

# Randomized, Controlled Study of the Safety and Immunogenicity of Peru-15, a Live Attenuated Oral Vaccine Candidate for Cholera, in Adult Volunteers in Bangladesh

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**Background.** A live oral *Vibrio cholerae* O1 El Tor vaccine candidate, Peru-15, was studied for safety, immunogenicity, and excretion in phase 1 (inpatient) and phase 2 (outpatient) studies of Bangladeshi adults.

**Methods.** The study was conducted among adults, by use of a double-blind, randomized, placebo-controlled design. A single dose of Peru-15 ( $\sim 2 \times 10^8$  cfu) or placebo (buffer only) was given in standard bicarbonate and ascorbic acid buffer.

**Results.** Study treatment did not elicit any major adverse events in the volunteers, during either the inpatient or the outpatient phases, and there were no reports of diarrhea. *V. cholerae* was isolated from the stool of only 1 volunteer and was found to be genetically identical to the vaccine strain. Vibriocidal antibody responses were seen in 30 (75%) of 40 vaccine recipients and in 3 (10%) of 30 placebo recipients. Peripheral blood immunoglobulin (Ig) A and IgM antibody-secreting cell responses to lipopolysaccharide were seen in the majority of vaccine recipients (response rate, 78%–88%). Seroconversion for lipopolysaccharide-specific IgA antibodies was seen in 88% of vaccine recipients. The response in vaccine recipients was significantly higher than that in placebo recipients, in all of the immunological assays ( $P = .036$  to  $<.001$ ). A lower immunological response against cholera toxin B subunit was detected.

**Conclusions.** The safety and immunogenicity of this Peru-15 vaccine candidate indicates the usefulness of future studies in Bangladesh, where cholera is endemic.

A serious need exists for a simple, easily administered vaccine to protect children in developing countries from the life-threatening consequences of cholera. Live vaccines based on attenuated mutants of *Vibrio cholerae* offer much promise in this regard. Peru-15 is a *V. cholerae* O1 El Tor, Inaba strain that has been engineered to be nontoxigenic (it lacks the *ctxA* and *rtxA* genes, which encode cholera toxin [CT] A subunit and the

RTX toxin, respectively), nonrecombinational (it lacks the *recA* gene and the attachment site for the CTX phage), nonmotile, and *ctxB* positive (it makes the immunogenic, nontoxic CTB subunit) [1]. It has been found to be safe and immunogenic in several studies [1, 2] and has also been found to be protective against *V. cholerae* O1 El Tor cholera in North American volunteers in experimental challenge studies [3]. Since the vaccine is immunogenic and protective in naive North

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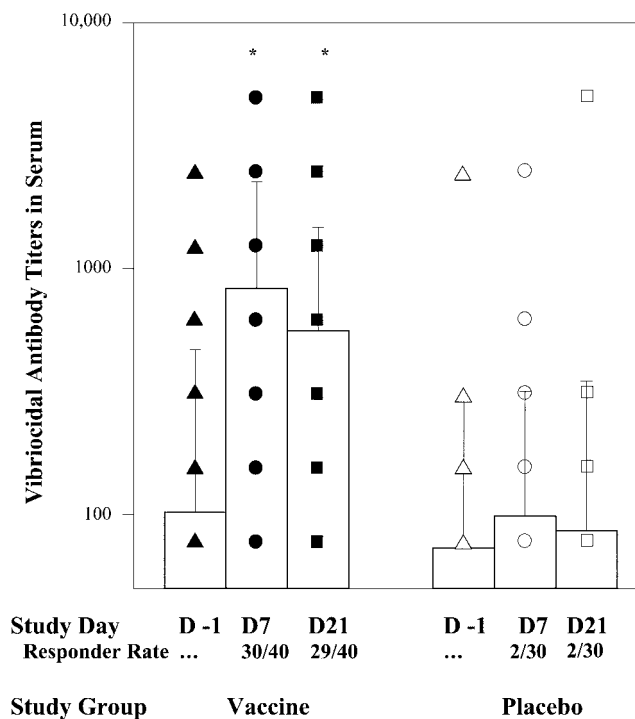
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**Figure 1.** Vibriocidal antibody responses in groups receiving Peru-15 (black symbols) or placebo (white symbols). Bars indicate the geometric means of titers, and lines indicate the SEs. Symbols denote individual values before ( $\blacktriangle$ ) and 7 ( $\bullet$ ) and 21 ( $\blacksquare$ ) days after immunization. Responder rates are the no. of participants with  $\geq 4$ -fold increases in titers/no. studied. Individual titers for 40 vaccine recipients and 30 placebo recipients are shown; because of overlapping values, fewer nos. of individual titers are seen. Asterisks indicate statistically significant differences in responses in vibriocidal antibody titers before (D -1) and 7 (D7) or 21 (D21) days after immunization.  $*P \leq .001$ , vs. preimmunization levels (Wilcoxon signed rank test).

