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Delayed Dosing of Oral Rotavirus Vaccine Demonstrates Decreased Risk of Rotavirus Gastroenteritis Associated With Serum Zinc: A Randomized Controlled Trial

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Background. Rotavirus is the world's leading cause of childhood diarrheal death. Despite successes, oral rotavirus vaccines are less effective in developing countries. In an urban slum of Dhaka, we performed active diarrhea surveillance to evaluate monovalent G1P[8] rotavirus vaccine (RV1) efficacy and understand variables contributing to risk of rotavirus diarrhea (RVD).

Methods. We performed a randomized controlled trial of monovalent oral rotavirus vaccine (RV1). Seven hundred healthy infants received RV1 or no RV1 (1:1) using delayed dosing (10 and 17 weeks) and were followed for 1 year. Intensive diarrhea surveillance was performed. The primary outcome was ≥1 episode of RVD. Nutritional, socioeconomic, and immunologic factors were assessed by logistic regression best-subsets analysis for association with risk of RVD and interactions with vaccine arm.

Results. Incidence of all RVD was 38.3 cases per 100 person-years. Per-protocol RV1 efficacy was 73.5% (95% confidence interval [CI], 45.8%–87.0%) against severe RVD and 51% (95% CI, 33.8%–63.7%) against all RVD. Serum zinc level (odds ratio [OR], 0.77; $P = .002$) and lack of rotavirus immunoglobulin A (IgA) seroconversion (OR, 1.95; $P = .018$) were associated with risk of RVD, independent of vaccination status. Water treatment and exclusive breastfeeding were of borderline significance. Factors not associated with RVD included height for age at 10 weeks, vitamin D, retinol binding protein, maternal education, household income, and sex.

Conclusions. In an urban slum with high incidence of RVD, the efficacy of RV1 against severe RVD was higher than anticipated in the setting of delayed dosing. Lower serum zinc level and lack of IgA seroconversion were associated with increased risk of RVD independent of vaccination.

Clinical Trials Registration. NCT01375647.

Keywords. rotavirus diarrhea; zinc; oral vaccine; underperformance; developing countries.

Rotavirus is the leading cause of child death from diarrhea. In 2008, prior to vaccine introduction, rotavirus diarrhea (RVD) caused approximately 453 000 deaths, most in South Asia and sub-Saharan Africa [1]. Three oral live attenuated rotavirus vaccines are now licensed and having tremendous impact: the 3-dose pentavalent human-bovine vaccine (RV5), the 2-dose monovalent G1P[8] vaccine (RV1), and the 3-dose human-bovine G9P[11] vaccine (116E). In phase 3 trials, RV5 and RV1 have robust efficacy of >85% in high-income countries, as measured by protection from severe RVD or related hospitalization [2, 3]. In contrast, efficacy is markedly lower in developing countries: in multiple clinical trials, oral rotavirus vaccine efficacy ranges from 18% to 61% in Africa and Asia [4–7].

Although lower rotavirus vaccine efficacy in developing countries is well established, little is understood about the biologic basis of vaccine underperformance. Previous efforts have postulated mechanisms related to the vaccine itself, including dosing schedule and inoculum, and factors impacting the child's ability to respond to vaccination [8]. The latter includes factors that prevent the vaccine from replicating in the intestine or blunt infant immune responses, such as breast milk and maternal antibody interference, enteropathy, and enteric coinfections [9–11]. Micronutrient deficiencies may also contribute; zinc specifically plays an extensive role in host defense and gut health, and deficiency has been associated with diarrheal morbidity and mortality [12, 13].

To assess factors related to RV1 performance, we enrolled a 700-child birth cohort in an urban slum of Dhaka, Bangladesh, in the Performance of Rotavirus and Oral Polio Vaccines in Developing Countries (PROVIDE) study: a randomized controlled trial of 2-dose RV1 vaccine using a delayed dosing schedule at 10

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and 17 weeks of age (compared to the Expanded Programme on Immunization [EPI]–recommended schedule of 6 and 10 weeks). With a primary outcome of any RVD post-vaccination to 1 year, we conducted biweekly home-based diarrhea surveillance for RVD. To inform public health interventions and vaccine development efforts, we determined RV1 efficacy in this population, assessed additional factors for associations with risk of RVD, and examined possible interactions with the vaccine.

