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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

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ABSTRACT

Background: Strategies are needed to improve oral rotavirus vaccine (RV), which provides suboptimal protection in developing countries. Probiotics and zinc supplementation could improve RV immunogenicity by altering the intestinal microbiota and immune function.

Methods: Infants 5 weeks old living in urban Vellore, India were enrolled in a randomized, double-blind, placebo-controlled trial with a 4-arm factorial design to assess the effects of daily zinc (5 mg), probiotic (10^{10} *Lactobacillus rhamnosus* GG) or placebo on the immunogenicity of two doses of RV (Rotarix®, GlaxoSmithKline Biologicals) given at 6 and 10 weeks of age. Infants were eligible for participation if healthy, available for the study duration and without prior receipt of RV or oral poliovirus vaccine other than the birth dose. The primary outcome was seroconversion to rotavirus at 14 weeks of age based on detection of VP6-specific IgA at ≥20 U/ml in previously seronegative infants or a fourfold rise in concentration.

Results: The study took place during July 2012 to February 2013. 620 infants were randomized equally between study arms and 551 (88.9%) completed per protocol. Seroconversion was recorded in 54/137 (39.4%), 42/136 (30.9%), 40/143 (28.0%), and 37/135 (27.4%) infants receiving (1) probiotic and zinc, (2) probiotic and placebo, (3) placebo and zinc, (4) two placebos. Seroconversion showed a modest improvement among infants receiving probiotic (difference between groups 1, 2 and 3, 4 was 7.5% (97.5% Confidence Interval (CI): −1.4%, 16.2%), p = 0.066) but not zinc (difference between groups 1, 3 and 2, 4 was 4.4% (97.5% CI: −4.4%, 13.2%), p = 0.272). 16 serious adverse events were recorded, none related to study interventions.

Conclusions: Zinc or probiotic supplementation did not significantly improve the low immunogenicity of rotavirus vaccine given to infants in a poor urban community in India. A modest effect of combined supplementation deserves further investigation.

Trial registration: The trial was registered in India (CTRI/2012/05/002677).

1. Introduction

Rotavirus diarrhea is a major cause of infant and child mortality globally, with an especially heavy burden in India [1]. Recent

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estimates put the annual burden in India at 11 million episodes of rotavirus gastroenteritis, resulting in at least USD (2013) $170 million direct costs and 79,000 deaths [2].

Oral rotavirus vaccines, in common with other oral vaccines, have lower immunogenicity and efficacy when given to children in low-income countries compared with high-income countries [3,4]. Experience with the two internationally licensed vaccines – Rotarix and Rotateq – in 36 low-income countries confirms the drop in effectiveness with increasing under-five mortality rate in a country [5]. This lower effectiveness is associated with diminished immunogenicity and potentially a more limited herd-effect [6–8]. Thus, although rotavirus vaccination results in substantial health benefits in low-income countries, reflecting the high burden of disease, these benefits are more limited than if the vaccine were to perform at the levels seen in high-income countries. In India, a newly licensed (since 2014) locally manufactured vaccine (Rotavac) is being introduced to the routine immunization schedule. Seroconversion and efficacy of this vaccine are about 50%, consistent with estimates for Rotarix and Rotateq from low-income countries in Asia and Africa [8–10]. This compares with approximately 90% seen in high-income countries.

The reasons for the diminished performance of oral vaccines in low-income countries are not clearly established, although they may include high levels of maternal antibody, micronutrient deficiencies, early life exposure to enteric pathogens or to the vaccine target, and differences in FUT2 secretor and blood group antigen status [11,12]. Development of practical strategies to enhance the immune response and efficacy of rotavirus vaccines are urgently needed.

The intestinal microbiota is known to play a central role in the development and homeostasis of local mucosal immunity, and may be important in determining the adaptive immune response to live oral vaccination [13]. Additionally, recent work in animal models has highlighted the significance of the microbiota and associated products (e.g. bacterial lipopolysaccharide) for the replication of enteric viruses [14]. In children in low-income countries the intestinal microbiota may be altered because of exposure to a fecally contaminated environment, infection with pathogens, frequent use of antibiotics or because of malnutrition. In addition, environmental enteropathy following repeated exposure to pathogens is common [15]. These changes have been hypothesized to affect the immunogenicity of oral vaccines, although their significance is unclear [16,17]. Probiotics have the potential to change the intestinal microbiota and release bacterial products that interact directly with lymphoid tissue, thereby altering the replication and immune response to oral vaccines [18]. In a study among Finnish infants who received a candidate oral rotavirus vaccine, probiotic (*Lactobacillus* strain GG) administration before and after vaccination resulted in modestly higher serum IgA titers [19]. Studies of other oral vaccines including cholera, typhoid and poliovirus vaccines have had mixed findings [18,20]. We recently reported an increase in IgG antibody responses in children given *Lactobacillus* GG for four weeks following an acute rotavirus infection, indicating that antibody responses to natural infections as well as to vaccines may be influenced by probiotic administration [21].