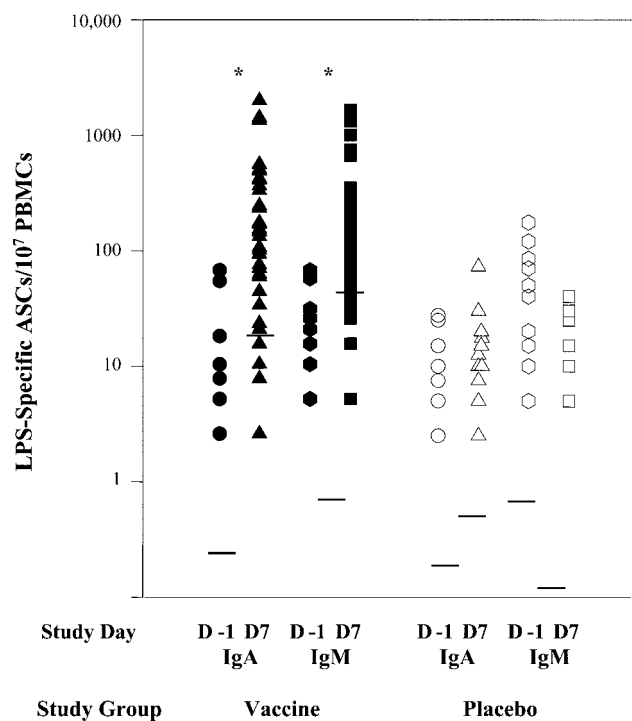
American volunteers, it is expected that it will also be protective in children who have not been primed previously with *V. cholerae* O1. A killed cholera vaccine was found to be less immunogenic in Bangladeshi children  $< 5$  years of age [4]. The live oral cholera vaccine CVD 103-HgR was significantly less immunogenic in children in Indonesia, compared with the response in naive North American participants who received the same dose [5]. We have initiated a series of studies of the live oral cholera vaccine candidate Peru-15 in Bangladesh, in age groups ranging from adults to infants. The data on safety, immunogenicity, and excretion of the vaccine strain in phase 1/phase 2 studies of Bangladeshi adults are presented here.

## SUBJECTS, MATERIALS, AND METHODS

**Setting.** The study population included adults living in an urban slum in the Mirpur area in Dhaka city in Bangladesh. The inpatient volunteer facility was set up at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B).

Conditions to limit the infectivity of the strain, the safety of the vaccine strain and of the study agent, and the safety of the individuals was assured in this facility. The participants were housed 1 day before intake of the study agent, for a total of 12 days. The outpatient facility was at the urban field site in Mirpur,  $\sim 10$  km from the ICDDR,B. Informed consent was obtained from the study participants. The research was approved by the Ethical Review Committee of the International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh.

**Eligibility.** Inclusion criteria included being a healthy adult 18–45 years of age and willingness to participate. Exclusion criteria included any chronic disease or recent illness, immunosuppressive conditions during the past 6 months, and pregnancy [2]. Those with a history of diarrheal illness during the past 6 weeks, febrile illness during the past week, or antibiotic treatment during the past 14 days were excluded. Food handlers, those cooking for or looking after infants or young children, and those who received any enteric vaccine during the past month were also excluded. Adults found to be positive for enteric pathogens on screening of stools 3–5 days before enrollment were not included [6]. Of the 140 individuals who consented to participate,



**Figure 2.** Antibody-secreting cell (ASC) responses to *Vibrio cholerae* O1, Inaba lipopolysaccharide (LPS) in participants receiving Peru-15 (black symbols) or placebo (white symbols). The responses in the IgA and IgM isotypes to LPS are shown before (D -1) and 7 days after (D7) immunization. Symbols denote the individual nos. of ASCs per  $1 \times 10^7$  peripheral blood mononuclear cells (PBMCs), and horizontal lines indicate the geometric means.  $*P \leq .001$ , day 7 vs. preimmunization (Wilcoxon signed rank test).

