Safety, Immunogenicity, and Transmissibility of Single-Dose Live Oral Cholera Vaccine Strain CVD 103-HgR in 24- to 59-Month-Old Indonesian Children

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Recombinant A^-B^+ *Vibrio cholerae* O1 strain CVD 103-HgR is a safe, highly immunogenic, single-dose live oral vaccine in adults in industrialized countries. Safety, excretion, immunogenicity, vaccine transmissibility, and environmental introduction of CVD 103-HgR were investigated among 24- to 59-month-old children in Jakarta. In 81 households, 1 child was randomly allocated a single dose of vaccine (5×10^9 cfu) and another, placebo. Additionally, 139 unpaired children were randomly allocated vaccine or placebo. During 9 days of follow-up, diarrhea or vomiting did not occur more often among vaccinees than controls. Vaccine was minimally excreted and was isolated from no controls and from 1 (0.6%) of 177 unvaccinated family contacts. A 4-fold or higher rise in serum vibriocidal antibody was observed in 75% of vaccinees (10-fold rise in geometric mean titer over baseline). Of 135 paired placebo recipients or household contacts, 5 had vibriocidal seroconversions. Moore swabs placed in sewers and latrines near 97 households failed to detect vaccine. These observations pave the way for a large-scale field trial of efficacy.

The return of cholera to South America in explosive epidemic form in 1991, after a century of absence [1], has focused attention on the lack of a satisfactory cholera vaccine. The venerable inactivated whole cell parenteral vaccine confers only limited ($\sim 50\%$) protection for a short time (generally 4–6 months) [2, 3]. Consequently, public health authorities advise that the parenteral vaccine should play no role in the control of epidemic or endemic cholera [4, 5]. In contrast, the World Health Organization has targeted the development of a new, improved cholera vaccine as a high priority [6, 7]. To be a practical public health tool, a new cholera vaccine should be oral, single-dose, safe, and highly efficacious (even in young children) [8]. Moreover, ideally, protective efficacy should commence within a few days of administration and should persist long-term (at least 5 years).

Oral vaccines consisting of inactivated vibrios given with or without B subunit of cholera toxin conferred moderate (50%–52%) protection against cholera for 3 years in a field trial in Bangladesh [9, 10]. While constituting an advance over the parenteral inactivated vaccine, the two oral inactivated vaccines tested in Bangladesh suffer from some notable drawbacks: Young children derived only short-term protection lasting <1 year [10]; protection in adults did not persist beyond 3 years; multiple (three, perhaps as few as two) spaced doses were necessary to confer protection [9, 10]; and protection was lower in persons of blood group O (an important host risk factor for cholera gravis) and against El Tor rather than classical biotype infection.

It is anticipated that genetically engineered strains of *Vibrio cholerae* O1 used as live oral vaccines may overcome the drawbacks of earlier generations of cholera vaccines and may, with a single dose, provide a high level of long-term protection among all age groups, without causing adverse reactions [8, 11–13]. However, the use of recombinant live vaccines raises certain other questions that must be answered, such as to what extent such strains are excreted, whether the vaccine strain is readily transmitted to contacts, and whether the vaccine persists in the environment.

Strain CVD 103-HgR, presently the leading live oral cholera vaccine candidate, was derived from wild-type classical *V. cholerae* O1 569B (classic biotype, Inaba serotype) using recombinant DNA techniques [13–15]. Ninety-four percent of the gene encoding the A (ADP-ribosylating) subunit of cholera enterotoxin was deleted (leaving expression of the nontoxic B subunit intact), and a gene encoding resistance to Hg^{++} was inserted into the *hlyA* locus of the chromosome to provide a marker to differentiate CVD 103-HgR from wild-type vibrios [13–15]. The safety and immunogenicity of CVD 103-HgR have been previously documented in randomized, placebo-controlled, double-blind clinical trials and open studies in adults in industrialized countries [14, 16–19] and adults and school-age children in developing countries [20–23]. Herein we report the first clinical studies of CVD 103-HgR in preschool children in a less-developed country. In addition to safety and immunogenicity, excretion and transmissibility of the vaccine strain were investigated and bacteriologic studies were done to detect the vaccine strain in the environment around the dwellings of vaccinated subjects.