Zinc deficiency is very common among Indian children [22]. Zinc plays a key role in the functioning of the adaptive immune system, and deficiency is associated with depressed T cell function [23]. Studies have examined the effect of supplementation with zinc on the response to vaccination, including oral poliovirus vaccine (OPV) and inactivated oral cholera vaccine [24–28]. In a study in rural Pakistan, supplementation with 10 mg zinc daily from birth to 18 weeks of age had no impact on seroconversion after 4 doses of trivalent OPV [25]. Zinc supplementation did increase serum vibriocidal antibody titers in children and adults following administration of inactivated oral cholera vaccine, although this effect was not apparent in infants 6–9 months old [26–28]. We are not aware of any studies of zinc supplementation in children and the response to rotavirus vaccine.

Taken together, published studies on probiotic and/or zinc supplementation are insufficient for public health action to improve the efficacy of oral rotavirus vaccines in low-income countries. We therefore investigated their effect on the immune response to oral rotavirus vaccine (Rotarix) in a four-arm placebo-controlled randomized controlled trial with a factorial design among infants in India.

2. Materials and methods

2.1. Study design and participants

Children were randomly assigned (1:1:1:1) in a factorial design to one of four treatment arms: (1) probiotic supplement and zinc supplement, (2) probiotic supplement and zinc placebo, (3) probiotic placebo and zinc supplement, (4) probiotic placebo and zinc placebo, using a blocked randomization procedure with varying block size of 4 and 8 by generated on a computer by an independent statistician. An independent pharmacist packaged test products by subject ID. Study staff assigned sequential subject IDs to eligible consenting participants and remained blinded to assignment throughout the study.

Children were recruited from Chinnallapuram, a densely populated urban area of Vellore, India. Children were eligible for enrolment if they were between 35 and 41 days of age, weighed 3.2 kg or more, available for 11 weeks of follow-up, and had no medical condition that precluded study involvement. Those having received any OPV beyond a birth dose or rotavirus vaccine prior to enrolment were not eligible for participation. Written informed consent was obtained from parents or legal guardians before recruitment.

The Institutional Review Boards (IRB) of the Christian Medical College (CMC), the US Centers for Disease Control and Prevention and the Western IRB (Washington, USA) approved the study protocol. An independent data safety and monitoring board provided study oversight. The trial was registered in the clinical trial registry of India (CTRI/2012/05/002677).

2.2. Interventions

Children received Rotarix (containing $10^{6.5}$ $CCID_{50}$ of the RIX4414 human rotavirus strain; Glaxosmithkline Biologicals) at 6 and 10 weeks of age. Children received other vaccines according to the routine immunization schedule, including BCG at birth, trivalent oral poliovirus vaccine (OPV) at birth, 6, 10 and 14 weeks of age, and pentavalent vaccine against diphtheria, pertussis, hepatitis B and *Hemophilus influenzae* B at 6, 10 and 14 weeks. A birth dose of hepatitis B vaccine was also available in tertiary hospitals with coverage in the study population at this time of about 35%. Routine vaccine doses administered at 6 and 10 weeks were given at the same time as Rotarix by study staff. Each child received daily, a 5 ml suspension containing 5 mg zinc sulphate (Zinc sulphate heptahydrate 1 mg/ml prepared by CMC Pharmacy) or its placebo (CMC pharmacy) and the probiotic strain LGG (*Lactobacillus rhamnosus* GG gelatin capsule with 10^{10} organisms; i-Health Inc, Cromwell, CT) or its placebo (i-Health Inc, Cromwell, CT), from a week before the first dose of rotavirus vaccine at 6 weeks to a week after the second dose at 10 weeks of age. Zinc supplement was administered orally and probiotics by emptying the contents of the gelatin capsule into 5 ml of expressed breast milk before feeding. The dose and timing of these interventions were based on considerations of likely therapeutic effects, safety and past studies of these interventions in infants [21,29].

