Safety and Immunogenicity of Oral Killed Whole Cell Recombinant B Subunit Cholera Vaccine in Barranquilla, Colombia[1]

ALBERTO CONCHA,[2] ALEJANDRO GIRALDO,[3] ELIZABETH CASTAÑEDA,[3] MANCEL MARTÍNEZ,[3] FERNANDO DE LA HOZ,[3] FABIO RIVAS,[3] ARIEL DEPETRIS,[4] ANN-MARI SVENNERHOLM,[5] & DAVID A. SACK[6]

♦♦♦

In January and February 1992, an assessment was conducted of the safety and immunogenicity of two doses of a new oral cholera vaccine prepared from the recombinant B subunit of the toxin and from killed whole cells (rBS/WC) in 1 165 individuals between the ages of 12 months and 64 years in Barranquilla, Colombia. This was a randomized, double-blind placebo-controlled study. Participants received two doses of either the vaccine or a placebo (killed Escherichia coli *K12) over a two-week interval. Few symptoms were detected during the three days following administration of the initial dose and even fewer following the second. Sera obtained upon administration of the first dose and two weeks after administration of the second were tested for* Vibrio cholerae *01 Inaba vibriocidal antibodies and antitoxins. Geometric mean titers (GMT) of vibriocidal antibodies were found to increase two-fold in subjects receiving the vaccine. In the paired samples taken from vaccinated subjects, two-fold or greater increases were observed in 44% and four-fold or greater increases were observed in 34%, as compared to similar increases in 9.2% and 2.2% of the sera taken from those receiving the placebo ($P < 0.05$). The GMTs of IgG and IgA antitoxins, as determined by ELISA, increased by factors of 4 and 3.2, respectively, in those receiving the vaccine, as compared to factors of 1.1 and 1.1 in those given the placebo ($P < 0.001$ for IgG, $P < 0.01$ for IgA). Approximately 80% of the paired samples from the vaccinated group showed an increase of both IgG and IgA antitoxins ≥ 1.5, as compared to only about 20% of those in the placebo group ($P < 0.000001$). Belonging to the O blood group did not significantly affect the immune response. Children under age four tended to show a weaker vibriocidal antibody response and a stronger antitoxin response than older subjects. The two doses of oral vaccine were found to be safe and without attributable side-effects. The vibriocidal antibody and antitoxin responses were similar to those obtained previously with the conventional oral killed whole cell B subunit cholera vaccine.*

A reduction in the incidence and severity of cholera achieved in Bangladesh with oral killed whole cell B subunit (BS/WC) cholera vaccine (*1–4*) sparked interest in its potential use in South America. However, the results obtained

[1]Reprint requests and other correspondence should be sent to Dr. Alberto Concha, Carrera 115 #6–140 C20, Cali, Colombia; fax 57-2-8839134; tel. 57-2-554 2483/58. This contribution is an edited version of an article previously published in Spanish in the *Boletín de la Oficina Sanitaria Panamericana*, Vol. 119, No. 2, August 1995, under the title "Seguridad e inmunogenicidad de la vacuna oral anticólera de células enteras muertas y de al subunidad B recombinante de la toxina en Barranquilla, Colombia."

[2]Ministry of Health, Santa Fe de Bogotá, Colombia.
[3]National Institute of Health, Santa Fe de Bogotá, Colombia.
[4]Pan American Health Organization, Santa Fe de Bogotá, Colombia.
[5]University of Göteborg, Göteborg, Sweden.
[6]Johns Hopkins University, Baltimore, Maryland, U.S.A.

in Bangladesh could not be extrapolated directly to this region because the vaccine had been modified and the epidemiologic picture differed (*2, 3*). The main difference between the new vaccine and the earlier one resulted from use of a recombinant B subunit in place of the native B subunit used previously. From an epidemiologic standpoint, the at-risk population in South America was immunologically naive and included a higher percentage of people with blood group O (considered a risk factor for cholera and cholera vaccine failure) than did the Bangladesh population (*5*).

For the above reasons, it was deemed advisable to perform additional studies before developing policies regarding use of the new rBS/WC vaccine in South America. The aim of the study reported here was to assess the vaccine's safety and immunogenicity prior to conducting a phase III field trial of its efficacy in Colombia.