Materials and Methods

Overview of the Studies

El Tor cholera occurs throughout the year in Jakarta, with seasonal increases in June–August and in December and January [24, 25]. In July and August 1991, pairs of children 24–59 months of age within the same household were randomly allocated so that 1 child received, with buffer, a single dose of CVD 103-HgR vaccine containing 5×10^9 cfu while the other received placebo. A second round of vaccination was carried out in January and February 1992. Because few households with pairs of age-eligible children were available, for this round of vaccination the protocol was modified so that (except for a small number of household pairs) only 1 child 24–59 months old per household was entered into the study and was randomly allocated vaccine or placebo. Double-blind clinical surveillance detected adverse reactions, and a rise in serum vibriocidal antibody was the main outcome of immunogenicity. All participating children and many family members had multiple stool cultures to detect excretion of the vaccine strain. Blood specimens were obtained from most family members who gave stool cultures. In a proportion of households, environmental bacteriologic studies were carried out to detect the vaccine strain.

Study Site

The two studies were carried out in villages (kelurahans) in North Jakarta, located within the catchment area of the Infectious Diseases Hospital, North Jakarta.

Subjects, Eligibility, Recruitment, and Allocation

Study 1. With active participation of the kelurahan civic leaders, a census was undertaken to identify and describe the study to households with 2 children 24–59 months of age. Families who expressed an interest in participating were invited to a meeting where the study was again explained, questions were answered, and the vaccination schedule was given. Interested families who did not attend the meeting were sent an informational letter that included vaccination date and time and a consent form. For each household, one child was administered a coded preparation labeled N and the other O (one signified vaccine and the other placebo). The older child in each pair was inoculated first. The randomized pairs of coded preparations and lists denoting the preparation to be administered to each child were generated by the manufacturer. By maintaining bacteriologic and serologic surveillance of all subjects in this randomized, double-blind, placebo-controlled study, it was possible to detect evidence of transmission of the vaccine strain to placebo recipients.

Study 2. The experience in study 1 and subsequent censuses in other villages revealed that because of successful family planning programs, there were few households with pairs of children within the 24- to 59-month age group. Accordingly, the study design was modified to include households having 1 child 24–59 months old. Age-eligible children were recruited as in study 1. On the day of vaccination, after a physical examination and other measures to verify eligibility, age-eligible children from single-subject households were randomized to receive a number-coded preparation (about one-half of which corresponded to packets of vaccine and the remainder placebo). A separate set of packets was available for the few pairs that were recruited for the second study; allocation followed the procedure of the first study except that these packets had number codes.

Vaccination was carried out in the village office near a local health center. Children with chronic health problems, receiving antibiotic therapy, or with acute illness on the scheduled day of vaccination were excluded.

Vaccines and Placebo

Packets of vaccine contained 5×10^9 cfu of lyophilized, filtered CVD 103-HgR [22]. Placebo (5×10^8 inactivated *Escherichia coli* K12 organisms) was of the type extensively used in evaluation of inactivated and live oral cholera vaccines [16, 17, 20–26]. Each packet of vaccine or placebo also contained 18.8 mg of aspartame to sweeten the vaccine cocktail and make it more palatable to children. The sachet of buffer that accompanied each packet of vaccine or placebo held 1.24 g of NaHCO₃ and 0.88 g of ascorbic acid. The buffer powder was stirred into a cup containing 50 mL of nonchlorinated water, after which the vaccine (or placebo) packet was emptied into the buffer solution, stirred again, and immediately given to the child. Parents had been instructed that the children should refrain from eating or drinking for 1 h before and after vaccination. Children were considered vaccinated if they drank ≥50% of the suspension.