The first dose of test products was administered at the clinic following randomization while subsequent doses were taken at home. Compliance, defined as receiving at least 80% of supplement doses and not missing more than 6 consecutive days of supplementation, was assessed during biweekly home visits by field workers who counted empty capsules and visually inspected the remaining suspension in bottles. On home visits, the field workers supervised the administration of supplements.

A 3 mL venous blood sample was obtained at the first dose of vaccine and 28 days after the second dose in trace-element free tubes. Stool samples were collected just prior to each vaccination and 4 and 7 days later.

2.3. Laboratory methods

Serum was tested for anti-VP6 IgA antibodies to rotavirus using an antibody-sandwich enzyme immunoassay [30]. Poliovirus-specific neutralizing antibodies to serotype 3 were measured with a modified microneutralisation assay [31]. Shedding of rotavirus was assessed using real-time PCR targeting the VP6 gene. Serum zinc levels were measured using atomic absorption spectroscopy. Further details of the laboratory methods are provided in the Appendix. A companion paper describes the characterization of the intestinal microbiota using PCR for pathogen gene targets and 16 S ribosomal RNA gene sequencing in a nested case-control study of infants stratified by their serological response to rotavirus vaccine [32].

2.4. Outcomes

The primary outcome was seroconversion to rotavirus measured at 14 weeks of age among infants receiving probiotic compared with placebo (groups 1, 2 compared with 3, 4) and among infants receiving zinc compared with placebo (groups 1, 3 compared with 2, 4). Seroconversion was defined as detection of serum anti-VP6 IgA at a concentration $\geq$20 U/ml at 14 weeks of age in a subject seronegative at 6 weeks or a fourfold rise in anti-VP6 IgA concentration between 6 and 14 weeks. Secondary outcomes were seroconversion to rotavirus among infants receiving probiotics and zinc (group 1) compared with placebos only (group 4), geometric mean concentration (GMC) of rotavirus antibodies by study group, and seroconversion to poliovirus serotype 3 at 14 weeks of age. Seroconversion to poliovirus was defined as a fourfold rise in neutralizing antibody titre or detection of antibodies at a titre $\geq$1:8 at 14 weeks of age among previously seronegative infants. Serotype 3 poliovirus was chosen because immunogenicity of trivalent OPV is lowest for this serotype, giving more power to detect an effect of the interventions compared with serotypes 1 and 2. In a subset of infants chosen retrospectively for a nested case–control study [32], shedding of rotavirus was measured in stool samples collected at the time of the first (6-week) vaccine dose, and at 4 and 7 days later to assess vaccine virus replication ('take'). All infants who seroconverted to rotavirus with sufficient stool sample volume for pathogen assessment and an approximately equal number who failed to seroconvert, matched by study arm, were included in this subset. Rotavirus shedding was defined as the presence of >100 viral copies per reaction either 4 or 7 days after vaccination. Infants were excluded from analyses of rotavirus shedding if one or more stool samples were of insufficient volume or if they shed at >100 viral copies per reaction on the day of vaccination.

2.5. Statistical methods

For the primary comparison, assuming 50% seroconversion to rotavirus for the control group and that 15% of infants fail to complete per-protocol, enrolment of 280 subjects per intervention (arms 1 and 2, or 1 and 3) and control group (arms 3 and 4, or 2 and 4) would provide 86% power to detect a 15% difference in the proportion seroconverting for a two-sided Chi-square test with alpha = 0.025 (under the null hypothesis of no interaction between zinc and probiotics). To meet this target we aimed to recruit a total of 620 subjects one week prior to the first immunization visit allowing for additional drop-outs in the first week of enrolment.

The primary per protocol analysis included children who received two doses of both rotavirus vaccine and OPV, were 80% or more compliant with supplements, received no unplanned vaccination between study visits and provided paired serum samples to assess seroconversion. Supportive intention-to-treat (ITT) analysis included all enrolled subjects who were randomized, received at least one rotavirus vaccine dose and had paired serum samples. The 95% Confidence Intervals (CIs) for proportions were based on the Wilson score method without continuity correction. We also calculated 97.5% CIs for the difference in the proportion seroconverting among those receiving a supplement or placebo using the Newcombe-Wilson method without continuity correction [33]. Significance testing was performed with a Fisher Exact test with $\alpha = 0.025$ for the primary outcome because of the two interventions being tested. For the calculation of the GMC of anti-VP6 antibody samples below the limit of detection were assigned a value of 3.5 U/ml.