METHODS

Study Design and Participants

As part of the PROVIDE study, we performed a randomized, open-label, controlled trial of live oral G1P[8] rotavirus vaccine (RV1) in a birth cohort of 700 children from the Mirpur urban slum in Dhaka, Bangladesh. Infants meeting inclusion and exclusion criteria were enrolled in the first week of life. Detailed study methods including consenting and eligibility criteria, as well as results from PROVIDE on the association of environmental enteropathy, enteric infection, and small intestinal bacterial overgrowth on health outcomes, are published elsewhere [11, 14–16]. The study was approved by the ethical review boards of the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), the University of Vermont, and the University of Virginia. The study was registered at Clinicaltrials.gov (NCT01375647).

Randomization and Masking

Children were randomized using permuted blocks with random block size selection (4 or 8) and assigned to 1 of 2 treatment groups: 50% (n = 350) to receive RV1 vaccine at weeks 10 and 17, and 50% no rotavirus vaccine. All clinical investigators and laboratories were masked to vaccine arm, but medical officers were not.

Procedures

The study was conducted from May 2011 through November 2013. Children were enrolled from birth to age 7 days in the home by trained field research assistants, following comprehensive consenting procedures according to International Council on Harmonisation Good Clinical Practice guidelines. Mothers were administered a baseline survey at enrollment for demographics, household socioeconomic, water, and sanitation data.

There were 10 clinic visits in the first year of life for anthropometry, phlebotomy, and vaccinations [14]. Children received the Bangladesh EPI vaccines, including trivalent oral polio vaccine at weeks 6, 10, and 14. All acute illnesses were evaluated by medical officers, and RV1 dosing was delayed in children presenting with fever at scheduled vaccination visits (n = 1). Breastfeeding was not withheld. Vaccine cold chain was reviewed before administration. Children with severe malnutrition (weight-for-age z score < −3 SD) were referred for specialized care.

Complete diarrhea surveillance was conducted throughout the first year of life [14]. Field research assistants visited households twice weekly to determine diarrheal episodes through a structured questionnaire. Diarrhea was defined as ≥3 abnormally loose stools in 24 hours, per the mother, with distinct episodes separated by >72 hours diarrhea-free. Severe diarrhea was defined as Vesikari score ≥11 [17]. One diarrheal stool sample was collected during each episode. Mothers brought children into the clinic for further assessment and treatment of diarrheal illness. Diarrheal stool specimens were tested for rotavirus antigen by PosSpecT enzyme-linked immunosorbent assay (ELISA; Oxoid Ltd, Hampshire, United Kingdom).

Blood specimens for immunogenicity and micronutrients were collected at weeks 6 and 18 into trace-metal free Vacutainer tubes and cryovials (Grenier Bio-One and ThermoScientific/Nunc, respectively). Plasma was evaluated for rotavirus-specific immunoglobulin A (IgA) antibodies as described [18]. In brief, a capture enzyme immunoassay (EIA) was performed using the rotavirus SA11 antigen; results were expressed as units per milliliter determined by positive control reference serum. Seropositive was defined as rotavirus IgA ≥ 20 U/mL; seroconversion was defined as seropositivity at week 18 following a seronegative result pre-vaccination (week 6). Vitamin D and retinol binding protein were assessed using commercial ELISA kits (Immunodiagnostics Systems Ltd, Tyne, United Kingdom and R&D Systems, Minneapolis, Minnesota), and serum zinc was analyzed by flame atomic absorption spectrophotometer using WinLab32 software.

Outcomes

The primary outcome for intention-to-treat (ITT) efficacy analysis was 1 or more episodes of RVD from birth to 1 year. RVD was defined as diarrhea positive for rotavirus antigen by ELISA. Secondary outcomes were severe RVD, all-cause diarrhea (diarrhea of any etiology) and all-cause severe diarrhea in the first year of life, and any and severe RVD post-vaccination, from 18 to 52 weeks of age. Missed diarrheal stool specimens were assumed negative for rotavirus. Rates of missingness are reported [14].

