

Peru-15, a live attenuated oral cholera vaccine, is safe and immunogenic in Bangladeshi toddlers and infants

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Abstract

A live oral *Vibrio cholerae* O1 El Tor vaccine, Peru-15 was tested in a double-blind, randomized placebo controlled study for safety and immunogenicity in Phase I and Phase II studies in 240 Bangladeshi children aged 9 months–5 years of age. Two different doses (2×10^7 and 2×10^8 cfu) were tested. Vaccination did not elicit adverse events and the strain was genetically stable. Vibriocidal antibody responses developed in 42/50 (84%) toddlers (2–5 years) and 35/50 (70%) of younger children (9–23 months) and overall 77/100 (77%) who received the high dose. LPS-IgA-antibody responses were seen in 60% of toddlers and 34% of infants; 40% responded with IgA antibodies to cholera toxin. The responses to the reduced dose was lower. These studies demonstrate that Peru-15 at a dose of 2×10^8 cfu is safe and immunogenic in children in Bangladesh.

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Keywords: Peru-15; Immunogenicity; Bangladeshi children

1. Introduction

A single dose, easily administered vaccine that can confer protection from cholera is needed for both children and adults in developing countries. One candidate, Peru-15 is a live oral vaccine that is based on an attenuated mutant of a *Vibrio cholerae* O1, El Tor Inaba strain. The vaccine has been

found to be safe, immunogenic, and efficacious in North American volunteers [1–3] and safe and immunogenic in Bangladeshi adults [4]. Peru-15 genetically engineered to be non-toxinogenic and non-recombinational. It is non-motile and ctxB positive [2] and genetically stable [1,4]. Since the vaccine is immunogenic in Bangladeshi and naïve North American volunteers it may likewise be immunogenic in children who have not been primed with *V. cholerae* O1. After satisfactory immunogenicity and safety studies in adults [4], we initiated studies in descending age groups from toddlers to infants 9 months of age to evaluate safety, immunogenicity and excretion of the vaccine strain in Phase I/Phase II studies in Bangladesh.

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2. Subjects and methods

2.1. Study participants

The study participants were children from an urban slum in the Mirpur area in Dhaka city in Bangladesh. The inpatient facility was adjacent to the ICDDR,B hospital. The outpatient facility was at the Mirpur field site, in an urban neighborhood about 10 km from the ICDDR,B and participants were recruited from an area of about 1 km around this facility.

2.2. Eligibility

Inclusion criteria included being healthy, aged between 5 years and 9 months of age. Exclusion criteria included any chronic disease, or any recent illness, immunosuppressive conditions in the past 6 months that may compromise the immune system. Those with history of diarrheal illness (passage of loose or watery stool $\geq 3/24$ h) in the past 2 weeks, febrile illness in the last week or antibiotic treatment within 2 weeks or who received any enteric vaccine within 1 month were excluded. Children whose stool sample was found to be positive for common enteric pathogens including enterotoxigenic *E. coli* were also not included [5]. For this purpose, stools were tested 3–5 days prior to immunization for the enteric pathogens using routine microscopic examination and microbiological culture. Of the eligible children (Tables 1 and 2), 240 were sequentially recruited into the study based in the order in which they were screened and then randomized into vaccine and placebo groups. Of the children screened 234 were not found to be eligible because of high parasitic load in stool and/or other enteric pathogens in stool ($n = 183$), history of recent illness ($n = 50$) or undernutrition (≤ -2 S.D.) ($n = 1$). Similar criteria were used for inpatient and outpatient subjects. Consent was obtained from guardians or parents to allow their child to participate in the study.

2.3. Study design

The trial was individually randomized, double blinded and placebo controlled. Randomization was carried out by the

International Vaccine Institute and sent to the vaccine formulation team. The sample size calculation was carried out at a power of 90% and significant difference level at 95% and was based on observing a statistically significant difference if 70% of vaccine recipients and 10% of placebo recipients developed a four-fold rise in vibriocidal antibodies after receiving the study agent [1]. The study was monitored by an independent Data Safety Monitoring Board (DSMB).