3. Results

We recruited 620 infants between 18 July 2012 and 22 February 2013 and assigned them equally among the four study groups (Fig. 1). A total of 563 infants received supplements or placebo, both doses of rotavirus vaccine, and provided paired serum samples. Of these, 551 completed the study per protocol. There were no significant differences between the groups at enrolment with regard to age, sex, type of house, mother's education or seroprevalence of polio and rotavirus antibodies (Tables 1–3).

3.1. Rotavirus vaccine immunogenicity

At six weeks of age, prior to the first dose of rotavirus vaccine, 167 (30.3%) infants were seropositive for rotavirus IgA (Table 2). Among infants who completed the study per protocol, 96 (35.2%) of 273 who received probiotic and 77 (27.7%) of 278 who received probiotic placebo seroconverted to rotavirus (absolute difference of 7.5% in the probiotic arms (97.5% CI: −1.4%, 16.2%), Fisher's p = 0.066). In the same per-protocol dataset, 94 (33.6%) of the 280 infants who received zinc supplementation and 79 (29.2%) of 271 who received zinc placebo seroconverted to rotavirus (4.4% difference (97.5% CI: −4.4%, 13.2%), Fisher's p = 0.272). A total of 54 (39.4%) of 137 infants receiving both probiotic and zinc seroconverted to rotavirus compared with 37 (27.4%) of 135 infants receiving both placebos (12.0% difference (95% CI 0.8, 22.8), Fisher's p = 0.04). In a logistic regression of the effect of zinc or probiotic supplementation on seroconversion the interaction term between the two interventions was not significant (p = 0.396). The probability of seroconversion did not show a significant difference according to whether the infant already had rotavirus-specific antibodies at 6 weeks of age (27.5% among infants with serum anti-VP6 IgA $\geq$20 U/ml compared with 33.1% for those <20 U/ml, p = 0.199). The intention to treat analysis gave similar results (Supplementary Table 1). The geometric mean concentration of rotavirus IgA did not show significant differences by study arm (Table 2).

Rotavirus shedding was measured in 288 infants meeting the inclusion criteria for this subset analysis, of whom 284 completed the study per-protocol. Selection of equal numbers of infants