Medical Supervision

Physicians visited the children daily for 9 days in their households to elicit and document complaints, do physical examinations, and record axillary temperatures. The clinical follow-up,

as well as the administration of vaccine (or placebo), was double-blind with neither the clinical staff, the children, nor their parents knowing the identity of the preparation given to any child. Diarrhea was defined as passage of at least three loose stools within 24 h. Vomiting was defined as one or more episodes of emesis. An axillary temperature $\geqslant 37.5°C$ signified fever. Any child who passed three or more loose stools in a 24-h period was given oral rehydration with glucose and electrolytes.

Bacteriology

Clinical. Stool samples or rectal swabs were obtained daily from the children on the 5 days after vaccination. During study 1, an attempt was also made to obtain stool or rectal swabs from family members in the households as a means of monitoring for transmission of the vaccine strain to contacts. Stool or swabs were transported to the laboratory in Cary-Blair medium and inoculated directly onto thiosulfate–citrate–bile salts–sucrose (TCBS) agar or were enriched for 5–6 h in alkaline peptone water before subculture onto TCBS agar [27]. Suspected colonies were confirmed as *V. cholerae* by biochemical tests and as O1 serogroup by agglutination with specific antiserum. Serotyping was done by agglutination with specific Inaba and Ogawa antisera, and preliminary biotyping was based on resistance to polymyxin B (El Tor isolates are resistant, classical are not), hemagglutination of chicken erythrocytes (El Tor strains are positive), and the CAMP test (El Tor strains are positive) [28].

Environmental. Most dwellings had concrete slab latrines that drained into open sewers. Other households had open privies. Moore swabs (4.0-cm-thick rolls of gauze attached to nylon string) [29, 30] were placed in open sewers (near where the household drain entered the sewer) or privies adjacent to 27 dwellings where pairs of children were vaccinated during study 1. In study 2, Moore swabs were similarly placed near 70 additional dwellings. The Moore swabs were placed on the evening or night of the same day as vaccination and remained in situ for 36–40 h. After retrieval, the swabs were inoculated into 250 mL of alkaline peptone enrichment broth and incubated for 6 h at 37°C before subculture onto TCBS agar. Incubation and processing were done in the same manner as for the stool cultures.

Serology

Venous blood (3–4 mL) was collected on the day of vaccination and 9 and 28 days thereafter. Serum Inaba vibriocidal antibody, the primary measurement of immune response, was assayed by microtiter method [31, 32], and a fourfold or greater rise in titer was considered significant (i.e., seroconversion) [32]. Serum vibriocidal antibodies are a marker for the elicitation of protective intestinal immune responses against *V. cholerae* O1 [2]. IgG cholera antitoxin was measured by ELISA in serum specimens diluted 1:50 [33]; if the net optical density (OD) of the prevaccination specimen was $\geqslant 1.00$, sera were tested at 1:400 dilution. A $\geqslant 0.20$ rise in net OD of the postvaccination over the prevaccination specimen was considered significant (i.e., seroconversion) [33].

Statistical Tests and Analyses

Overall rates of adverse reaction and seroconversion were compared by χ^2 test with Yates's correction or by Fisher's exact test. Adverse reaction rates and seroconversion rates among the household pairs of children were analyzed by McNemar's matched pairs analysis. Before their analysis, serum Inaba vibriocidal antibody titers were transformed to logarithms to better approximate normality. The relationship between age of subject and occurrence of fever was examined by logistic regression analysis. The relationship between age group (24–35, 36–47, and 48–59 months) and the fold rise in reciprocal geometric mean titer (GMT) of serum Inaba vibriocidal antibody was examined using analysis of covariance (covariate = day 0 Inaba vibriocidal titer).