The study was carried out using six separate groups of participants studied sequentially from the toddlers (2–5 years old) to the infants (for the sake of simplicity those between 23 and 9 months old) from the reduced to the full dose of Peru-15 and from the inpatient to the outpatient phases (Table 1). The DSMB approved the progression of the study from one phase to the next.

2.4. Study agents, allocation and administration

The freeze dried vaccine was supplied by AVANT Immunotherapeutics Inc. (Needham, MA, US). Vaccine dose was either a 10 times reduced dose of 2×10^7 cfu or a full dose of 2×10^8 cfu. The vaccine was formulated in 5 ml of chlorine free bottled water and then mixed with 45 ml of buffer (for toddlers) or 20 ml (for infants) of a bicarbonate and ascorbic acid buffer (2.5 g of bicarbonate and 1.65 g of ascorbic acid were reconstituted in 100 ml of water; this preparation was used for formulation of the vaccine for toddlers and infants). The placebo consisted of 50 or 25 ml of buffer only in the two age groups. The vaccine formulation team prepared and blinded the vaccine and the placebo according to the randomization list. The study agents were administered within an hour of preparation and the participants were not allowed to eat or drink for 60 min before and after the intake of the study agents.

2.5. Follow-up for adverse events

Participants stayed at the inpatient facility for 12 days, starting 1 day prior to vaccination. Clinical monitoring was carried out 1 h before and after intake of the study agent(s)

Table 1
Study subjects in the different phases of the Phase I/Phase II trial on Peru-15

Participants and study agent	Inpatient group (Phase I) ^a ($n = 120$)		Outpatient (Phase II) ^d ($n = 120$)
	Reduced dose ^b ($n = 60$) (2×10^7 cfu/dose)	Full dose ^c ($n = 60$) (2×10^8 cfu/dose)	Full dose ($n = 120$) (2×10^8 cfu/dose)
Toddlers			
Vaccine group	20	20	30
Placebo group	10	10	30
Infants			
Vaccine group	20	20	30
Placebo group	10	10	30

The Peru-15 Phase I/Phase II trial was carried out using six separate groups of participants studied sequentially, first in the toddlers (2–5 years) and then in the infants (9–23 months). The study was conducted in the ^ainpatient toddler group given the ^breduced dose, followed by the ^cinpatient toddler group given the full dose and then the ^doutpatient older group given the full dose. Following the completion of the study a similar sequence was followed in the infants. The DSMB approved each phase of the study, following which the next phase was initiated.

Table 2
Demographic and anthropometric data of toddlers and infants in the vaccine and placebo groups in the study

Characteristics	Toddler ^a (n = 120)		Infant ^b (n = 120)	
	Vaccine (n = 70)	Placebo (n = 50)	Vaccine (n = 70)	Placebo (n = 50)
Male, no./total (%)	32/55 (58.2)	23/55 (41.8)	37/64 (57.8)	27/64 (42.2)
Female, no./total (%)	38/65 (58.5)	27/65 (41.5)	33/56 (58.9)	23/56 (41.1)
Age, mean (S.D.) (years)	48.7 (8.2)	49.6 (8.4)	21.2 (2.6)	21.7 (1.9)
Weight, mean (S.D.) (kg)	13.5 (2.1)	13.6 (2.4)	10.1 (1.6)	10.6 (1.6)
Height, mean (S.D.) (cm)	94.6 (6.7)	94.1 (6.9)	81.6 (6.9)	83.6 (5.7)

^aOf the 263 participants screened among the toddlers, 157 were eligible and ^bof the 265 infants screened, 137 were eligible. From amongst these participants, 120 were enrolled per each age group.

and then twice daily. Physicians observed participants for the occurrence of symptoms possibly related to its ingestion including vomiting, diarrhea, headache, fever, and abdominal cramps [4]. Observation was continued after the participants were released through daily visits by study staff, for up to 21 days to the subject's home. For the outpatient phase, clinical monitoring was performed at the study site and once daily at their homes for the duration of the study.

2.6. Safety precautions

During the inpatient phase only, children were under enteric precautions and all feces were passed into disposable biohazard bags. Feces were decontaminated in bleaching solution [4]. On day 6 of the study, all children were given erythromycin (50 mg/kg) in four divided doses/day for a period of 4 days for clearance of vaccine strain.