Statistical Analysis

The trial was designed with at least 90% power to detect 50% vaccine efficacy at $\alpha = .05$, assuming rotavirus infection in 26% of non-vaccinated children by 1 year. Primary analysis was by ITT: all randomized subjects were included regardless of whether they adhered to the protocol vaccine regimen or terminated study participation prior to 1 year. Secondary per-protocol (PP) analyses were performed including all children who had 365 days of follow-up and, if assigned to the vaccine arm, received both doses of rotavirus vaccine within the protocol-specified window.

Vaccine efficacy was calculated using the standard formula: $(AR_{UNVAX} - AR_{VAX}) / (AR_{UNVAX} \times 100)$, with AR_{VAX} the attack rate in vaccinated and AR_{UNVAX} the attack rate in unvaccinated individuals. For dichotomous clinical outcomes, proportions of children with diarrhea along with Wilson 95% confidence intervals (CIs) and absolute risk differences in vaccinated and unvaccinated groups were calculated [19, 20].

Considering the study size and limitation on outcome events, a limited pool of 12 explanatory variables was selected a priori for analysis by best-subsets multivariable logistic regression to determine a model for risk of RVD based on the literature, biologic plausibility, and data availability. The best subset of p variables was chosen for the minimum Mallows' Cp statistic for which $Cp < p$ [21]. From this main effects model, all 2-way interactions were tested by change in deviance from the reduced model (likelihood ratio test). Model diagnostics included inspection of residuals, influential cases, and linearity in the logit for continuous variables. The Hosmer-Lemeshow goodness of fit test was performed. Raw P values (Wald test) for regression coefficients were adjusted for multiplicity post hoc using a stepdown Holm procedure [22]. The final model for RVD was then applied to the clinical outcome of severe RVD. Analyses were performed using SAS software version 9.3 (SAS Institute, Cary, North Carolina).

RESULTS

Study Population

After a community-wide survey of Mirpur, Dhaka, Bangladesh, 700 mother-child pairs were consented and enrolled within 7 days of birth (median age, 5 days [range, 1–7 days]) (Figure 1).