MATERIALS AND METHODS

Study Design

A two-month field trial was planned in order to assess the vaccine's safety and immunogenicity when administered in two doses—the second dose to be given two weeks after the first—to a representative population sample randomly selected from the neighborhood of Los Olivos in Barranquilla, Colombia. The double-blind trial was placebo-controlled. Steps were taken to ensure follow-up of all participants, the taking of blood samples, and compliance with ethical criteria.

Population and Sample Selection

The study was conducted in January and February 1992 in a population characterized by its socioeconomic homogeneity. The densely populated neighborhood consisted mainly of one- and two-room homes, many of them lacking modern water or sanitation facilities. At the outset of the study, maps of the neighborhood were prepared and information was gathered on the number of inhabitants per house (the average being six), the numbers of households and families per block, and the ages of household members. All 130 neighborhood blocks were numbered; and, beginning with one randomly chosen block, one household was selected from every seventh block until a sample of 250 households was obtained.

To obtain participation of the families selected, several teams of motivators were employed. Each family was visited by one team, which explained the study and asked each participant—or his or her parents if the participant was under age 18—to sign an individual consent form. In addition, the teams entered information about the members of each family on a form, all the recorded data then being entered into a computer. With the information thus obtained, vaccination forms were prepared that listed all eligible and consenting individuals with unique identifiers and addresses.

Aside from the need to obtain an appropriate sample size, the only additional selection criteria were that the participants had to be between the ages of 12 months and 64 years and to have resided in the neighborhood for at least two months. Exclusion criteria were confirmed or possible pregnancy, illness requiring bed rest, and a known mental illness limiting the person's ability to give consent. The sample also excluded all individuals who had diarrhea at the time either of the two doses was administered, in order to facilitate detection of new diarrhea episodes following vaccination.

Sample size was determined by considering the need to have enough participants to detect serologic responses and side-effects. It was felt that 5% of the

group receiving the placebo was likely to report the existence of side-effects, and that a further increase of 5% in the vaccinated subjects would have clinical importance. Detection of such a 5% difference ($\alpha = 0.05$; statistical power or potency $= 1 - \beta = 90\%$) would require monitoring 560 participants in each of the two groups. Due to the possible loss of subjects between doses, the projected sample size was designed to exceed this figure by at least 30%.

Regarding serologic responses, it was expected that the vaccine would appear to stimulate antibody production in at least 25% of those given the vaccine as compared to 1% of those given the placebo. Detection of this difference would require a sample of 24 individuals per group ($\alpha = 0.01$; $1 - \beta = 90\%$). The Epi Info computer program, version 5, was used to calculate the sample size (*6*).

Vaccine Administration

From all blocks containing participating families, households were randomly selected to receive either the vaccine or a placebo. Subsequently, the vaccination team visited each home, confirmed the participants' identities, and administered the agent.

The oral vaccine made from killed whole cells of *V. cholerae* (10^{11} vibrios per dose) and from the recombinant B subunit of the toxin (1 mg per dose) was prepared at Sweden's National Bacteriologic Laboratory in Stockholm. The vaccine was maintained in multidose bottles and was refrigerated until administered (*7, 8*).

The placebo consisted of killed whole cells of nonenterotoxigenic *Escherichia coli* K12 with an optical density equal to that of the vaccine. Killed bacteria were included because the placebo had to be innocuous but at the same time needed to have certain characteristics (appearance, taste, and odor) similar to those of the vaccine, something not easily attainable with a chemical solution. Both agents were administered double blind; the vaccination team knew the two only as "vaccine A" and "vaccine B."

Each 3 mL dose of vaccine or placebo was dispensed by a pumping device into a plastic drinking glass containing buffer solution (Samarin, 50 g/L) capable of protecting the gastric-acid sensitive B subunit (*9*). Every 100 mL of the buffer solution contained 2.66 g sodium bicarbonate, 1.6 g tartaric acid, 535 mg citric acid, 150 mg sodium carbonate, 50 mg sodium potassium tartrate, and 2.5 mg colloidal silicon dioxide. The vaccine dose was identical for each participant, but the volume of buffer varied in accordance with age (1–2 years, 10 mL; 3–5 years, 40 mL; 6–11 years, 75 mL; ≥12 years, 150 mL). One member of the vaccination team observed the ingestion of each dose. No restrictions were imposed on intake of food or liquid, either during or after vaccination.

