled study among Indian infants. *Vaccine.* 2018;36:273–79.
 130. Marie C, Ali A, Chandwe K, Petri WA Jr., Kelly P. Pathophysiology of environmental enteric dysfunction and its impact on oral vaccine efficacy. *Mucosal Immunol.* 2018;11(5):1290–98. doi:10.1038/s41385-018-0036-1.
 131. Church JA, Parker EP, Kosek MN, Kang G, Grassly NC, Kelly P, Prendergast AJ. Exploring the relationship between environmental enteric dysfunction and oral vaccine responses. *Future Microbiol.* 2018;13:1055–70. doi:10.2217/fmb-2018-0016.
 132. Liu TC, VanBuskirk K, Ali SA, Kelly MP, Holtz LR, Yilmaz OH, Sadiq K, Iqbal N, Amadi B, Syed S, et al. A novel histological index for evaluation of environmental enteric dysfunction identifies geographic-specific features of enteropathy among children with suboptimal growth. *PLoS Negl Trop Dis.* 2020;14:e0007975. doi:10.1371/journal.pntd.0007975.
 133. Kosek MN, Investigators M-EN. Causal pathways from enteropathogens to environmental enteropathy: findings from the MAL-ED birth cohort study. *EBioMedicine.* 2017;18:109–17. doi:10.1016/j.ebiom.2017.02.024.
 134. Naylor C, Lu M, Haque R, Mondal D, Buonomo E, Nayak U, Mychaleckyj JC, Kirkpatrick B, Colgate R, Carmolli M, et al. Environmental enteropathy, oral vaccine failure and growth faltering in infants in Bangladesh. *EBioMedicine.* 2015;2(11):1759–66. doi:10.1016/j.ebiom.2015.09.036.
 135. Mahfuz M, Das S, Mazumder RN, Masudur Rahman M, Haque R, Bhuiyan MM, Akhter H, Sarkar MSA, Mondal D, Muaz SSA, et al. Bangladesh Environmental Enteric Dysfunction (BEED) study: protocol for a community-based intervention study to validate non-invasive biomarkers of environmental enteric dysfunction. *BMJ Open.* 2017;7(8):e017768. doi:10.1136/bmjopen-2017-017768.
 136. Rogawski ET, Platts-Mills JA, Colgate ER, Haque R, Zaman K, Petri WA, Kirkpatrick BD. Quantifying the Impact of Natural Immunity on Rotavirus Vaccine Efficacy Estimates: A Clinical Trial in Dhaka, Bangladesh (PROVIDE) and a Simulation Study. *J Infect Dis.* 2018;217(6):861–68. doi:10.1093/infdis/jix668.
 137. Taniuchi M, Sobuz SU, Begum S, Platts-Mills JA, Liu J, Yang Z, Wang XQ, Petri WA Jr., Haque R, Hout ER. Etiology of diarrhea in Bangladeshi infants in the first year of life analyzed using molecular methods. *J Infect Dis.* 2013;208:1794–802. doi:10.1093/infdis/jit507.
 138. Mokomane M, Tate JE, Steenhoff AP, Esona MD, Bowen MD, Lechile K, Pernica JM, Kasvosve I, Parashar UD, Goldfarb DM. Evaluation of the Influence of gastrointestinal coinfections on rotavirus vaccine effectiveness in Botswana. *Pediatr Infect Dis J.* 2018;37(3):e58–e62. doi:10.1097/INF.0000000000001828.
 139. Praharaj I, Platts-Mills JA, Taneja S, Antony K, Yuhass K, Flores J, Cho I, Bhandari N, Revathy R, Bavdekar A, et al. Diarrheal Etiology and Impact of Coinfections on rotavirus vaccine efficacy estimates in a clinical trial of a monovalent human-bovine (116E) oral rotavirus vaccine, Rotavac, India. *Clin Infect Dis.* 2019;69:243–50. doi:10.1093/cid/ciy896.
 140. VIEW-hub.org. RV-Vaccine Access: #Children without Access. [accessed 2020 Sep17]. <https://view-hub.org/map/?set=children-without-access&group=vaccine-access&category=rv>.