2.7. Follow-up and test methods for Immune responses

Blood (1.5 and 3 ml for infants and toddlers, respectively) was collected for immunological studies prior to (day –1) and 7 and 21 days subsequent to immunization. Blood grouping was carried out prior to immunization.

2.8. Vibriocidal antibody and LPS and rCTB specific antibody assays

Vibriocidal antibody assays were carried out with *V. cholerae* O1, Inaba T-19479 using sera (1:10 starting dilution) collected on days –1, 7 and 21 in duplicates [6]. The vibriocidal titer was defined as the reciprocal of the highest serum dilutions causing a greater than 50% reduction of the optical density (OD) at 595 nm compared to the control wells without serum. Titer ≥ 2 -fold from the pre-immune titer was used to signify seroconversion [7]. The responses were studied in the vaccine recipients given the full dose of Peru-15 to *V. cholerae* O1, Ogawa strain X25049. Sera from adult Peru-15 vaccines given the full dose of Peru-15 in an earlier study were also tested for responses to both serotypes [4].

Pre- and post-immunization sera were tested for IgA antibodies specific for *V. cholerae* O1, Inaba LPS and rCTB, by ELISA [7,8]. A study subject with a ≥ 2 -fold in antibody titer was considered a seroconvertent.

2.9. Excretion of vaccine strain

Fecal excretion of vaccine strain was tested using stools and/or rectal swabs. Stool samples from participants in the inpatient phase (two times/day for 10 days after immunization) were tested qualitatively and quantitatively for Peru-15 [1]; in the outpatient component of the inpatient group (on days 14 and 21) and in the outpatient group (on days 0, 1, 3, 7, 14 and 21) only qualitative cultures were carried out. *V. cholerae* were confirmed by agglutination with monoclonal antibody specific for *V. cholerae* O1 Inaba serotype and motility [9,10]. The sensitivity of isolation of Peru-15 was found to be $\sim 5 \times 10^2$ cfu/g stool. Colony blot hybridization using probes for *ctxA*, *ctxB* genes and the *attRS1* [2] was used to confirm identity of vaccine strain.

2.10. Data management and statistical analyses

Data management was carried out by Microsoft Visual FoxPro Version 6.0 (Microsoft Corporation, Redmond, WA, US) based program set up for the study. After data checking and verification by IVI, monitoring by an independent clinical monitor from AVANT, the study was locked and data sets analyzed. The results were analyzed sequentially in the order in which they had been completed and unblinded. Statistical analyses were carried out using the SigmaStat computer program (Jandel Scientific, San Rafael, CA). Paired samples were assessed by the Wilcoxon signed rank test, non-paired samples by Mann–Whitney *U* test. Proportions were compared using the χ^2 or the Fisher's Exact test as appropriate.

3. Results

3.1. Adverse events to Peru-15

Of the 240 children, 119 were male and 121 were female; 120 were in the 2–5 years range (toddlers) and 120 were 9–23 months of age range (infants) (Table 2). The vaccine was well tolerated in both age groups in the inpatient and outpatient phases (Table 3). The surveillance for side-effects revealed only mild symptoms, and occurred in similar rates in subjects receiving vaccine and placebo. Complaints of headache, vomiting and abdominal cramp were observed in only a few

Table 3
Symptoms in the participants receiving study agents

Sign/symptom ^a	Toddler (n = 120)		Infant (n = 120)	
	Vaccine ^b (n = 70)	Placebo (n = 50)	Vaccine (n = 70)	Placebo (n = 50)
No symptoms	67 (96)	50 (100)	68 (97)	49 (98)
Symptoms	3 (4)	0 (0)	2 (3)	1 (2)
Abdominal Cramp	1 (1.4)	0 (0)	0 (0)	1 (2)
Vomiting	1 (1.4)	0 (0)	2 (2.8)	0 (0)
Headache	1 (1.4)	0 (0)	0 (0)	0 (0)
Diarrhea	0 (0)	0 (0)	0 (0)	0 (0)
Fever	0 (0)	0 (0)	0 (0)	0 (0)

^a The symptoms recorded in all cases were mild, and none of the study participants showed more than 1 symptom within 4 days of receiving Peru-15.