Monitoring Side-effects

During the three days following vaccination, several teams of nurses—who likewise were unaware of how the agents had been distributed—made daily visits to each vaccinated subject in order to record any reported or presenting symptoms. Precoded forms were used to solicit information on the occurrence of diarrhea, nausea, vomiting, and abdominal pain, and to record other symptoms not specifically related to the gastrointestinal tract. Since no vaccine (or placebo) was administered to any participant complaining of symptoms on the day of vaccination, it was assumed that all symptoms reported to the surveillance teams were new.

Serology

The teams took blood samples at the time the initial vaccination was administered and again two weeks following

administration of the second dose. Fingerstick capillary blood (0.1 mL) was drawn using calibrated pipettes and was placed in vials containing 0.9 mL of saline. The diluted blood was refrigerated and sent to the laboratory for centrifuging; the serum was then separated and frozen for subsequent serologic analysis. In addition, red blood cells from the second samples were analyzed to determine each participant's ABO and Rh blood groups (*10*). None of the laboratory technicians who quantified the vibriocidal antibodies and antitoxin were aware of which samples were obtained from vaccine versus placebo recipients.

Vibriocidal antibody titers were determined using a standard microtitering method with an Inaba strain (*V. cholerae* 01 T19479) (*10*), a procedure that involves mixing a series of double dilutions of each serum with a standardized inoculum of the bacterium and a guinea pig complement. The titer obtained in each case corresponded to the highest serum dilution completely inhibiting visible growth of the *V. cholerae* 01. If growth was observed in the first well, it was assumed that the serum had a titer of 1:10, half the titer of the dilution of the first well (1:20). A twofold increase between the titers obtained with the first and second samples was taken to indicate a positive result.

IgG and IgA ELISA titers were determined by means of a microtiter method using low-binding plates, as previously described (*10*). High-binding plates (Immulon II, Dynatech) had been used initially, but these yielded falsely high baseline titers. The titers recorded were estimated from the interpolated serum dilution having an optical density of 0.4 above the baseline value. The paired sera (drawn before and after the two doses of vaccine or placebo) were analyzed simultaneously on the same plate. A standard high-titer serum was also included on each plate for quality control. A titer increase equalling or exceeding 1.5 was taken to show seroconversion.

Statistical Methods

The proportions of vaccine and placebo recipients manifesting various side-effects were analyzed for statistical significance by means of the chi-square (χ^2) test. The incidence of each symptom noted during the three-day period following administration of each dose was calculated, and relative risks, with 95% confidence intervals, were determined. The significance of the different proportions of placebo and vaccine recipients exhibiting a change of antibody titer was tested with the chi-square test. The observed vibriocidal, IgG, and IgA titers were log transformed before comparing titers of the vaccine and placebo recipients, and differences in their geometric mean titers (GMTs) were assessed using Student's *t*-test.

Scientific and Ethical Analysis

The protocol for the study was prepared following a meeting organized by PAHO that was held in Washington, D.C., on 3–4 May 1991. The meeting, attended by consultants and officials from both PAHO and WHO, established priorities for evaluating cholera vaccines in the Region of the Americas. The resulting protocol and available resources were then examined by a committee of experts made up of consultants and officials from both organizations. The protocol was also reviewed by national and regional health authorities, as well as by the ethical evaluation committees of PAHO and Colombia's National Health Institute in Santa Fe de Bogotá. In addition to official authorizations, the Colombian institute asked a group of consulting Colombian physicians and immunologists to conduct an independent review of the protocol.

RESULTS

Population Characteristics

The study, which took in 40 blocks of the Los Olivos neighborhood, initially involved preliminary visits to 515 households, 568 families, and 3 028 individuals in order to explain the purpose and scope of the study and request their consent. On the average, each block contained 12.88 households and 14.20 families, there being an average of 5.88 individuals per selected household. A total of 1 933 individuals (64%) agreed to participate in the study and signed individual consent forms; 1 313 (43% of the original study population) received the initial dose of either vaccine or placebo, and 1 165 (88.7% of these) received the second dose. Of the individuals who initially gave their consent to participate in the study, 620 decided not to do so as a result of a political campaign oriented against the vaccination project.