Results

Clinical tolerance. In study 1, parents from 170 households with age-eligible pairs of children identified during the census expressed interest in participating. On the day of vaccination, 86 pairs of children presented, of which 80 remained eligible after physical examination and age verification and were entered into the study and vaccinated. Of the 160 children (80 pairs) who were offered vaccine or placebo suspensions, 75 pairs ingested at least 50% of the suspension (the cutoff for being considered vaccinated). In fact, 132 (83%) ingested the full volume. Any child from a pair who ingested the cocktail while the sibling did not was followed to detect adverse reactions. These surveillance data are included in the overall analysis of reactogenicity.

In study 2, parents of 244 age-eligible children expressed interest for their offspring to participate. On the allotted day, 185 children were brought to the vaccination site. Thirty children were judged ineligible by the screening pediatricians, leaving 155 age-eligible children who were offered vaccine; 130 (84%) ingested the entire volume of vaccine or placebo suspension while 149 (96%) ingested at least 50%.

In total, 81 eligible pairs of 24- to 59-month-olds were vaccinated (75 during study 1 and 6 during study 2) and followed for adverse reactions. Two additional children in study 1, representing the single members within a pair who ingested the vaccine cocktail (whereas their paired sibling did not), were also kept under clinical surveillance. Finally, in study 2, 139 additional children, 1 per household, received vaccine or placebo.

The overall occurrence of diarrhea, vomiting, abdominal cramps, and fever among these 303 recipients of vaccine or placebo is summarized in table 1. Neither during the first 3 days of clinical follow-up (when diarrheal reactions were most likely to occur) nor during the entire 9-day period of follow-up were gastrointestinal adverse reactions noted significantly more often in vaccinees. The diarrheal reactions observed in the vaccinees and controls were mild.

Fevers were observed significantly more often in the vac-

Table 1. Adverse reactions among 24- to 59-month-old Indonesian children who received a single 5 × 10⁹ cfu dose of CVD 103-HgR or placebo, during 9 days of follow-up after immunization.

| | | No. (%) with reaction | | | |
Group (n)	Mean age*	Diarrhea	Vomiting	Abdominal cramps	Fever
All children					
Vaccinees (155)	42.5 ± 11.1	18 (11.6)	16 (10.3)	18 (11.6)	28 (18.1)
Placebo (148)	44.3 ± 11.4	12 (8.1)	10 (6.7)	13 (8.7)	14 (9.4)
P†	0.17	0.69	0.37	0.53	0.045
Household pairs					
Vaccinees (81)	41.6 ± 11.6	7 (8.6)	5 (4.9)	10 (12.3)	15 (18.5)
Placebo (81)	43.3 ± 11.9	4 (4.9)	4 (5.6)	7 (8.6)	10 (12.3)
P‡	0.93	0.53	1.00	0.30	0.55

* Months ± SD.
† Unpaired t test for continuous data. χ^2 with Yates's correction (two-tailed test) for comparisons of proportions.
‡ Paired t test for continuous data. χ^2 with Yates's correction (two-tailed test) for comparisons of proportions.

cinees (18.1%) than in the placebo recipients (8.7%; P = .045; table 1). The fevers were low-grade (the maximum temperature elevation was 39.0°C in a vaccinee and 39.4°C in a control) and clustered in the youngest children; fevers were recorded in 29% of children 24–35 months, 16% of those 36–47 months, and 10% of those 48–59 months of age. The relationship between age and occurrence of fever in the vaccine group was statistically significant (logistic regression, P = .02). In contrast, the occurrence of fever in placebo recipients was unrelated to age, being observed in 9% of children aged 24–35 and 48–59 months and 12% of children aged 36–47 months. The fevers demonstrated a curious distribution over time. During the first 5 days of follow-up there was no difference in the occurrence of fever: 14 (9%) of 155 vaccinees versus 12 (8.1%) of 148 controls. However, during the last 4 days of clinical follow-up, fever was observed in 14 vaccinees (9%) and only 1 control (0.7%; P = .002). Analysis of the individual cases of fever that occurred among the vaccinees between days 6 and 9 of follow-up after vaccination revealed that 12 of the 14 subjects had clearcut intercurrent infections. These included upper respiratory infections in 8 children, pharyngitis in 2, and shigellosis in 1.