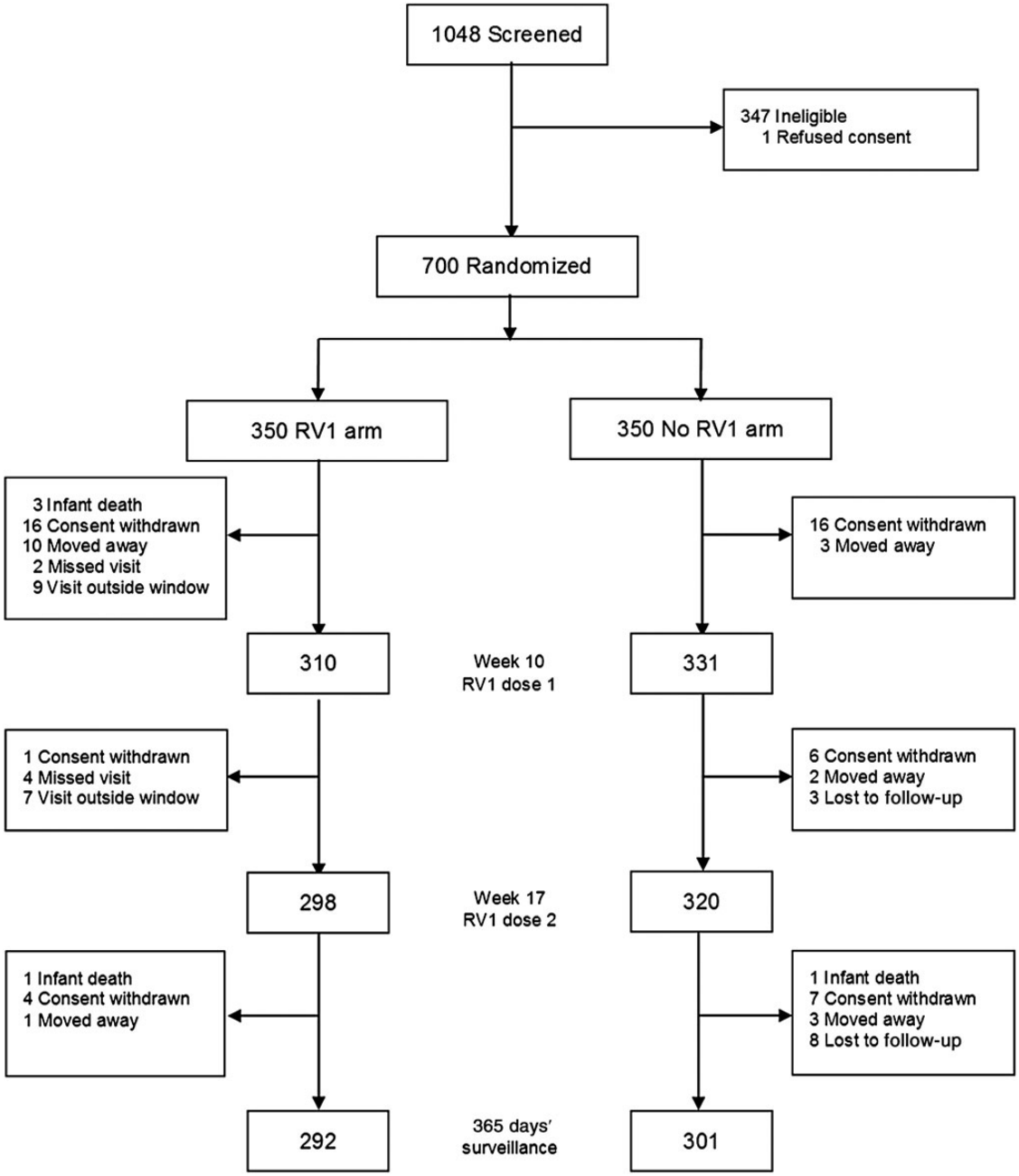

Figure 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram. Abbreviation: RV1, monovalent G1P[8] rotavirus vaccine.

Table 1. Characteristics by Randomization Arm

Characteristic	Randomization Arm	
	RV1 Arm (n = 350)	No RV1 Arm (n = 350)
Child features		
Sex, male	182 (52.0)	186 (53.1)
Median age at enrollment, d	5 (1–7)	5 (1–7)
Median weight at enrollment, kg	2.7 (1.7–4.1)	2.8 (1.9–4.0)
Median length at enrollment, cm	48.5 (43.1–55.4)	48.8 (44.5–54.6)
Median height-for-age *z* score at 10 wk[a]	−0.98 (−3.54 to 2.67)	−0.89 (−4.36 to 1.69)
Median weight-for-age *z* score at 10 wk[a]	−0.91 (−4.53 to 1.83)	−0.86 (−3.61 to 1.40)
Exclusive breastfeeding at 18 wk[b]	154 (50.0)	161 (53.5)
Home birth	100 (28.6)	81 (23.1)
Maternal features		
Median age at enrollment, y	24 (18–40)	24 (18–41)
Vaginal delivery	277 (79.1)	263 (75.1)
Median height, cm[c]	150 (137–187)	150 (134–167)
Median postpartum weight, kg[c]	48.0 (30.2–80.0)	47.0 (30.0–77.0)
Other children ≤5 y old in home	92 (26.3)	96 (27.4)
Mother education class 9+	54 (15.4)	55 (15.7)
Household and socioeconomic features		
Median total monthly income, 1000 taka	10 (3–77)	10 (3–70)
Piped municipal water	339 (96.9)	339 (96.9)
Toilet or septic tank	195 (55.7)	172 (49.1)
One-room home	251 (71.7)	256 (73.1)
Median No. of household members	5 (1–16)	4 (2–18)
Any water treatment	209 (59.7)	211 (60.3)
Immunogenicity		
RV IgA seroconversion[d]	80 (26.8)*	50 (17.2)*
RV IgA geometric mean titer, U/mL[e]	12.2**	4.3**
Median serum micronutrients at 18 wk		
Zinc, μg/dL[f]	75 (44–173)	77 (55–150)
Vitamin D, nmol/L[g]	58.8 (15.1–146.1)	58.1 (13.2–139.9)
Vitamin A, μg/mL[g]	26.1 (6.0–99.8)	25.8 (4.8–99.9)

Data are presented as No. (%) or median (range).

Abbreviations: IgA, immunoglobulin A; RV, rotavirus; RV1, monovalent G1P rotavirus vaccine.