The participants' age distribution was compared to that of the general Barranquilla population, with no significant differences being found. As Table 1 shows, roughly half the participants were between 15 and 64 years old. Sixty-two percent of the participants had blood group O.

Initially, the group scheduled to receive the placebo contained 95 more participants than the group scheduled to receive the vaccine, because the randomly selected placebo group households tended to be larger than the vaccine group households. After 620 consenting individuals withdrew from the study, the number actually receiving the first dose of placebo came to exceed the number receiving the vaccine by 105.

Symptom Surveillance

Tables 2 and 3 show the numbers of participants reporting symptoms during the three days following their first and second vaccinations, respectively. Incidences of abdominal pain and nausea were somewhat higher among those receiving the vaccine, while headache was reported somewhat more frequently by those receiving the placebo. All of the symptoms reported were minor, and none required medical attention. The incidence of diarrhea following the initial dose was about 2%, with no significant difference being detected between the groups.

As Table 3 shows, the incidence of reported symptoms was much lower in the three-day period following receipt of the second dose of both agents. Moreover, most of those who complained of diarrhea had this symptom for only one day. Although three became sick for a period of two or three days and were given oral

Table 1. Breakdown by age group of participants who received two doses of rBS/WC cholera vaccine or placebo. Barranquilla, Colombia, 1992.

Age group (in years)	First dose				Second dose			
	Vaccine		Placebo		Vaccine		Placebo	
	No.	%	No.	%	No.	%	No.	%
1–4	91	15.1	93	13.1	87	16.4	84	13.2
5–14	203	33.6	250	35.3	186	35.1	236	37.2
15–64	310	51.3	366	51.6	257	48.5	315	49.6
Total	604	100	709	100	530	100*	635	100*
Vaccine + placebo (No.)	1 313				1 165			

* The second dose was received by 87 8% and 89.6% of those who received the first doses of vaccine and placebo, respectively.

Table 2. Symptoms reported in the three-day period following administration of the first dose of cholera vaccine or placebo.

	Age group															
	1–4 years				5–14 years				15–64 years				Total			
	Vaccine		Placebo		Vaccine		Placebo		Vaccine		Placebo		Vaccine		Placebo	
Symptom	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No	(%)
Diarrhea	3	(3)	3	(3)	5	(2)	2	(1)	6	(2)	3	(1)	14	(2)	8	(1)
Nausea	2	(2)	1	(1)	4	(2)	1	(0.4)	9	(3)	5	(1)	15	(2)	7	(1)*
Abdominal pain	6	(7)	2	(2)	17	(8)	8	(3)†	24	(8)	18	(5)	47	(8)	28	(4)‡
Vomiting	0		0		0		0		1	(0 3)	0		1	(0.2)	0	
Headache	0		4	(4)	2	(1)	13	(5)	14	(5)	23	(6)	16	(3)	40	(6)§
Dizziness	0		1	(1)	0		1	(0.4)	8	(3)	7	(2)	8	(1)	9	(1)

* $\chi^2 = 4.5$; $P = 0.33$ for the placebo (A) as compared to the vaccine (B); RR = 0.4, 95% CI: 0.16–0.96.
† $\chi^2 = 5.97$; $P = 0\ 014$ for A as compared to B; RR = 0.4, 95% CI. 0.17–0 85.
‡ $\chi^2 = 9$; $P = 0.0025$ for A as compared to B; RR = 0.5, 95% CI: 0.32–0.79.
§ $\chi^2 = 6.1$; $P = 0\ 013$ for A as compared to B; RR = 5.2, 95% CI: 1.2–22.7.

Table 3. Symptoms reported in the three-day period following administration of the second dose of cholera vaccine or placebo. No significant differences were found between the symptoms caused by the vaccine and the placebo.