 141. Chen MY, Kirkwood CD, Bines J, Cowley D, Pavlic D, Lee KJ, Orsini F, Watts E, Barnes G, Danchin M. Rotavirus specific maternal antibodies and immune response to RV3-BB neonatal rotavirus vaccine in New Zealand. *Hum Vaccin Immunother.* 2017;13:1126–35. doi:10.1080/21645515.2016.1274474.
 142. Bines JE, At Thobari J, Satria CD, Handley A, Watts E, Cowley D, Nirwati H, Ackland J, Standish J, Justice F, et al. Human neonatal rotavirus vaccine (RV3-BB) to target rotavirus from birth. *N Engl J Med.* 2018;378(8):719–30. doi:10.1056/NEJMoa1706804.
 143. Boniface K, Byars SG, Cowley D, Kirkwood CD, Bines JE. Human neonatal rotavirus vaccine (RV3-BB) produces vaccine take irrespective of histo-blood group antigen status. *J Infect Dis.* 2020;221(7):1070–78. doi:10.1093/infdis/jiz333.
 144. Cowley D, Sari RM, Handley A, Watts E, Bachtar NS, At Thobari J, Satria CD, Bogdanovic-Sakran N, Nirwati H, Orsini F, et al. Immunogenicity of four doses of oral poliovirus vaccine when

- co-administered with the human neonatal rotavirus vaccine (RV3-BB). *Vaccine*. 2019;37(49):7233–39. doi:10.1016/j.vaccine.2019.09.071.
145. Fix A, Kirkwood CD, Steele D, Flores J. Next-generation rotavirus vaccine developers meeting: Summary of a meeting sponsored by PATH and the Bill & Melinda Gates Foundation (19–20 June 2019, Geneva). *Vaccine*. 2020. doi:10.1016/j.vaccine.2020.11.034.
 146. Wen X, Wen K, Cao D, Li G, Jones RW, Li J, Szu S, Hoshino Y, Yuan L. Inclusion of a universal tetanus toxoid CD4(+) T cell epitope P2 significantly enhanced the immunogenicity of recombinant rotavirus ΔVP8* subunit parenteral vaccines. *Vaccine*. 2014;32:4420–27. doi:10.1016/j.vaccine.2014.06.060.
 147. Groome MJ, Koen A, Fix A, Page N, Jose L, Madhi SA, McNeal M, Dally L, Cho I, Power M, et al. Safety and immunogenicity of a parenteral P2-VP8-P[8] subunit rotavirus vaccine in toddlers and infants in South Africa: a randomised, double-blind, placebo-controlled trial. *Lancet Infect Dis*. 2017;17(8):843–53. doi:10.1016/S1473-3099(17)30242-6.
 148. Groome MJ, Fairlie L, Morrison J, Fix A, Koen A, Masanya M, Jose L, Madhi SA, Page N, McNeal M, et al. Safety and immunogenicity of a parenteral trivalent P2-VP8 subunit rotavirus vaccine: a multisite, randomised, double-blind, placebo-controlled trial. *Lancet Infect Dis*. 2020;20(7):851–63. doi:10.1016/S1473-3099(20)30001-3.
 149. Esona MD, Foytich K, Wang Y, Shin G, Wei G, Gentsch JR, Glass RI, Jiang B. Molecular characterization of human rotavirus vaccine strain CDC-9 during sequential passages in Vero cells. *Hum Vaccin*. 2010;6(3):247–53. doi:10.4161/hv.6.3.10409.
 150. Jiang B, Wang Y, Glass RI. Does a monovalent inactivated human rotavirus vaccine induce heterotypic immunity? Evidence from animal studies. *Hum Vaccin Immunother*. 2013;9(8):1634–37. doi:10.4161/hv.24958.
 151. Wang Y, Azevedo M, Saif LJ, Gentsch JR, Glass RI, Jiang B. Inactivated rotavirus vaccine induces protective immunity in gnotobiotic piglets. *Vaccine*. 2010;28(33):5432–36. doi:10.1016/j.vaccine.2010.06.006.
 152. Resch TK, Wang Y, Moon SS, Joyce J, Li S, Prausnitz M, Jiang B. Inactivated rotavirus vaccine by parenteral administration induces mucosal immunity in mice. *Sci Rep*. 2018;8:561. doi:10.1038/s41598-017-18973-9.