When the 81 pairs of 24- to 59-month-olds were analyzed separately using a matched pairs analysis, no gastrointestinal or febrile adverse reactions were observed significantly more frequently in vaccinees than in controls (table 1).

Immunogenicity. The rate of seroconversion of serum Inaba vibriocidal antibody among vaccinees and placebo recipients for whom blood samples were available for testing is summarized in table 2, as is the GMT before and the peak GMT after vaccination. Overall, 75% of vaccinees manifested seroconversions; the peak GMT after vaccination was 10-fold above the baseline GMT, and 17% of children reached reciprocal titers ≥2560. Of placebo recipients, 3% demonstrated seroconversions. The propensity to seroconvert was not related to age (table 2). Although the peak

GMT of vibriocidal antibody rose with age, so did the baseline GMT, undoubtedly reflecting cumulative contact with cholera antigens in this endemic area. The mean fold rise in GMT was highest in the oldest stratum of children within this cohort (analysis of covariance, P = .018; table 2). CVD 103-HgR elicited similar seroconversion rates in girls (74%) and boys (76%).

Baseline serum antitoxin levels were exceedingly high, with an arithmetic mean net OD of 0.92 in the vaccine group and 0.96 in the placebo group. Despite the high baseline titers, significant rises in serum antitoxin were observed in 31 (25%) of 125 vaccinees and in 7 (6%) of 120 placebo recipients whose sera were available for testing.

Excretion of vaccine strain by vaccinees. Of the 162 vaccinees from whom stool cultures were obtained, the vaccine strain was recovered from only 4 (2.5%), 2 in study 1 and 2 in study 2. One child's stool cultures also yielded *V. cholerae* of a serogroup other than O1. Wild-type *V. cholerae* O1 strains (biotype El Tor, serotype Ogawa) were isolated from 3 vaccinees (1 in study 1 and 2 in study 2).

The available classical Inaba *V. cholerae* O1 isolates were hybridized with DNA probes that detect the cholera toxin A subunit gene and the Hg⁺⁺ gene. The isolates were negative with the former and positive with the latter probe. In contrast, the wild-type El Tor Ogawa isolates hybridized with the toxin probe and were negative with the Hg⁺⁺ probe.

Transmissibility of the vaccine. Transmission of the vaccine strain to placebo recipients and other household contact controls was sought, directly by culturing stool to detect the vaccine strain and indirectly by collecting blood specimens to look for seroconversion of vibriocidal antibody. Neither CVD 103-HgR nor wild-type El Tor *V. cholerae* was isolated from stool cultures of any of the 81 placebo recipients among the household pairs. CVD 103-HgR was recovered from the stool culture of 1 (0.6%) of 174 other family contacts (a mother). Wild-type *V. cholerae* El Tor Ogawa was isolated

Table 2. Serum Inaba vibriocidal antibody and serum IgG antitoxin responses following ingestion of a single 5×10^9 cfu dose of CVD 103-HgR live oral cholera vaccine or placebo by 24- to 59-month-old Indonesian children.

Group (n)	Vibriocidal antibody					IgG antitoxin		
	Seroconversion rate*	GMT before vaccination[†]	Peak GMT[†]	Rise in GMT	No. (%) with titer >2560	Seroconversion rate*	Mean before vaccination[‡]	Mean peak[‡]
Vaccinees								
All ages (125)	94 (75)	26 (3.2 ± 1.4)	243 (5.5 ± 1.9)	10-fold	21 (17)	31 (25)	0.92 ± 0.57	1.13 ± 0.60
24–35 months (41)	32 (78)	16 (2.8 ± 1.1)	131 (4.9 ± 1.7)	8-fold	4 (10)	8 (20)	1.10 ± 0.67	1.26 ± 0.60
36–47 months (36)	25 (69)	24 (3.2 ± 1.2)	176 (5.2 ± 1.7)	7-fold	3 (8)	9 (25)	0.95 ± 0.44	1.12 ± 0.49
48–59 months (48)	37 (77)	39 (3.7 ± 1.6)	523 (6.3 ± 1.9)	13-fold	14 (29)	14 (29)	0.75 ± 0.52	1.04 ± 0.67
Placebo, all ages (120)	4 (3)	21 (3.2 ± 1.1)	23 (3.1 ± 1.1)	—	0	7 (6)	0.95 ± 0.62	0.93 ± 0.58