[a] n = 318 RV1 arm, n = 326 No RV1 arm.

[b] n = 308 RV1 arm, n = 301 No RV1 arm.

[c] n = 330 RV1 arm, n = 339 No RV1 arm.

[d] n = 299 RV1 arm, n = 291 No RV1 arm.

[e] n = 289 RV1 arm, n = 269 No RV1 arm.

[f] n = 300 RV1 arm, n = 301 No RV1 arm.

[g] n = 305 RV1 arm, n = 294 No RV1 arm.

*$P = .005$, χ^2 test.

**$P = 2.4 \times 10^{-8}$.

Children were randomized to receive the 2-dose RV1 vaccine at 10 and 17 weeks of age or not. Population characteristics by randomization arm are shown in Table 1. There were no differences in characteristics between arms, except in rotavirus IgA seroconversion (26.8% seroconverted among vaccinated vs 17.2% in unvaccinated children; $P = .005$) and rotavirus IgA geometric mean titer (GMT) at week 18 (12.2 U/mL in vaccinated vs 4.3 U/mL in unvaccinated children; $P = 2.4 \times 10^{-8}$). There were 92 dropouts, 12% (n = 43) in the RV1 arm and 14% (n = 49) in the control arm ($P = .53$).

Rotavirus Diarrhea Incidence and Vaccine Efficacy

Under intense surveillance, incidence of RVD in unvaccinated children in the densely populated urban slum was 38.3 cases per 100 person-years, higher than previously reported in developing countries [4, 5, 7] (Table 2). In the primary analysis, vaccinated children vs unvaccinated had significantly less RVD (19.1% vs 32.6%) and severe RVD (4% vs 11.1%) in the first year of life (Table 3). Overall, by ITT analysis, vaccine efficacy was 41.2% (95% CI, 23.6%–54.8%) against all RVD and 64.1% (95% CI, 35.1%–80.1%) against severe RVD. The number of children

Table 2. Incidence of Rotavirus Diarrhea in Performance of Rotavirus and Oral Polio Vaccines in Developing Countries Study Compared With Other Cohorts

	Rotavirus Diarrhea			Severe Rotavirus Diarrhea		
Cohort	Cases, No.	Person-years	Incidence[a]	Cases, No.	Person-years	Incidence[a]
PROVIDE (urban, unvaccinated)	121	315.9	38.3	41	315.9	13
Rural Bangladesh and Vietnam [4]	109	1143.4	9.5	71	1156.9	6.1
Sub-Saharan Africa [5]	294	2556.3	11.5	129	2585.9	5.0
South Africa and Malawi [7]	NA	NA	NA	70	NA	8.0

Abbreviations: NA, not available; PROVIDE, Performance of Rotavirus and Oral Polio Vaccines in Developing Countries study.

[a] Incidence per 100 person-years.

needed to treat to prevent 1 case of RVD, derived as the reciprocal of the absolute risk difference, was 8 (95% CI, 6–15). There were 3 cases of RVD and 2 cases of severe RVD between week 6, first dose of the EPI-recommended RV1 dosing regimen, and week 10 when the first dose of RV1 was given in PROVIDE. Efficacy estimates against all-cause diarrhea were 1.3% (95% CI, −4.8% to 7.1%) for any diarrhea and 12.7% (95% CI, −7.5% to 29.1%) for severe diarrhea.

PP analysis of efficacy post-vaccination (18–52 weeks) was performed. PP efficacy against all RVD was 51% (95% CI, 33.8%–63.7%) and 73.5% (95% CI, 45.8%–87.0%) against severe RVD (Table 3), while efficacy against any and severe all-cause diarrhea was −3.1% (95% CI, −9.2% to 2.7%) and 22.1% (95% CI, −3.0% to 41.1%), respectively.

Best Subset of Factors Associated with Rotavirus Diarrhea and Vaccine Interactions

There were 555 children with complete data for all 12 explanatory variables in the best-subsets modeling of risk of RVD post-vaccination to 1 year. The explanatory variable pool consisted of rotavirus vaccine arm, household income, sex, delivery status (cesarean vs vaginal), mother's education (below class 9 vs class 9 and above), household water treatment (yes/no), exclusive breastfeeding at 18 weeks (yes/no), rotavirus IgA seroconversion (IgA < 20 U/mL at 6 weeks and ≥20 U/mL at 18 weeks), height-for-age *z* score (HAZ) at 10 weeks, and retinol binding protein, vitamin D, and serum zinc at 18 weeks.