**Table 1. Anthropometric and other relevant data on participants in the study.**

Characteristic	Vaccine recipients (n = 40)	Placebo recipients (n = 30)
Male, no./total (%)	23/36 (63.9)	13/36 (36.1)
Female, no./total (%)	17/34 (50.0)	17/34 (50.0)
Age, mean (SD), years	26.3 (6.7)	26.4 (6.8)
Weight, mean (SD), kg	48.3 (8.5)	47.0 (10.0)
Height, mean (SD), cm	155 (7.8)	153 (7.0)

**NOTE.** Of the 140 potential participants screened, 84 were found to be eligible, and 70 were enrolled in the study.

84 met inclusion criteria; 70 adults were sequentially recruited in the order in which they were screened and then were randomized into vaccine and placebo groups.

**Study design.** The trial had a double-blind, placebo-controlled design in which randomization was performed by the International Vaccine Institute (IVI), Seoul, South Korea, and was sent to members of the vaccine formulation team at ICDDR,B. The sample-size calculation was performed at a power of 90% and significant difference level of 95%, on the basis of earlier studies involving Peru-15 [2]. The study was monitored by an independent data safety monitoring board.

**Study agents, allocation, and administration.** The freeze-dried vaccine was supplied by AVANT Immunotherapeutics. Each vaccine dose contained  $\sim 2 \times 10^8$  cfu of Peru-15. The vaccine was formulated in 5 mL of chlorine-free bottled water and then mixed with 95 mL of a buffer containing bicarbonate (2.5 g) and ascorbic acid (1.65 g) (AVANT). The placebo consisted of 100 mL of buffer only. The vaccine formulation team prepared the vaccine and the placebo in accordance with the randomization code at the inpatient or the outpatient facility, and the study agents were administered within 1 h of preparation at these sites. For the outpatient phase, the participants were given the study agents at the field site. The participants fasted for 60 min before and after intake of the study agent.

**Follow-up for adverse events.** All participants were clinically monitored 1 h before and after intake of the study agent.

At the inpatient site, clinical monitoring was performed twice daily; for the outpatient phase, clinical monitoring was performed at the participants' homes once daily for 10 days. Clinical symptoms that were monitored included vomiting, diarrhea, headache, fever, abdominal cramp, and gas. After the participants went home, monitoring was continued during visits by study staff every day for up to 21 days, when the study was terminated. On day 6 of the study, all participants were given 200 mg of doxycycline, followed by 100 mg of doxycycline twice daily for a total of 4 days, to eliminate shedding of the vaccine strain.

**Safety precautions.** During the inpatient phase, individuals were kept under enteric quarantine conditions, and all stool was passed into disposable biohazard bags. After samples had been collected for microbiological analyses, stool was decontaminated by soaking it in bleaching powder [2] for 1 h, after which it was flushed down a toilet. Extensive safety measures were maintained in the inpatient facility.

**Follow-up and test methods for immune responses.** Blood (10 mL) was collected for immunological studies before immunization (day -1) and 7 and 21 days after immunization. *V. cholerae* O1, Inaba lipopolysaccharide (LPS) [7] and recombinant CTB (rCTB) [8] were used. Blood grouping was performed for all participants at day -1.

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood and assayed for LPS- and rCTB-specific antibody-secreting cells (ASCs) of the different isotypes by the enzyme-linked immunospot (ELISPOT) technique [7]. A 2-fold or greater increase in vaccine-specific ASCs/ $10^7$  PBMCs between pre- and postimmunization samples was considered to be a significant response; only frequencies  $>10$  ASCs/ $10^7$  MNCs in postimmunization specimens were considered to be significant. The antigen-specific ASC response was compared with the serum response by use of 4-field table analyses [7].