^b Data are numbers and (%).

children. There was no diarrhea or fever in the subjects and most importantly no serious adverse events were noted. There were no differences in the rates of any adverse events between children given the low or high dose nor between vaccine and placebo recipients ($P = 1.00$).

3.2. Excretion of Peru-15

Only 9 children in the inpatient component excreted detectable levels of Peru-15 in stool. Of these 9 children, 1 toddler and 1 infant receiving the reduced dose shed the vaccine; 6 others receiving the full dose while 1 was a toddler in the placebo group.

The excretion of Peru-15 was observed between the second to the fourth day of intake at a concentration of 1 to 6×10^2 cfu/g stool and was cultured between one and four times. Peru-15 was cultured from a toddler in the placebo group in the inpatient phase on the fourth day of the study. The nine *V. cholerae* O1 Inaba isolates were confirmed to be the vaccine strain since they were non-motile, negative for *attRS1*, *RS1*, and *ctxA* but positive for *ctxB*.

V. cholerae was not isolated from anyone in the outpatient component.

3.3. Vibriocidal antibody responses

The baseline antibody titer to *V. cholerae* O1 Inaba in the toddlers and infants ranged from a minimum of 5 to a maximum of 5120 (GM = 20) and was similar in the two age groups ($P = 0.850$). Of the 40 children given the low dose of the vaccine, 40% in the toddler and 30% in the infant component developed a ≥ 4 -fold vibriocidal antibody response. No immune responses were detected in the placebo recipients in either age group (Table 4).

Vibriocidal antibody responses to the high dose were 84% in the toddlers (Fig. 1) and 70% in the infants. Collectively, 77% of children responded ≥ 4 -fold increases in titers versus 6% (5/80) of placebo recipients.

A maximal immune response was seen 7 days after the vaccination in the toddlers (82%) and the infants (62%) although titers at day 21 were also significantly higher compared to the pre-immune level ($P < 0.001$) but decreased significantly when compared to the response at day 7 in

Table 4
Vibriocidal antibody responses to the reduced dose of Peru-15 in toddlers and infants

Group	Toddlers	Infants
Vaccine (n = 20 in each group) ^a		
Responder ^b	8	6
Responder frequency ^c	40%	30%
Placebo (n = 10 in each group)		
Responder	0	0
Responder frequency	0%	0%

^a The reduced dose consisted of 2×10^7 cfu of Peru-15. Placebo was composed of buffer. Only 20 children in each group were given the reduced dose of the vaccine.

^b Increase in ≥ 4 -fold at day 7 or day 21 compared to pre-immune levels.

^c Responder frequency indicates number of responders per total number of subjects studied.

both age groups ($P < 0.001$). The individual maximal fold increase in titer among seroconvertents was up to 1024-fold seen within 7 days after immunization. The highest average fold increase of titer (16-fold in the toddler and 5-fold

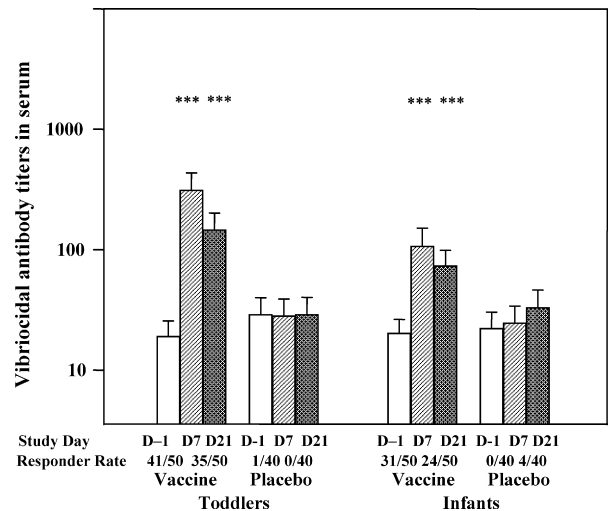


Fig. 1. Vibriocidal antibody responses in groups receiving Peru-15 or placebo. The bars indicate geometric mean titers and lines, the standard errors of the mean. Asterisks indicate statistically significant differences in responses in vibriocidal antibody titers prior to (day -1; □), 7 days (day 7; ▨) or 21 days (day 21; ▩) after immunization ($P < 0.001$). The Wilcoxon Signed Rank test was used for statistical analyses.