In the multivariable best-subsets logistic regression analysis, the most parsimonious main effects model (minimum Cp < *p*) was the 5-variable model including vaccination arm, serum zinc, IgA seroconversion, exclusive breastfeeding, and water treatment. No 2-way interactions were statistically significant; no variable impacted the effect of rotavirus vaccine on risk of RVD (interactions with vaccine arm $P > .33$). The Hosmer-Lemeshow goodness-of-fit test for the main effects model was performed ($P = .67$). Based on model diagnostics, removing the 5 cases most poorly fit by the model (change in deviance $P < .005$ with largest leverage) strengthened the odds ratio (OR) point estimates by approximately 10% for vaccination arm, seroconversion, and serum zinc. There was no expectation these cases were different from the study population, so results include all cases.

Variables most strongly associated with risk of RVD were not receiving RV1 (OR, 2.84 [95% CI, 1.87–4.30]), serum zinc level (OR, 0.77 [95% CI, .66–.91]), and lack of IgA seroconversion (OR, 1.95 [95% CI, 1.12–3.39]). Absence of water treatment and not exclusively breastfeeding were also associated with increased risk of RVD (ORs of 1.50 and 1.46, respectively; Table 4). The effects of RV1 and serum zinc retained statistical significance after adjusting for multiple testing.

Table 3. Rotavirus Diarrhea Incidence and Vaccine Efficacy, Intention-to-Treat and Per-Protocol Analyses

Analysis	All Subjects, % (95% CI)	RV1 Arm, % (95% CI)	No RV1 Arm, % (95% CI)	Risk Difference, % (95% CI)	*P* Value	RR (95% CI)	Efficacy % (95% CI)
Year 1 ITT analysis (n = 700)							
RVD	25.8 (22.7–29.2)	19.1 (15.3–23.6)	32.6 (27.8–37.6)	13.4 (7.0–19.8)	4.0×10^{-5}	1.70 (1.31–2.21)	41.2 (23.6–54.8)
Severe RVD	7.6 (5.8–9.8)	4.0 (2.4–6.6)	11.1 (8.2–14.9)	7.1 (3.2–11.2)	3.0×10^{-4}	2.78 (1.54–5.02)	64.1 (35.1–80.1)
All-cause diarrhea	85.7 (82.9–88.1)	85.1 (81.0–88.5)	86.3 (82.3–89.5)	1.1 (–4.1 to 6.4)	.66	1.01 (.95–1.08)	1.3 (–4.8 to 7.1)
Severe all-cause diarrhea	33.7 (30.3–37.3)	31.4 (26.8–36.5)	36.0 (31.1–41.2)	4.6 (–2.4 to 11.5)	.20	1.14 (.93–1.41)	12.7 (–7.5 to 29.1)
Post-vaccination per-protocol analysis (n = 593)							
RVD	25.6 (22.3–29.3)	16.8 (12.9–21.5)	34.2 (29.1–39.8)	17.4 (10.5–24.2)	6.6×10^{-7}	2.04 (1.51–2.75)	51.0 (33.8–63.7)
Severe RVD	7.4 (5.6–9.8)	3.1 (1.6–5.8)	11.6 (8.5–15.7)	8.5 (4.4–12.9)	5.0×10^{-5}	3.77 (1.85–7.71)	73.5 (45.8–87.0)
All-cause diarrhea	88.7 (85.9–91.0)	90.1 (86.1–93.0)	87.4 (83.1–90.7)	–2.7 (–7.8 to 2.5)	.30	0.92 (.92–1.03)	–3.1 (–9.2 to 2.7)
Severe all-cause diarrhea	25.5 (22.1–29.1)	22.3 (17.9–27.4)	28.6 (23.8–33.9)	6.3 (–.71 to 13.2)	.08	1.28 (.97–1.70)	22.1 (–3.0 to 41.1)

Abbreviations: CI, confidence interval; ITT, intention-to-treat; RR, relative risk; RV1, monovalent G1P[8] rotavirus vaccine; RVD, rotavirus diarrhea.