	Age group															
	1–4 years				5–14 years				15–64 years				Total			
	Vaccine		Placebo		Vaccine		Placebo		Vaccine		Placebo		Vaccine		Placebo	
Symptom	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Diarrhea	3	(3.4)	0		1	(0.5)	1	(0.4)	0		1	(0.3)	4	(0.8)	2	(0.3)
Nausea	1	(1.1)	0		1	(0.5)	0		0		0		2	(0.4)	0	
Abdominal pain	0		0		3	(1.6)	1	(0.4)	7	(2.7)	11	(3.5)	10	(1.9)	12	(1.9)
Vomiting	1	(1.1)	0		1	(0.5)	1	(0.4)	1	(0.4)	0		3	(0.6)	1	(0.2)
Headache	0		0		3	(1.6)	3	(1.3)	4	(1.6)	6	(1.9)	7	(1.3)	9	(1.4)
Dizziness	0		0		1	(0.5)	1	(0.4)	3	(1.2)	0		4	(0.8)	1	(0.2)

rehydration salts, they did not become dehydrated. No participant had nausea or abdominal pain for more than a day.

Immunogenicity

Although not all participants agreed to have blood drawn, it was possible to obtain blood samples from a sufficiently large number to permit immunogenicity analysis. Out of a total of 955 paired serum specimens taken from individuals who received both doses, 383 were randomly selected for analysis of vibriocidal antibody titers (Table 4); and of these, 162 were used to investigate antitoxin titers (Tables 5 and 6). The vibriocidal antibody GMTs increased roughly two-fold among those receiving the vaccine but did not vary significantly among those receiving the placebo ($P < 0.000001$). Among those receiving the placebo, 2% and 9% showed respective ≥4-fold and ≥2-fold increases in their vibriocidal antibody titers. By comparison, 34% and 44% of the vaccine recipients had such titer increases ($\chi^2 = 71.9$, df = 4, $P < 0.000001$ for the overall difference between vaccine and placebo recipients when grouping all titer rises ≥16).

Although the IgG antitoxin GMTs increased roughly four-fold among those

Table 4. Geometric means of the vibriocidal antibody titers before and after administration of the two doses of cholera vaccine or placebo.

Age group (in years)	Serum samples (No.)		Prevaccination titers		Postvaccination titers	
	Vaccine	Placebo	Vaccine	Placebo	Vaccine	Placebo
1–4	35	33	11.3	13.4	15.5	14.9
5–14	59	66	16.0	15.5	32.0*	15.2
15–64	98	92	25.8	17.9	62.0*	20.1
Total	192	191	19.1	16.2	39.3*	17.2
Proportional change from prevaccination titer:					2.1	1.1

* $P < 0.001$.

Table 5. Geometric means of the IgG antitoxin titers before and after administration of the two doses of cholera vaccine or placebo.

Age group (in years)	Serum samples (No.)		Prevaccination titers		Postvaccination titers	
	Vaccine	Placebo	Vaccine	Placebo	Vaccine	Placebo
1–4	10	8	305	288	1 466*	329
5–14	21	32	250	240	824*	301
15–64	49	42	148	222	609*	240
Total	80	82	186	234	741*	269
95% CI for the total			171–203	219–251	662–836	248–292
Proportional change from the prevaccination titer:					4.0	1.1

* $P < 0.001$.

Table 6. Geometric means of the IgA antitoxin titers before and after administration of the two doses of cholera vaccine or placebo.

Age group (in years)	Serum samples (No.)		Prevaccination titers		Postvaccination titers	
	Vaccine	Placebo	Vaccine	Placebo	Vaccine	Placebo
1–4	10	8	13	19	78*	19
5–14	21	32	17	13	46*	16
15–64	49	42	17	22	49*	25
Total	80	82	16	18	51*	20
95% CI for the total			15–17	16–19	47–56	12–22
Proportional change from the prevaccination titer:					3.2	1.1

* $P < 0.01$.

receiving the vaccine, they remained almost unchanged among the placebo recipients ($P = 0.000001$) (Table 5). Marked increases (>1.5 times) in IgG antitoxin titers were observed in 88% of the former and 22% of the latter ($P < 0.000001$). Similarly, the overall IgA antitoxin GMTs increased by 3.2 times versus 1.1 times in the sera of those receiving the vaccine and placebo, respectively ($P < 0.000001$), while the seroconversion rates were 80% and 21% respectively ($P < 0.000001$). Overall, an IgG or IgA antitoxin response was observed in 93.7% of those who re-

ceived the vaccine, as compared to 30% of those who received the placebo ($P < 0.000001$) (Table 6).