**Vibriocidal antibody assays and ELISAs.** Vibriocidal assays were performed with *V. cholerae* O1, Inaba T-19479 as the target strain and serum samples from participants, by use of standard procedures [9]. The vibriocidal titer was defined as

**Table 2. Symptoms reported by volunteers receiving Peru-15 vaccine or placebo.**

Sign/symptom <sup>a</sup>	Inpatient		Outpatient		Overall	
	Vaccine (n = 20)	Placebo (n = 10)	Vaccine (n = 20)	Placebo (n = 20)	Vaccine (n = 40)	Placebo (n = 30)
No symptoms	18 (90)	10 (100)	20 (100)	20 (100)	38 (95)	30 (100)
Symptoms	2 (10)	0 (0)	0 (0)	0 (0)	2 (5)	0 (0)
Gas	1 (5)	0 (0)	0 (0)	0 (0)	1 (2.5)	0 (0)
Abdominal cramp	1 (5)	0 (0)	0 (0)	0 (0)	1 (2.5)	0 (0)
Diarrhea	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Fever	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

**NOTE.** Data are no. (%) of participants.

<sup>a</sup> The symptoms recorded in all cases were mild, and none of the study subjects showed  $>1$  symptom or any symptom within 4 days of receiving Peru-15.

the reciprocal of the highest serum dilution causing a >50% reduction of the optical density at 595 nm, compared with that of the control wells without serum. A  $\geq 4$ -fold increase in titer in comparison with the preimmune titer was used to signify seroconversion [7].

For LPS- and CTB-specific antibody responses, pre- and postimmunization samples from study subjects were tested for the presence of antibodies specific for LPS and rCTB, by ELISA [1, 7, 10]. A study subject with a  $\geq 2$ -fold increase in antigen-specific antibody response in serum was considered to be a responder [10].

**Microbiological studies.** At the inpatient facility, the first 2 stool specimens or rectal swabs collected each day from each participant were cultured to detect excretion of the vaccine strain, by use of both quantitative and qualitative methods, for 11 days, followed by surveillance at home up to day 21 [2]. For the outpatient phase, stool was collected for analysis before and on the day of immunization and 1, 3, 7, 14, and 21 days after immunization, for qualitative counts only. Thus, higher numbers of stool samples were cultured during the inpatient than during the outpatient phase of the study. Isolated *V. cholerae* O1, Inaba [11] were tested for motility [12] and by colony blot hybridization techniques for the *ctxA* and *ctxB* genes and the *attRS1* sequence [1, 13]. The absence of *ctxA* and *attRS1* and motility in combination with the presence of the *ctxB* gene was used to confirm the identity of the vaccine strain. Peru-15 strain was used as control to determine the sensitivity of the procedures.

**Data management and statistical analyses.** Data were entered into a computerized data-management system using a Visual FoxPro (version 6.0; Microsoft)-based program set up for the study. Data were transferred electronically to the IVI. After data checking and verification and monitoring by an independent clinical monitor, the study was locked, and data sets were sent to the different investigators. Data analyses were performed after the study was unblinded.

Results are expressed as geometric mean (GM), SD, and SE. Statistical analyses were performed using SigmaStat (version 2.03; Jandel Scientific). Paired samples were assessed by the Wilcoxon signed rank test, and unpaired samples were assessed by the Mann-Whitney *U* test. Proportions were compared using the  $\chi^2$  or Fisher's exact test, as appropriate.  $P \leq .05$  was considered to be significant.

## RESULTS

**Adverse events associated with Peru-15.** Of the 70 participants, 36 were male and 34 were female; 21 were blood type O, 18 were type A, 25 were type B, and 6 were type AB. Anthropometric characteristics were very similar between placebo and vaccine recipients (table 1). The vaccine was well tolerated by the 40

participants during the inpatient and outpatient study phases (table 2); surveillance for adverse events revealed only mild symptoms. Complaints of headache and abdominal cramp were reported by only a few participants, and there was no diarrhea or fever reported. No serious adverse events were noted. Very few symptoms were attributable to the vaccine within 4 days of intake of the study agent (table 2) or during the entire 21-day surveillance period. Two vaccine recipients vomited after the first dose of doxycycline, which was given on the sixth day after immunization. There were no significant differences in the rates of any adverse events between the vaccine and placebo recipients.

**Excretion of Peru-15.** Only 1 subject excreted the vaccine strain during the inpatient phase, on the fourth day of intake, at a concentration of  $6 \times 10^2$  cfu/g of stool. The excreted isolate was confirmed to be the vaccine strain, since it was nonmotile; negative for *attRS1*, *RS1*, and *ctxA*; and positive for *ctxB*. *V. cholerae* was not isolated from anyone during the outpatient phase.