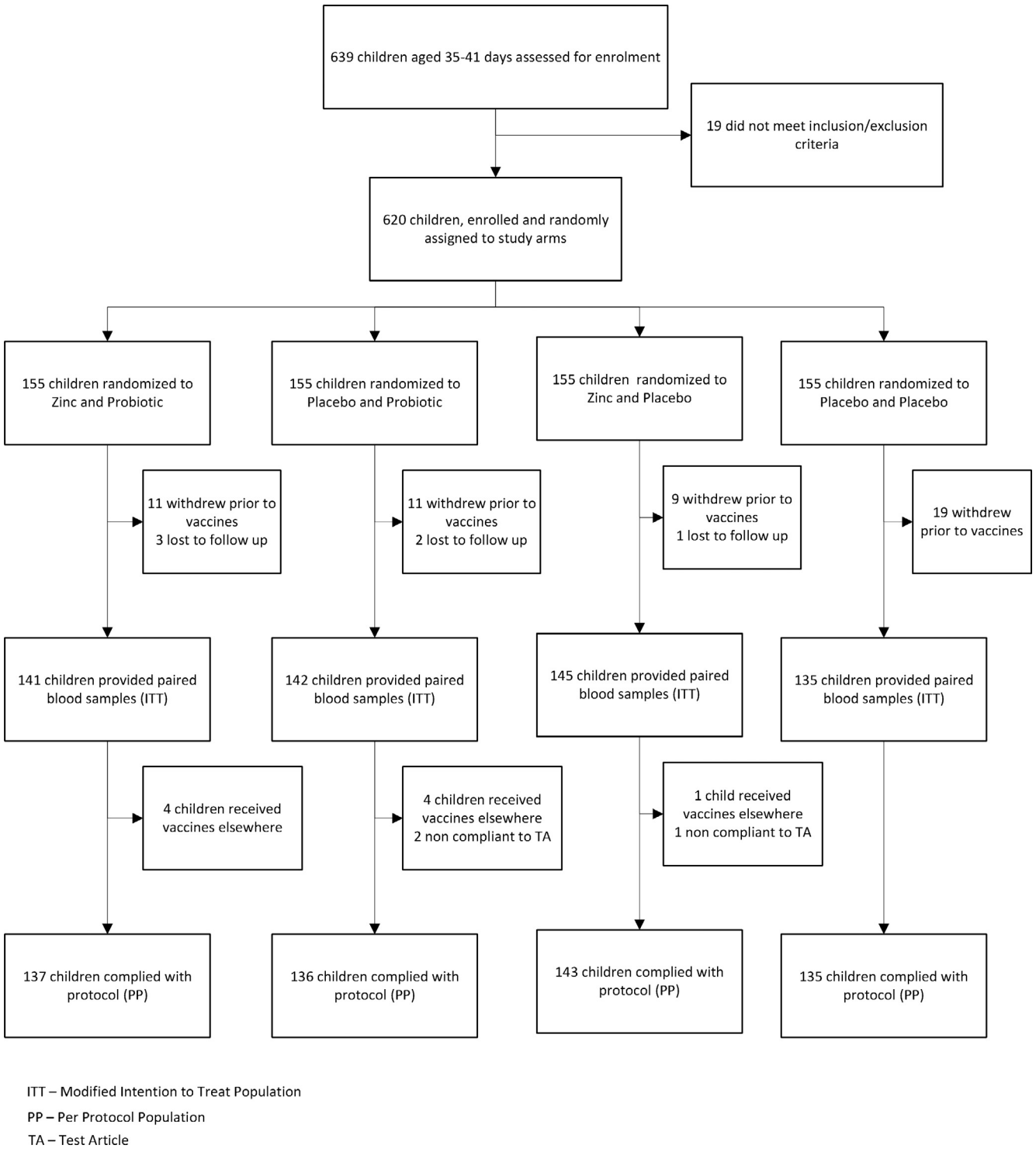

Fig. 1. Flow diagram of study recruitment and follow-up.

according to seroconversion status for the shedding assay resulted in 90 of the per-protocol subset coming from the arm that received zinc and probiotic, 66 probiotic only, 61 zinc only and 67 placebo-only. In total, shedding was observed in 41 (14.4%) infants on day 4, 49 (17.3%) on day 7, and 67 (23.6%) at 4 and/or 7 days after the first (6-week) vaccine dose. Seroconversion was strongly correlated with rotavirus shedding, occurring in 46/67 (68.7%) of infants in who shed rotavirus at 4 and/or 7 days and 98/217 (45.2%) of non-shedders (Fisher's $p = 0.001$). We did not compare shedding by study arm because of the purposive sampling of equal numbers of infants who seroconverted or not.

3.2. Oral poliovirus vaccine immunogenicity

At 6 weeks of age 263 (47.8%) infants had detectable ($\geq$1:8) neutralizing antibodies to type 3 poliovirus. Among infants who completed the study per protocol 219 (80.5%) receiving probiotic seroconverted to poliovirus serotype-3 compared with 228

Table 1
Baseline characteristics of the study participants.

Characteristic	Probiotic and zinc (N = 155)	Probiotic alone (N = 155)	Zinc alone (N = 155)	Placebo (N = 155)	Total (N = 620)
Age in weeks	5.1 (0.3)	5.1 (0.3)	5.1 (0.3)	5.2 (0.3)	5.1 (0.3)
Currently any breastfeeding	154 (99.4)	154 (99.4)	155 (100)	155 (100)	618 (99.7)
Height in cm	52.8 (2.0)	52.8 (1.9)	53 (2.3)	52.8 (2.0)	52.9 (2.0)
Weight in kg	4.0 (0.5)	4.0 (0.5)	4.0 (0.5)	4.0 (0.5)	4.0 (0.5)
Female	75 (48.4)	80 (51.6)	87 (56.1)	84 (54.2)	326 (52.6)
Mother's education					
None	14 (9)	5 (3.2)	14 (9)	10 (6.4)	43 (6.9)
– Primary	30 (19.4)	23 (14.8)	31 (20)	19 (12.3)	103 (16.6)
– Secondary	87 (56.2)	72 (46.5)	72 (46.5)	90 (58.1)	321 (51.8)
– Higher secondary	12 (7.7)	38 (24.5)	27 (17.4)	21 (13.6)	98 (15.8)
– Degree/diploma	12 (7.7)	17 (11)	11 (7.1)	15 (9.6)	55 (8.9)
Type of house					
– Permanent (pucca[a])	85 (54.8)	93 (60.0)	92 (59.4)	90 (58.1)	360 (58.1)
– Mixed	59 (38.1)	52 (33.6)	47 (30.3)	54 (34.8)	212 (34.2)
– Temporary (kutcha[a])	11 (7.1)	10 (6.4)	16 (10.3)	11 (7.1)	48 (7.7)