 153. Conner ME, Crawford SE, Barone C, O’Neal C, Zhou YJ, Fernandez F, Parwani A, Saif LJ, Cohen J, Estes MK. Rotavirus subunit vaccines. *Arch Virol Suppl*. 1996;12:199–206.
 154. Wang Y, Vlasova A, Velasquez DE, Saif LJ, Kandasamy S, Kochba E, Levin Y, Jiang B. Skin vaccination against rotavirus using microneedles: proof of concept in gnotobiotic piglets. *PLoS One*. 2016;11:e0166038. doi:10.1371/journal.pone.0166038.
 155. Crawford SE, Labbe M, Cohen J, Burroughs MH, Zhou YJ, Estes MK. Characterization of virus-like particles produced by the expression of rotavirus capsid proteins in insect cells. *J Virol*. 1994;68:5945–52. doi:10.1128/JVI.68.9.5945-5952.1994.
 156. Crawford SE, Estes MK, Ciarlet M, Barone C, O’Neal CM, Cohen J, Conner ME. Heterotypic protection and induction of a broad heterotypic neutralization response by rotavirus-like particles. *J Virol*. 1999;73(6):4813–22. doi:10.1128/JVI.73.6.4813-4822.1999.
 157. Jiang B, Estes MK, Barone C, Barniak V, O’Neal CM, Ottaiano A, Madore HP, Conner ME. Heterotypic protection from rotavirus infection in mice vaccinated with virus-like particles. *Vaccine*. 1999;17(7–8):1005–13. doi:10.1016/S0264-410X(98)00317-X.
 158. O’Neal CM, Crawford SE, Estes MK, Conner ME. Rotavirus virus-like particles administered mucosally induce protective immunity. *J Virol*. 1997;71:8707–17. doi:10.1128/JVI.71.11.8707-8717.1997.
 159. Fernandez FM, Conner ME, Hodgins DC, Parwani AV, Nielsen PR, Crawford SE, Estes MK, Saif LJ. Passive immunity to bovine rotavirus in newborn calves fed colostrum supplements from cows immunized with recombinant SA11 rotavirus core-like particle (CLP) or virus-like particle (VLP) vaccines. *Vaccine*. 1998;16:507–16. doi:10.1016/S0264-410X(97)80004-7.
 160. Blazevic V, Lappalainen S, Nurminen K, Huhti L, Vesikari T. Norovirus VLPs and rotavirus VP6 protein as combined vaccine for childhood gastroenteritis. *Vaccine*. 2011;29(45):8126–33. doi:10.1016/j.vaccine.2011.08.026.
 161. Malm M, Heinimäki S, Vesikari T, Blazevic V. Rotavirus capsid VP6 tubular and spherical nanostructures act as local adjuvants when co-delivered with norovirus VLPs. *Clin Exp Immunol*. 2017;189(3):331–41. doi:10.1111/cei.12977.
 162. Tan M, Huang P, Xia M, Fang PA, Zhong W, McNeal M, Wei C, Jiang W, Jiang X. Norovirus P particle, a novel platform for vaccine development and antibody production. *J Virol*. 2011;85:753–64. doi:10.1128/JVI.01835-10.
 163. Xia M, Huang P, Jiang X, Tan M. Immune response and protective efficacy of the S particle presented rotavirus VP8* vaccine in mice. *Vaccine*. 2019;37(30):4103–10. doi:10.1016/j.vaccine.2019.05.075.
 164. Liu MA. A comparison of plasmid DNA and mRNA as vaccine technologies. *Vaccines (Basel)*. 2019;7:37. doi:10.3390/vaccines7020037.
 165. Desselberger U. Potential of plasmid only based reverse genetics of rotavirus for the development of next-generation vaccines. *Curr Opin Virol*. 2020;44:1–6. doi:10.1016/j.coviro.2020.04.004.
 166. Holmgren J, Parashar UD, Plotkin S, Louis J, Ng SP, Desautiers E, Picot V, Saadatian-Elahi M. Correlates of protection for enteric vaccines. *Vaccine*. 2017;35:3355–63. doi:10.1016/j.vaccine.2017.05.005.