* No. (%) with 4-fold or greater rises. Postvaccination blood specimens were not available from some vaccinated children.
[†] Reciprocal geometric mean titer (log GMT ± SD).
[‡] Arithmetic mean net optical density units ± SD.

from another family contact (0.6%). Of the members of the household pairs who received placebo and whose sera were available for testing, only 1 (1.5%) of 68 manifested seroconversions of vibriocidal antibody; similarly, only 4 (6%) of 67 family contacts who provided serum for testing showed significant rises.

Environmental bacteriology to detect CVD 103-HgR. Moore swabs from 26 (96%) of 27 household environments sampled in study 1 yielded non-O1 *V. cholerae*, demonstrating the sensitivity of the method. In study 2, *V. cholerae* of serogroups other than O1 were recovered from the environment (wastewater or privy) near 20 (29%) of 70 dwellings. However, in no instance in either study did a Moore swab culture yield the vaccine strain or *V. cholerae* O1.

Discussion

This report summarizes experiences with a single 5×10^9 cfu dose of CVD 103-HgR in 24- to 59-month-old children living in North Jakarta. Because we were evaluating in young children a recombinant live vaccine derived from a known pathogenic parent strain of *V. cholerae* O1, we were particularly concerned about the occurrence of gastrointestinal adverse reactions. It is reassuring that in this randomized, placebo-controlled, double-blind study, neither diarrhea, vomiting, nor abdominal cramps was observed significantly more often among vaccinees. Surprisingly, febrile reactions were noted significantly more often in vaccinees than controls (18.1% vs. 8.1%; $P = .045$). These fevers, which were mostly low-grade, clustered in the youngest children during days 6–9 after vaccination. It is not readily apparent why an attenuated *V. cholerae* O1 vaccine would be associated with low-grade fevers in this age group, particularly with a late

onset. In previous randomized, placebo-controlled trials in adults and 5- to 9-year-old Indonesian children, febrile reactions were not observed [14, 16–23]. Moreover, fever is not a prominent symptom of cholera, although it has been observed in 10%–20% of volunteers with experimental cholera [34]. We believe that this is a chance finding not biologically attributable to vaccine, since careful examination of the clinical records of each of the children who manifested fever during the latter third of the clinical follow-up (when fever was seen more often in vaccinees) revealed an intercurrent infection in 12 of 14 vaccinees. The fevers in vaccinees were not correlated with diarrhea ($P = .46$) or other gastrointestinal adverse effects. A similarly designed reactogenicity and immunogenicity study of CVD 103-HgR in 24- to 59-month-olds that was recently completed in Costa Rica showed no difference in the rate of occurrence of fever between vaccinees and controls (unpublished data).

A single dose of CVD 103-HgR was quite immunogenic. Seventy-five percent of vaccinees manifested significant rises in vibriocidal antibody (the best correlate of protection) [2, 35–38], and overall the vaccinees demonstrated a mean 10-fold rise in GMT over baseline (table 2). For the purpose of comparison, it is of interest to note that among children 2–5 years of age in Bangladesh who ingested three doses of inactivated whole cell *V. cholerae* O1 vaccine (either alone or combined with B subunit), 36% manifested 4-fold rises in serum Inaba vibriocidal antibody and overall they exhibited a mean 4-fold increase in titer over baseline; after a single dose of vaccine, only 18% exhibited 4-fold rises [26].