Data are mean (SD) or n (%).
[a] Pucca house has brick walls and concrete/tiled roof, kutcha house has wall and roof of mud, tin, asbestos or thatched leaves.

Table 2
Rotavirus-specific IgA before and after immunization with oral rotavirus vaccine (Rotarix) given at 6 and 10 weeks of age (per-protocol).

	Probiotics and zinc (N = 137)	Probiotic alone (N = 136)	Zinc alone (N = 143)	No supplement (N = 135)
IgA ≥ 20 U/mL pre-vaccination (at 6 weeks of age)	35 (25.5%)	44 (32.4%)	42 (29.4%)	46 (34.1%)
IgA ≥ 20 U/mL post-vaccination (at 14 weeks of age)	71 (51.8%)	75 (55.2%)	71 (49.7%)	69 (51.1%)
IgA GMC pre-vaccination (at 6 weeks of age)	9.3 (7.5, 11.6)	11.3 (9.0, 14.2)	10.8 (8.4, 13.9)	12.2 (9.5, 15.7)
IgA GMC post-vaccination (at 14 weeks of age)	23.4 (17.5, 31.3)	25.4 (19.2, 33.6)	23.9 (17.8, 32.1)	26.0 (19.3, 34.9)
Seroconversion (all infants)	54 (39.4%)	42 (30.9%)	40 (28.0%)	37 (27.4%)
Seroconversion (IgA ≥ 20 U/mL pre-vaccination)	15 (42.9)	9 (20.5)	11 (26.2)	11 (23.9)
Seroconversion (IgA < 20 U/mL pre-vaccination)	39 (38.2)	33 (35.9)	29 (28.7)	26 (29.2)

Data are n (%) or GMC (95% confidence interval); GMC includes samples below the limit of detection assigned a value of 3.5 U/ml.

Table 3
Poliovirus serotype 3 serum neutralising antibodies before and after immunisation with trivalent oral poliovirus vaccine (Biopolio) given at 6 and 10 weeks of age (per-protocol).

Antibody	Probiotics and zinc (N = 136[a])	Probiotic alone (N = 136)	Zinc alone (N = 143)	No supplement (N = 135)
Titre ≥ 8 pre-vaccination (at 6 weeks of age)	64 (47.1%)	72 (52.9%)	64 (44.8%)	63 (46.7%)
Titre ≥ 8 post-vaccination (at 14 weeks of age)	119 (87.5%)	117 (86.0%)	129 (90.2%)	121 (89.6%)
Seroconversion	107 (78.7%)	112 (82.4%)	116 (81.1%)	112 (83.0%)

[a] One pre-vaccination sample did not have laboratory data on poliovirus antibodies and is excluded from the per-protocol analysis; data are n (%).

(82.0%) receiving probiotic placebo (difference = −1.5% (95% CI −8.0, 5.0), P = 0.663). Among per protocol infants receiving zinc 223 (79.9%) seroconverted compared with 224 (82.7%) receiving zinc placebo (−2.7%, (95% CI −9.2, 3.8), P = 0.385). Combined supplementation with zinc and probiotics did not enhance seroconversion compared with placebo (−4.3% (95% CI −13.6, 5.1), P = 0.359). The intention to treat analysis gave similar results (Supplementary Table 2).

3.3. Impact of interventions on serum zinc and intestinal microbiota

The mean serum zinc level at 14 weeks of age was 53.9 mcg/dl among those supplemented and 54.6 mcg/dl among those who received a zinc placebo (P = 0.349). Serum zinc levels at this time-point did not differ according to whether the infant seroconverted to rotavirus (54.2 mcg/dl among those who seroconverted vs. 54.3 mcg/dl among those who did not, p = 0.819).