Table 5
Vibriocidal antibody responses to *V. cholerae* O1 Inaba and Ogawa serotypes in vaccine recipients receiving the full dose of Peru-15

Vibriocidal antibody response	Adult				Toddlers				Infants			
	Inaba		Ogawa		Inaba		Ogawa		Inaba		Ogawa	
	Day -1	Day 7	Day -1	Day 7	Day -1	Day 7	Day -1	Day 7	Day -1	Day 7	Day -1	Day 7
GM ^a	102	830	118	600	20	320	13	103	20	107	12	28
Fold increase ^b	8		5		16		8		5		2	
Responder frequency ^c n (%)	30 (75%)		28 (70%)		42 (84%)		33 (66%)		35 (70%)		17 (34%)	
P	<0.001 ^d , NS ^e				<0.001 ^d , NS ^e				<0.001 ^{d,e}			

^aGeometric mean of titers (GM) and ^bfold increase of GM of titers day 7 compared to pre-immune titers. ^cResponder frequency refers to numbers and % of vaccines responding with ≥ 4 -fold increases in titer at day 7 post-immunization compared with pre-immune titers to each serotype. ^dDifference in vibriocidal antibody titers to Inaba or Ogawa serotypes of *V. cholerae* O1 and ^ecomparison of proportion of responders to *V. cholerae* O1 Inaba and Ogawa serotypes. The Wilcoxon Signed Rank test^d and the Chi square test^e used for comparisons. $P \leq 0.05$ considered statistically significant. NS indicates no differences between groups ($P > 0.05$).

in the infants) was observed on day 7. The response was lower than in the placebo group was lower than in the vaccines (4–11-fold lower; $P = 0.004$ – <0.001). A placebo recipient, who shed Peru-15, did not seroconvert with vibriocidal antibodies.

Of those not responding to the vaccine after the full dose, 21% (5/23) had high baseline titers ranging from 640 to 2560, while 6% (5/77) of the responders had a pre-immune titer of 320 ($P = 0.047$) suggesting that more non-responders had high baseline titers.

Of the 240 study participants, 67 were of O, 55 were of A, 91 were of B and 27 were of the AB blood group ($n = 30$, 24, 35 and 11 in the O, A, B, AB, respectively, of the 100 children receiving the full dose of the vaccine). The vibriocidal antibody responses in the vaccines belonging to the different blood groups were 67, 92, 77 and 73% in the O, A, B and AB groups, respectively. Higher frequency of responders were seen in children in the A blood group compared to the O blood group ($P = 0.046$).

Peru-15 also induced vibriocidal antibody responses to the Ogawa serotype of *V. cholerae* O1 in the toddlers (responder frequency –66%) which was lower in magnitude compared to that seen to the Inaba serotype ($P = 0.018$) (Table 5). Amongst the infants a lower response rate (34%; $P < 0.001$) and magnitude of response ($P < 0.001$) was observed to the Ogawa compared to the Inaba serotype. Testing of serum from adult vaccine recipients however showed comparable response to the Inaba and the Ogawa serotypes ($P = NS$).

3.4. LPS and CTB specific IgA antibody responses in serum

About 60% of toddlers seroconverted with Inaba LPS-IgA antibodies within 7 days of immunization (Table 6). The magnitude of the response remained elevated for at least 21 days after immunization ($P < 0.001$); although levels were decreased compared to the magnitude seen at day 7 ($P = <0.001$). Of the infants, 34% responded with LPS

Table 6
IgA antibody responses to *V. cholerae* O1 Inaba lipopolysaccharide in sera of infants and toddlers