**Vibriocidal antibody responses.** The baseline antibody titer in the participants ranged from a minimum of 5 to a maximum of 2560. Overall, 75% of the participants responded with antibacterial antibodies in sera (figure 1). During the inpatient phase, 85% responded, and during the outpatient phase, 65% responded ( $P > .05$ ). A maximal response was seen within 7 days of intake of vaccine; the titers on day 21 were also significantly higher, compared with preimmunization levels ( $P < .001$ ). When responses on the 2 study days after vaccination were compared, it was found that the titer decreased significantly on day 21 ( $P < .001$ ), compared with that on day 7. The individual maximal fold increase in titer among those who responded was as high as 256-fold. The highest average fold increase in GM titer (8-fold) was seen by day 7.

Only 2 of 30 placebo recipients showed a 4-fold increase in titer; the response was lower in magnitude than that in the vaccine recipients (8-fold lower;  $P < .001$ ). Of the 10 vaccine recipients who did not experience seroconversion, 8 had high baseline titers, ranging from 320 to 2560. Of those not responding to the vaccine, 80% (8/10) had a high baseline titer, whereas only 10% (3/30) of the responders had a preimmune titer  $< 320$  ( $P < .001$ ).

**Vaccine-specific ASC responses.** Overall, 78% of vaccine recipients responded with LPS-specific IgA ASCs in the circulation (range, 0–1900 ASCs/ $10^7$  PBMCs) (figure 2). This was >150-fold higher than the levels before immunization ( $P < .001$ ). Response to LPS in the IgM isotype was also seen (responder frequency, 88%;  $P < .001$ ), whereas the IgG response was poor (data not shown). The CT-specific ASC responses in vaccine recipients were very modest, with response rates ranging from 7% to 27% in the IgA and IgG isotypes (table 3). The response in the IgM isotype was poor (data not shown).

**Table 3. Immunological response in blood to cholera toxin B subunit (CTB) in the study participants.**

Response	Vaccine recipients		Placebo recipients	
	Day 7	Day 21	Day 7	Day 21
<b>ASC</b>				
<b>IgA</b>				
Titer, GM (range)	0.57 (0–360)	...	0.110 (0–160)	...
Fold increase in titer	8.3	...	1.75	...
Responders, no./total	10/40	...	2/30	...
Responder frequency, %	25	...	6.66	...
<b>IgG</b>				
Titer, GM (range)	0.216 (0–310)	...	0.019 (0–15)	...
Fold increase in titer	8.3	...	0.826	...
Responders, no./total	11/40	...	1/30	...
Responder frequency, %	27.5	...	3.33	...
<b>Serum</b>				
<b>IgA</b>				
Titer, GM (range)	227.51 (47–1574)	247.17 (56–2654)	271.64 (28–1724)	306.20 (28–1988)
Fold increase in titer	1.12	1.22	1.20	1.35
Responders, no./total	3/40	7/40	2/30	5/30
Responder frequency, %	7.5	17.5	6.66	16.66
<b>IgG</b>				
Titer, GM (range)	2123.24 (451–11,289)	2552.70 (727–14,482)	2630.27 (536–12,250)	3019.95 (436–9091)
Fold increase in titer	1.04	1.25	1.14	1.31
Responders, no./total	0/40	5/40	1/30	5/30
Responder frequency, %	0	12.5	3.33	16.66

**NOTE.** Antibody-secreting cell (ASC) responses were studied before and 7 days after immunization only; serum antibody levels were tested on these days as well as 21 days after immunization. Responders were considered to be those with  $\geq 2$ -fold responses, compared with preimmunization levels. The baseline geometric mean (GM) nos. of CTB ASCs in the IgA and IgG isotypes were 0.07 and 0.03 ASCs/ $10^7$  peripheral blood mononuclear cells, respectively; the baseline GM serum titers in the IgA and IgG isotypes were 203 and 2037, respectively.

**Serum anti-LPS and anti-CTB responses.** Approximately 88% of vaccine recipients experienced seroconversion for LPS IgA antibodies in sera within 7 days of immunization (table 4). Although the magnitude of the response remained elevated for at least 21 days after immunization ( $P < .001$ ), the levels were decreased, compared with those seen on day 7 ( $P < .001$ ). The LPS-specific serum responses on day 7 showed a sensitivity of 96%, compared with the ASC responses.