Table 4. Multivariable Logistic Regression Main Effects Model for Risk of Rotavirus Diarrhea and Severe Rotavirus Diarrhea

Variable	Coefficient (SE)	Odds Ratio (95% CI)	Main Effect Raw *P* Value	Main Effect Adjusted *P* Value
Rotavirus diarrhea post–week 18 in year 1				
No RV1 arm (control)	1.04 (0.68)	2.84 (1.87–4.30)	9.6×10^{-7}	1.4×10^{-5}
Zinc, 18 wk (×10 ug/dL)	−0.26 (0.08)	0.77 (.66–.91)	.002	.024
Lack of RV IgA seroconversion	0.67 (0.28)	1.95 (1.12–3.39)	.018	.228
Absence of water treatment	0.40 (0.40)	1.50 (.99–2.24)	.051	.612
Stopped exclusive breastfeeding by week 18	0.38 (0.20)	1.46 (.97–2.18)	.066	.726
Severe rotavirus diarrhea post–week 18 in year 1				
No RV1 arm (control)	1.37 (0.39)	3.93 (1.83–8.47)	.0005	.008
Zinc, 18 wk (×10 ug/dL)	−0.12 (0.13)	0.88 (.69–1.13)	.325	1.000
Lack of RV IgA seroconversion	0.59 (0.50)	1.81 (.68–4.81)	.233	1.000
Absence of water treatment	0.54 (0.33)	1.72 (.92–3.30)	.099	1.000
Stopped exclusive breastfeeding by week 18	0.47 (0.33)	1.59 (.83–3.07)	.163	1.000

Raw *P* values from Wald test and adjusted using Holm stepdown procedure.

Abbreviations: CI, confidence interval; IgA, immunoglobulin A; RV, rotavirus; RV1, monovalent G1P[8] rotavirus vaccine; SE, standard error.

The final model selected for the RVD outcome was applied to severe RVD. Only lack of vaccine was significantly associated with increased risk of severe RVD (OR, 3.81 [95% CI, 1.78–8.82]; $P = .0008$; Table 4). The coefficients and effect size estimates for all variables were comparable to the all-severity RVD model, although cases of severe RVD (incidence rate, 7.4%) were insufficient to meet statistical significance.

DISCUSSION

Using a clinical primary endpoint of RVD, we performed a controlled efficacy trial of oral rotavirus vaccine given at 10 and 17 weeks after birth. The study was performed in a highly rotavirus-endemic, densely populated, urban setting in Bangladesh. PP analysis allowed comparison of our vaccine efficacy estimates with large phase 3 trials of RV1 [4, 7]. Following a delayed dosing regimen, the efficacy of RV1 vaccine to prevent severe RVD was 73.5% (95% CI, 45.8%–87.0%), higher than previously reported in developing countries, including 45.7% efficacy against severe RVD in Bangladesh [4]. Vaccine efficacy against RVD of any severity was 51% (95% CI, 33.8%–63.7%). Although past studies have suggested that rotavirus vaccine may protect against diarrhea from all etiologies [2, 4], we saw no impact of rotavirus vaccination on all-cause diarrhea.

We postulate our improved efficacy was due to several factors in our study design. Intense community-based diarrhea surveillance captured higher than expected incidence of RVD (including mild cases not requiring medical attention). This high incidence exposes the large burden of rotavirus disease and contributes toward higher vaccine efficacy. Additionally, although not directly tested, our delayed dosing schedule at 10 and 17 weeks may have minimized maternal antibody interference with RV1 vaccine. Although previous efficacy trials [4, 7] and immunogenicity studies [23, 24] have tested 2–3 doses of rotavirus vaccine administered between 6 and 16 weeks, this work is the only efficacy trial with delayed dosing (10 and 17 weeks). An additional trial, using a clinical endpoint to compare early vs late dosing schedules, would be necessary to confirm superiority of delayed dosing. With only 5 cases of RVD between the currently EPI-recommended start of vaccination (week 6) and week 10 (used here), the added risk of delaying dosing appears minimal.