Response to LPS	Toddlers (n = 120)				Infants (n = 120)			
	Vaccine (n = 50)		Placebo (n = 40)		Vaccine (n = 50)		Placebo (n = 40)	
	Day 7	Day 21	Day 7	Day 21	Day 7	Day 21	Day 7	Day 21
GM ^a (range)	91 (12–2649)	54 (11–439)	48 (1–170)	48 (15–228)	83 (12–2106)	70 (10–858)	46 (0–865)	51 (0–1310)
Fold increase in titer	2.9	1.7	1.1	1.1	1.5	1.2	1.1	1.2
Responders ^b	27	12	4	4	13	6	2	3
Responder frequency ^c (%)	27 (54)		12 (24)		4 (10)		4 (10)	
Responder frequency (%) (overall)	60		15		34		12.5	
P	$\leq 0.026^{\text{d,e}}$		<0.001 ^d		NS ^f		NS ^f	
					0.005 ^d		0.006 ^d	
							NS ^f	
							<0.001 ^d	

^aGeometric mean of titers (GM) and range shown (minimum and maximum titers). ^bThose with a ≥ 2 -fold increase in response after immunization compared to pre-immune level. ^cResponder frequency indicates number of responders per total number of subjects studied. ^dComparison of response before immunization and follow-up on day 7 or 21 after immunization. ^eComparison of response of vaccines compared to placebo recipients. ^fDifferences between different study days in placebo recipients ($P > 0.05$). The ^dWilcoxon Signed Rank test, the Mann–Whitney test, the ^eChi square or Fisher Exact test used for comparisons. $P \leq 0.05$ considered statistically significant. NS indicates no differences between groups ($P > 0.05$).

specific antibodies and increases by study day 7 ($P=0.005$); which remained unchanged 2 weeks later ($P=0.059$). The response in placebo recipients was lower (12.5–15%). Significant increases in response in vaccines compared to the placebo group were only observed in the toddlers ($P=0.026$).

The cholera toxin responses in vaccines were modest with response rate ranging from 46% in toddlers to 36% in infants. The responses in the infants were lower (36%) with no increases in the magnitude of antibody titers at either of the two post-immunization phases ($P=0.977$ – 0.113). Significant differences in magnitude or responder frequency to CTB were not seen compared to the placebo recipients amongst either the toddlers or the infants ($P=0.153$ – 0.926).

4. Discussion

This is the first report of the testing of Peru-15 in the pediatric group. A single dose of 2×10^8 cfu was found to be safe in both the toddlers and infants. A lower dose of 2×10^7 was much less immunogenic. Both the seroconversion rate and the magnitude of response to the full dose were appreciable.

Of the different advantages of Peru-15, one was the almost complete lack of reactogenicity when doses of 2×10^7 or 2×10^8 cfu were evaluated. Adverse events attributable to the vaccine were not detected. The high tolerability of Peru-15 is similar to that seen in previous studies both Bangladeshi and adult US vaccines [1,3,4]. The complaints of headache reported in US recipients of Peru-15 [1,3] was observed in only one child and was not associated with the vaccine candidate similar to the our previous findings in the adults [4].

Shedding of Peru-15 was low in the toddlers and infants although higher excretion rates have been seen in the US adults [1,3]. The low rate of excretion may be a positive attribute since it reduces the probability of transmission of the strain in the environment. However this did not effect the magnitude of the immune response or the seroconversion rate in the children as has been seen earlier in adults [1,4]. The *in vivo* passaged strain remained unchanged in phenotypic and genotypic properties evaluated. This is an important safety attribute for live vaccines especially meant for environments where enteric pathogens are common and the frequency of virulence genes to be acquired by strains by lysogenic bacteriophages is high [11]. One toddler in the placebo group in the inpatient phase also shed Peru-15, but did not respond with antibodies. This infection probably resulted from spread from vaccine recipients in the cohort and has also been seen in placebo recipients in studies on CVD-103HgR in children [12]. Peru-15 was not isolated from anyone in the outpatient phase. This is most likely due to the fact that culturing of stools for Peru-15 was carried out less frequently and on fewer study days compared to that carried out in the inpatient component.

There appeared to be an age related lowering of the response in the descending age groups in the serum antibody response to LPS. These responses were comparable to the

vibriocidal response to Peru-15 in Bangladeshi adults [4]. The reduced response to LPS in children may be because young children and infants are relatively less responsive to polysaccharide than protein antigens [13]. It will be important to measure the avidity of the immune response in the different age groups to determine if the response can be used to predict protective capacity of these antibody responses [14].