Although seroconversion was also seen for the IgM and IgG antibody types, the responses were of lower magnitude. The response to CTB was low in sera; the maximal responder fre-

quency was 20% in the IgA isotype, with even lower rates in the IgG isotype (table 3) and the IgM isotype (data not shown).

## DISCUSSION

This is the first study of Peru-15 to be conducted in a cholera-endemic country after several studies in the United States showed the vaccine candidate to be safe, immunogenic, and efficacious. This study is important because vaccine candidates need to be tested in heterogeneous populations and at different field sites, since response rates and efficacies can vary a great deal. Since

**Table 4. Immune response in serum from the study participants to *Vibrio cholerae* O1, Inaba lipopolysaccharide (LPS)**

Serum IgA response to LPS	Vaccine		Placebo	
	Day 7	Day 21	Day 7	Day 21
Titer, GM (range)	230.0 (24.0–3696.0)	138.0 (14.0–1808.0)	64.7 (9.0–887.0)	74.3 (9.0–1392.0)
Fold increase in titer	6.3	3.8	1.0	1.2
Responders, no.	35	30	0	2
Responder frequency, %	87.5	70.0	0	6.6
<i>P</i>	$<.001^{a,b}$	$<.001^{a,b}$	.088	.004 <sup>a</sup>

**NOTE.** The Wilcoxon signed rank test or the Mann-Whitney *U* test were used for comparisons, as appropriate. The baseline geometric mean (GM) titer in serum in the IgA isotype was 37.

<sup>a</sup> Statistically significant difference in responses before immunization and at follow up on day 7 or 21 after immunization.

<sup>b</sup>  $P \leq .036-.001$ , response in vaccine vs. placebo recipients.

an oral, live vaccine can give rapid immunity after a single dose, these traits would be most useful in Bangladesh, both for feasibility of delivery and as a public health tool during and before epidemics, especially to protect young children from cholera. Thus, Peru-15 needed to be studied in Bangladesh and to be studied first in adults, to test its safety and immunogenicity as well as the stability of the strain, before the vaccine could be studied in children in decreasing age groups.

After vaccination with Peru-15, the majority of recipients developed vibriocidal antibodies and experienced seroconversion for LPS-specific antibodies, as well as ASC responses in blood. Among the 10 vaccine recipients who did not respond with vibriocidal antibodies, 8 had high baseline titers ( $\geq 320$ ), which, in previous cholera vaccine studies, have been shown to be an impediment to the development of a vibriocidal response [14, 15].

Our study was designed to test the vaccine's applicability in Bangladeshi participants, and we did not preselect individuals on the basis of low baseline vibriocidal titers [5] or the presence of blood group O [16], both of which have been used as determinants of a "high take rate" of cholera vaccines. Of the 70 participants, 21 were blood type O, 18 were type A, 25 were type B, and 6 were type AB; the responder frequencies for these groups were 69%, 57%, 85%, and 83%, respectively, in vibriocidal antibodies. The results are, however, encouraging in this unbiased selection of participants, and we can expect similar seroconversion rates in immune responses when we move to large field studies in this population.

The response to LPS in the IgA isotype in ASCs and in sera suggests that the gut-associated immune response was stimulated by Peru-15. The IgA ASC responses showed >96% sensitivity, compared with the serum responses on day 7, suggesting that the serum responses could be used quite effectively to gauge mucosal responses in Peru-15 studies. However, we were not able to test intestinal secretions as an indicator for assessing the direct measures of the mucosal immune responses in the present study.

Peru-15 elicited relatively low levels of antibodies to CTB. In North American subjects, ~48% of vaccine recipients experienced seroconversion for the IgG isotype when a  $10^8$  or a  $10^9$  dose of a frozen preparation of Peru-15 was used [2]. However, in another study, conducted in Cincinnati, 28% of vaccine recipients responded with CT-specific antibodies when a lyophilized preparation of a dose of  $2 \times 10^8$  cfu—identical to the vaccine used in the present study—was administered [3]. In both of these studies, the antibody response to the toxin was lower than the vibriocidal antibody responses. The killed, whole-cell cholera vaccine containing CTB stimulates a more vigorous toxin-specific response in vaccine recipients and also cross-protects against diarrhea from the immunologically similar heat labile toxin-expressing strains of enterotoxigenic *Escherichia*

*coli* [17]. It is possible that Peru-15, which induces a lower response to CTB, will not be as efficient in this respect. However, since antibacterial immunity is thought to be more important than antitoxin immunity for protection [18], it should not be a major issue for a vaccine meant to protect mainly against O1 cholera and not against diarrhea caused by other toxigenic bacteria.