To further understand risk of RVD, we used multivariable best-subsets analysis to evaluate factors for association with risk of RVD, including nutritional, socioeconomic, hygiene, and immunologic variables. Beyond vaccination, only serum zinc and IgA seroconversion were strongly associated with protection from RVD, and both were independent of vaccination status. This suggests that, regardless of vaccine performance, improvement in these variables would decrease risk and overall burden of RVD. In the final model, only vaccination and serum zinc level retained significance.

The observation that serum zinc is associated with decreased risk of RVD (OR, 0.77; $P = .002$) recalls the importance of zinc in intestinal epithelial repair and immunologic response mechanisms critical for mucosal protection [12, 25]. Previous studies have demonstrated a clear benefit of both supplemental and therapeutic zinc in protection from diarrheal disease in infants [13, 26]; however, there have been mixed results regarding the benefits of zinc specifically in RVD [27, 28]. We did not find an association between zinc and all-cause diarrhea.

Relevant to interpretation of our zinc findings, there has been controversy about the value of serum zinc level as a biomarker of individual zinc status; however, the recently published review of zinc under the National Institutes of Health's Biomarkers of Nutrition for Development (BOND) program [29] supports the use of serum zinc for this purpose as it relates to clinical signs of zinc deficiency, is responsive to zinc supplementation interventions, and can predict functional responses to supplementation, particularly in populations where functional bioindicators (ie, low HAZ scores) suggest risk of zinc deficiency. With a stunting prevalence

exceeding 20% by 1 year of age, the PROVIDE cohort meets BOND's recommended threshold for populations in which serum zinc may be a particularly strong biomarker of zinc status, lending confidence to the interpretation of our findings.

As in the PROVIDE study, a disconnect has been noted in several trials in developing countries between development of rotavirus-specific IgA and vaccine efficacy [9]; however, our findings regarding immunologic responses raise concern. Less than one-third of PROVIDE children seroconverted following vaccination, and IgA GMTs were markedly lower in PROVIDE, even compared to another cohort in Bangladesh that found GMTs of 47 U/mL among vaccinated children [30]. Importantly, immunogenicity in PROVIDE was measured 1 week after the second dose of vaccine (week 18), whereas other studies examined IgA 1–2 months after the second dose. Another possible contributing factor may be the difference in antigenic lysate used in the capture EIA assay in PROVIDE (rotavirus SA11 antigen) vs other studies.

Although not reaching statistical significance at the $P \leq .05$ level, other potential risk factors for RVD warrant further investigation in larger studies. Lack of exclusive breastfeeding and absence of water treatment in particular, together with zinc supplementation and vaccination, might be envisioned as the core of a focused diarrhea-prevention public health plan offering both vaccination and improved baseline health to reduce the burden of RVD.

Our work has several limitations. Although we performed intense diarrhea surveillance, results may underreport the incidence of short-duration diarrheal episodes. We assumed these episodes were negative for rotavirus, which may downwardly bias our efficacy estimates. The Hawthorne effect, in which enrolled children receive higher-standard primary care, may be present and upwardly bias efficacy estimates. Our sample size and number of outcome events limited the explanatory variables in our best-subsets analysis; our results need confirmation in larger studies. Additionally, we measured post-vaccination immunogenicity earlier than comparable trials. Although our results may reflect poor primary response or poor boosting after the second dose of RV1, our data could have underestimated immunogenicity based on this earlier timepoint.

Additional data are necessary to fully understand the limitations of oral vaccine efficacy in developing countries and delineate the optimal response. Previously we described the association of concurrent enteroviruses, as well as environmental enteropathy (measured by fecal reg1B, neopterin, serum soluble CD14, and enteric infection), on RV1 failure [11, 16]. Future work will focus on the impact of maternal antibodies and blood group antigens, the role of enteric co-pathogens, the effect of zinc supplementation and whether zinc has a pathogen-specific effect in protection from diarrheal disease, and the role of asymptomatic infection in IgA seroconversion and rotavirus vaccine performance. Research toward the identification of more predictive immune correlates of protection for rotavirus is also under way (B. D. Kirkpatrick, personal communication). With high efficacy against severe RVD, our work demonstrates the importance of oral rotavirus vaccines in highly endemic settings and suggests that support of baseline health and sanitation are still critical components of a public health approach, including vaccination, to prevent morbidity and mortality due to rotavirus diarrhea.

Notes

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