Analysis of the titer changes by age group revealed that the vibriocidal antibody response in children between the ages of 1 and 4 was less than that observed in older age groups, whether expressed as a change in GMT or as the proportion of participants in each age group with a significant increase in titer (see Table 4). On the other hand, as may be seen in Tables 5 and 6, the average increases in the IgG and IgA antitoxin titers were greater in this younger age group.

No significant differences were detected between the participants with blood group O and those with group non-O in terms of their specific prevaccination titers by age group or in terms of their response to the vaccine. For example, the IgG antitoxin GMT for participants with blood group O versus group non-O was 301 versus 291 in the 1–4 year age group ($P = 0.73$), 252 versus 231 in the 5–14 year group ($P = 0.6$), and 186 versus 171 in the 15–64 year group ($P = 0.6$).

DISCUSSION

This study, which was conducted on an immunologically naive South American population, corroborated the results of previous studies that found the rBS/WC cholera vaccine prepared with the recombinant B subunit of the toxin (*8, 11*) and the previous vaccine made with the native B subunit (*7*) to be safe and immunogenic. Previous studies of the vaccine made with the recombinant B subunit employed relatively limited numbers of adult volunteers, whereas the present study took in a relatively larger group that included young children.

Few symptoms were reported following administration of either the vaccine or the placebo. Some of the reported symptoms were related to previously existing health problems, as indicated by their occurrence among those receiving the placebo. However, there was an increase in the incidence of mild abdominal pain and nausea following administration of the first dose of vaccine. Conversely, a somewhat higher incidence of headache was reported following administration of the first dose of placebo. (These could have been chance associations, in view of the many comparisons allowed by this type of analysis.) Few symptoms were reported following administration of the second dose of vaccine or placebo.

The antibody titers of a random sample of 383 paired serum specimens indicated the vaccine was as immunogenic as that in which the native B subunit was used (*7, 10*), and also as immunogenic as the same recombinant vaccine when it was administered to volunteers in Sweden and the United States (*8, 11*). In order to standardize procedures and ensure the achievement of comparable results in the future, the serologic methods employed in the present study were established through training offered by the Swedish laboratory and an exchange of specimens with that institution.

In this study, children 1–4 years old tended to show a greater antitoxin response and a lower vibriocidal antibody response than older participants. The similarity of this age-related immunogenicity to that previously found in Bangladesh suggests that the relatively poor vibriocidal antibody response among children is truly an age-related hyporesponsiveness not merely due to a lack of prior natural exposure, as might be concluded from the Bangladesh experience. It also suggests that it may be especially difficult to protect young children (<5 years of age) with killed oral vaccines.

The blood group O, a risk factor for cholera, did not notably influence either the prevaccination titers or the response to the vaccine. In Bangladesh, children

with blood group O had higher antitoxin titers before vaccination (*10*), but that was not the case in Colombia. Since the cholera toxin is similar to the thermolabile enterotoxin produced by *E. coli*, the high antitoxin titers recorded in Bangladesh could have been stimulated by either of the two, as it was not possible to distinguish between the two antigens. However, cholera had only recently appeared in the Barranquilla study area and was rare, while enterotoxigenic *E. coli* infection is common among children in South America (*12, 13*). The absence of a specific group O difference in the antitoxin titers of young Colombian children suggests that the difference observed in Bangladesh was the result of exposure to cholera at a young age and that blood group O is probably not a risk factor for enterotoxigenic *E. coli*.

Because the vaccine is believed to provide protection by way of mucosal immunity, levels of IgA as well as IgG antitoxin were measured. However, although serum IgA antitoxin responses were common, the titers were relatively low and the IgA increases observed in paired sera were less than those found for IgG. In view of the low incremental yield of the serum IgA assay, future studies might best conduct only IgG assays, particularly in view of a correlation found by another study between serum IgG and intestinal IgA (*14*).