It was important to test whether the attenuated vaccine strain remained genetically stable after human passage in an environment where a multitude of enteric pathogens can be cultured from the stool of even healthy individuals. This is a major issue in the safety of live strains in cholera-endemic countries where there is a possibility of strains reverting to virulence by acquiring lysogenic bacteriophages [19]. We found that the in vivo passaged strain remained unchanged in phenotypic and genotypic properties and was identical to Peru-15.

The strain could be cultured from only 1 individual during the study. When tested in the United States, Peru-15 was excreted by >50% of participants [2, 3]. The low excretion rate in the Bangladeshi adults, compared with that in North American subjects, could be due to the presence of neutralizing antibodies in the gut. In studies in Peru and Indonesia, there was a much lower frequency of shedding of both live cholera vaccines CVD103 HgR and CVD111, compared with that seen in individuals in the United States [5, 20, 21].

The lower shedding rate may be an added advantage in the use of the single-dose Peru-15 strain in developing countries, since this will reduce the introduction of the recombinant strain into the environment and reduce nosocomial transmission to potential contacts. This may be especially advantageous in households with immunocompromised family members who could become infected inadvertently. The low excretion rate of Peru-15 in Bangladeshi adults, however, did not appear to affect the immunogenicity of the vaccine, a result similar to those of earlier studies in which no relationship was found between fecal shedding and rate of seroconversion [2, 3]. However, it cannot be ruled out that a higher dose will result in a greater immune response, leading to high vaccine efficacy.

The results of this carefully monitored study of Peru-15 in Bangladeshi adults shows that the single-dose live vaccine candidate is safe in these subjects and is also well tolerated, with only minor adverse events. None of the 40 individuals ingesting the vaccine had any instance of diarrhea or fever. The vomiting observed in the 2 individuals ~6 days after intake of the vaccine appeared to be related to the intake of doxycycline rather than Peru-15, since the antibiotic has been shown to have such an effect in patients [22]. The adverse events observed even in this actively conducted surveillance showed that there were no significant differences between vaccine and placebo recipients.

The Bangladeshi vaccine recipients had antibacterial immune responses comparable to those of the North American

subjects. However, a greater fold increase in vibriocidal antibody response was seen in North Americans, as a result of at least a 14-fold lower baseline titer, compared with that seen in participants in the present study in Bangladesh [2]. The magnitude of the postimmunization titers in the 2 studies were also comparable (GM, ~800 in both groups). Thus, Peru-15, in a relatively low dose, generated similar immune responses in participants in 2 vastly different geographical settings. This is very encouraging, because, when CVD103HgR was tested in volunteers in Peru and Indonesia at similar doses, a lower magnitude of response was seen in the Peruvian and Indonesian “primed” subjects than in the participants from the United States [5, 21, 23]. Efficacy and immune responses to oral cholera vaccines have usually been lower in children in cholera-endemic countries than in adults and children in industrialized settings. Studies of the oral whole-cell killed cholera vaccine in Bangladesh have shown higher efficacy in adults than children [4], whereas varying immunogenicity results have been obtained in children from other populations [24, 25]. Ongoing studies of Peru-15 in children will be able to determine the usefulness of Peru-15 for further studies in Bangladesh.

This is the first study in which Peru-15 has been studied in a developing country endemic for cholera. The vaccine strain is of the El Tor biotype, which is the predominant biotype in the current cholera pandemic. Thus, we can expect Peru-15 to be more effective for protection than vaccines derived from the classical *V. cholerae* O1 biotype.

The results of these studies in adults are very promising, since satisfactory immune responses were seen. At the time of initiation of the study, we had not expected such high seroconversion rates in the adults, on the basis of the notion that preexisting antibodies in primed individuals can blunt or diminish immune responses in older individuals. We are hopeful that the vaccine may be useful for a variety of age groups and populations, including those living in cholera-endemic areas, refugees, and travelers.

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