The response to cholera toxin was low in the children as was seen in the adults in Bangladesh [4]. In studies with Peru-15 in adults in the US where frozen bacteria have been used, the response to CTB has been higher [1,2] while in Cincinnati where a similar lyophilized vaccine formulation as that used in Bangladesh was used the response to CTB was low (28% seroconverted) [3]. Since antibacterial immunity is considered to be a better marker of protection than antitoxin immunity [15], the low immune response to the toxin should not be a constraint to the use of the vaccine. Also the killed whole cell cholera vaccine, produced in Vietnam does not contain CTB but is efficacious [16].

The vibriocidal responses to *V. cholerae* O1 Ogawa was lower than that seen in the Inaba serotype in the youngest age group ($P<0.001$). The magnitude was lower than that seen in toddlers and adults, and only 50% of the infants who responded to the Inaba also responded to the Ogawa serotype. The higher frequency of responses in the older individuals could be due to previous exposure to *V. cholerae*, allowing for a broader immune response to the vaccine. In studies with CVD-103HgR, a *V. cholerae* O1 Inaba strain of the classical biotype, vaccination of adult Peruvians led to a higher response to the Inaba (76%) than the Ogawa serotype (45%) [17]. However, patients with cholera with Ogawa or Inaba strains are known to induce antibody responses to both serotypes [7,18] and even children respond to both serotypes after natural infection with one or the other serotype (Qadri et al., ongoing studies). The lower response in vibriocidal antibodies to the heterologous serotype in toddlers and especially in the infants receiving Peru-15 may suggest the need for a higher dose or multiple doses in this age group.

Studies with the parenteral killed whole cell cholera vaccines have shown that Inaba vaccines protected against both Ogawa and Inaba serotypes whereas the Ogawa vaccines protected better against the homologous serotype only [19]. Field trials will also be needed to determine if Peru-15 will protect against both serotypes.

The baseline vibriocidal titer was low in the children to the Peru-15 Inaba serotypes in comparison to the adults [4]. Thus there was less blunting of the post-immunization responses and appreciable changes in rates were not noted when those with high baseline titers prior to immunization were excluded from the analyses. However, we noticed that the magnitude of increase of the post-vaccination titer was higher in the adults compared to the toddlers and infants, presumably because of the previous priming through natural exposure.

More responders in vibriocidal antibodies were seen in children in the 'A' compared to those in the 'O' blood group. Earlier studies have shown a relationship between O

blood type and increased risk of severe cholera or protection [20,21]. The present study was designed to test the applicability of Peru-15 in Bangladesh in all types of individuals and we did not pre-select individuals on presence of blood group in contrast to studies which have been used as a determinants for testing suitability of cholera vaccines [3,22]. Although those in the 'A' blood group show a higher rate of response, this may be purely coincidental since the study was not designed to test such effects and was not seen in the adult participants receiving the vaccine [4].

Since an oral, live vaccine can give rapid immunity after a single dose, these traits would be most useful in Bangladesh as a public health tool during and before epidemics. Immune responses to Peru-15 were generated within 7 days of vaccination and a dose of 2×10^8 cfu could generate vibriocidal responses associated with conferring protection which could overcome the barrier of existing neutralizing antibodies. However, higher doses need to be tested to determine if dose escalation further enhances the immune response of children; especially those in the youngest age group since they showed lower vibriocidal responses to O1 Inaba as well as the Ogawa serotypes as well as lowered LPS specific antibodies. The administration of multiple doses will enable the determination if a boosting of the immune response is needed to enhance the duration of protection in young children. Other factors such as the effect of micronutrient supplementation which can influence immunogenicity of enteric vaccines in developing countries also need to be tested [23]. If found efficacious, the vaccine will also need to be tested for its suitability for including in the EPI scheme of vaccines.

We hope that studies in the near future will help to determine if Peru-15 which is of the current El Tor pandemic biotype will be useful for different age groups and populations. This may render Peru-15 more suitable than vaccines derived from the classical biotype since biotype related protection may also be important [24]. It is hoped that the vaccine will be useful for those living in other cholera endemic areas, refugees, and travelers: that is for both epidemic and endemic situations.

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