Among the subset of infants tested using 16 S ribosomal RNA sequencing [32], *Lactobacillus* was detected in 87.6% (92/105) and 79.0% (83/105) of stool samples at 6 and 10 weeks, respectively, among those receiving probiotics (either with or without zinc) compared with 17.5% (11/63) and 30.2% (19/63) among those receiving probiotic placebo (p-values <0.001). Among infants receiving the probiotic, detection of *Lactobacillus* in stool at 6 or 10 weeks was not significantly associated with seroconversion to rotavirus (p-values 0.835 and 0.897 respectively). We could not compare detection across study arms because of the purposive sampling of an equal number of infants who seroconverted or not across the study arms (see Parker et al. [32] for more details).

3.4. Safety

No immediate adverse events were recorded after the administration of supplements or vaccines. Sixteen serious adverse events occurred during the study none of which were considered related to the study interventions and all recovered. Solicited and reported adverse events were similar across the four arms.

4. Discussion

In a setting where the immunogenicity of oral vaccines is low, a 6 week course of probiotic (LGG) resulted in a small increase in the immunogenicity of rotavirus vaccine (35.2% seroconversion in probiotic arms 1 and 2 vs. 27.7% in placebo arms 3 and 4, p = 0.066). Infants who received combined supplementation with zinc and probiotics had the highest rates of seroconversion to rotavirus

(39.4% compared with 27.4% among infants receiving only placebo, p = 0.04). However, provision of zinc alone did not improve rotavirus vaccine immunogenicity, and there was no significant interaction between zinc and probiotics in their association with seroconversion.

Daily provision of LGG resulted in a substantially higher prevalence and abundance of *Lactobacillus* in stool samples collected at the time of vaccination. Among probiotic recipients, the abundance of *Lactobacillus* in stool showed a modest association with rotavirus shedding after the first dose of vaccine [32], consistent with the concept that probiotic bacteria may promote vaccine virus replication and immune response. Nonetheless, the relative abundance of *Lactobacillus* remained low (<1% of all sequences) compared with other intestinal bacteria (e.g. *Bifidobacterium*) following daily provision of 10^{10} organisms, and the extent of colonisation, if any, of the intestinal mucosa is not clear. Moreover, the presence of this organism did not change the broader composition or diversity of the bacterial microbiota [32]. This raises the question as to whether alternative or additional probiotic organisms, together with the use of prebiotic supplements, could have a greater impact.

The absence of an effect of zinc supplementation observed in our study must be interpreted with caution, since we did not observe an increase in serum zinc levels at the dosage used [22]. Supplementation with 5 mg/day was a cautious decision that was informed by considerations of the recommended daily allowance in this age group, the therapeutic dose recommendation for infants with diarrhoea and advice from paediatricians and the Institutional Review Board.

Overall, the levels of seroconversion to oral rotavirus vaccine were lower than expected, ranging from 27.4% to 39.4% across the study groups. Rotarix has previously been evaluated in the same population with two doses given at 8 and 12 weeks to children receiving routine vaccines at 6, 10 and 14 weeks per the national immunization schedule, and the levels of rotavirus immunogenicity were higher with 58% seroconversion [10]. Greater interference from maternal antibodies might have occurred in the current study given the slightly younger age of infants at the time of vaccination. However, a more significant factor is likely to be the co-administration of OPV, which can interfere with the response to oral rotavirus vaccines [34,35].

Our study is limited by the absence of an immune correlate of protection for rotavirus vaccines [36]. However, although immunogenicity does not consistently correlate with vaccine efficacy in developing countries, seroconversion is used as a marker of vaccine take and is required for licensure. Hence, even modest improvements in immunogenicity to oral rotavirus vaccines may translate into higher efficacy, which may have consequences for vaccine impact in countries where there is a high disease burden [9,37,38].

In conclusion, we detected a modest increase in seroconversion after 2 doses of Rotarix that appeared to be associated with receipt of probiotic. This finding points to a need for further studies of the effect of similar or alternative probiotic strains on rotavirus vaccine immunogenicity, including in children given vaccine on different schedules including where OPV is given separately or replaced with inactivated poliovirus vaccine.

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The authors affirm independence from funding agencies in the design, conduct and reporting of the trial.

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that JF is an employee of PATH which partially funded the study. None of the other authors have competing interests that may be relevant to the submitted work.

The preliminary results of this trial were presented at Vaccines for Enteric Diseases, November 2013 in Bangkok as an oral presentation.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2017.07.116.

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