A characteristic feature of CVD 103-HgR live oral cholera vaccine is that while it elicits excellent immune responses, it is minimally excreted. In these studies of those 24–59 months old, the vaccine strain was recovered from stool cul-

tures of only 2.5% of vaccinees. With such minimal excretion one would not anticipate frequent secondary transmission of the vaccine strain. This study was designed to incorporate conditions that would favor transmissibility: for example, the study was carried out in 24- to 59-month-olds, an age group in which fecal transmission occurs readily because of primitive personal hygiene practices, even in industrialized settings with high levels of sanitation [39, 40]; moreover, dwellings in the study villages typically do not have piped water or sewage disposal. Despite these conditions, neither by stool culture nor by seroconversion of vibriocidal antibody was there much evidence of secondary transmission of the vaccine strain to contacts. Since El Tor cholera is endemic in North Jakarta, one cannot exclude the possibility that some of the few seroconversions of vibriocidal antibody observed in placebo recipients and family contacts were due to ingestion of wild-type *V. cholerae* O1. Even if all the seroconversions among contacts were due to the live vaccine, this would represent a secondary transmission rate of only 6% under conditions highly favorable for such transfer.

Obtaining information on transmissibility of the vaccine strain was critical in order to design a large-scale field trial to determine the efficacy of a single dose of CVD 103-HgR in preventing cholera under conditions of natural challenge in North Jakarta. Earlier field trials elsewhere that examined the efficacy of parenteral and oral inactivated vaccines were designed so that individual subjects were randomly allocated to receive vaccine or placebo [41–45]. If CVD 103-HgR live cholera vaccine were readily transmitted to contacts, as is the Sabin attenuated poliomyelitis vaccine [46], it would be incorrect to design a field trial based on random allocation of individuals. With a readily transmissible vaccine strain, many placebo recipients inadvertently would be immunized; as a result, the incidence of cholera in the placebo group would be falsely low and this, in turn, would lower the calculated vaccine efficacy. Rather, to field test the efficacy of a readily transmitted live oral cholera strain, an innovative design would be required in which the unit of random allocation would be villages or extended households, not individuals. On the basis of the apparent low level of transmission of CVD 103-HgR even among preschool children, a large-scale, randomized, placebo-controlled, double-masked field trial, which follows the classic study design by which earlier cholera vaccines were tested, was initiated in June 1993 in 66,000 pediatric and adult subjects in North Jakarta. This World Health Organization–sponsored field trial will measure the efficacy of a single dose of CVD 103-HgR in preventing cholera in this high-risk area.

Recombinant DNA technology has revolutionized vaccine development, resulting in new or improved vaccines against many infectious diseases [47]. Nevertheless, a segment of the public and the scientific community remains suspicious of the products of recombinant DNA technology and of the release into the environment of genetically engi-

neered strains, including vaccines [48–52]. Although the US Food and Drug Administration does not regulate genetically engineered vaccines any differently than it does live vaccines prepared by nonrecombinant means such as chemical mutagenesis, there are advantages to using a live recombinant vaccine such as CVD 103-HgR that is not heavily excreted and that does not readily enter or persist in the environment. The sensitivity and utility of Moore swab environmental sampling to isolate *Salmonella typhi* [30, 53, 54] and *V. cholerae* O1 [29] from fecally contaminated surface waters is well-established. Moore swabs did not recover CVD 103-HgR from the environment around any of the 97 North Jakarta households sampled.

The international, multiagency Children's Vaccine Initiative [55] is promulgating the concept of immunizing infants by the oral route with single-dose, multiantigen vaccines. The remarkable immunogenicity of a single dose of CVD 103-HgR in pediatric populations in less-developed countries [22], and its efficacy thus far in experimental challenge studies in volunteers [14, 19], adds optimism for the generic notion of future multivalent single-dose oral vaccines. In this context, CVD 103-HgR itself is being investigated as a potential live vector vaccine to express foreign antigens [56].

Acknowledgments

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