Not all of the individuals residing in the neighborhood of Los Olivos participated in the study, and it is conceivable that the study participants were not representative of the general population. However, the participants' demographic characteristics were similar to those of the general population, indicating a low likelihood that this would be a constraint. In addition, the study subjects were not randomly selected until after they had agreed to participate, as a result of which the groups receiving the vaccine and the placebo are comparable.

To sum up, this study shows that the vaccine tested is safe and immunogenic, indicating its readiness for testing by means of a phase III efficacy study in a similar South American population.

Acknowledgments. The study reported here received financial support from the Pan American Health Organization and the World Health Organization. The vaccine itself was generously donated by the National Bacteriological Laboratory in Stockholm, Sweden. We gratefully acknowledge the cooperation of the families of Los Olivos that participated in the study and the encouragement and support of Dr. W. Munar and the Health Ministry in Colombia's Atlántico Department. We are also indebted to Mrs. Kierstin Andersson of the University of Göteborg, who helped to establish the serologic assays in Santa Fe de Bogotá, and to Dr. Akira Homma of PAHO and Dr. Nathaniel Pierce of WHO for their encouragement and scientific guidance.

REFERENCES

1. Holmgren J, Svennerholm AM, Clemens JD, Sack DA, Black R, Levine M. An oral B subunit-whole cell vaccine against cholera: from concept to successful field trial. *Adv Exp Med Biol* 1987;216B:1649–1660.
2. Clemens JD, Harris JR, Sack DA, Chakraborty J, Ahmed F, Stanton BF, et al. Field trial of oral cholera vaccines in Bangladesh: results of the one year of follow-up. *J Infect Dis* 1988;158(1):60–69.
3. Clemens J, Sack DA, Harris JR, vanLoon F, Chakraborty J, Ahmed F, et al. Field trial of oral cholera vaccines in Bangladesh: results from three-year follow-up. *Lancet* 1990;335(8684):270–273.
4. Pan American Health Organization. Status of new cholera vaccines. *Bull Pan Am Health Organ* 1991;25(3):278–280.
5. Clemens JD, Sack DA, Harris JR, Chakraborty J, Khan MR, Huda S, et al. ABO blood groups and cholera: new observations on specificity of risk and modification of vaccine efficacy. *J Infect Dis* 1989; 159(4):770–773.

6. Dean JG, Dean JA, Burton AH, Dicker RC. Epi Info, Version 5.01b: a word processing, database, and statistics program for epidemiology on microcomputers. Atlanta, Georgia: Centers for Disease Control and Prevention; 1991.
7. Clemens JD, Stanton BF, Chakraborty J, Sack DA, Khan MR, Huda S, et al. B subunit-whole cell and whole cell only oral vaccines against cholera: studies on reactogenicity and immunogenicity. *J Infect Dis* 1987;155(1):79–85.
8. Jertborn M, Svennerholm AM, Holmgren J. Safety and immunogenicity of an oral recombinant cholera B subunit-whole cell vaccine in Swedish volunteers. *Vaccine* 1992;10(2):130–132.
9. Clemens J, Jertborn M, Sack DA, Stanton B, Holmgren J, Khan MR, et al. Effect of neutralization of gastric acid on immune responses to an oral B subunit killed whole cell cholera vaccine. *J Infect Dis* 1986; 154(1):175–178.
10. Sack DA, Clemens JD, Huda S, Harris JR, Khan MR, Chakraborty J, et al. Antibody responses following immunization with killed oral cholera vaccines during the 1985 field trial in Bangladesh. *J Infect Dis* 1991;164(2):407–411.
11. Sánchez JL, Trofa AF, Taylor DN, Kuschner RA, DeFraites RF, Craig SC, et al. Safety and immunogenicity of the oral, whole cell/recombinant B subunit cholera vaccine in North American volunteers. *J Infect Dis* 1993;167(6):1446–1449.
12. Trujillo H, Jaramillo C, Restrepo M, Mejía GI, Zapata CT, Ramírez R, et al. Rotavirus y otros enteropatógenos en la etiología de la diarrea aguda en Medellín, Colombia, 1982. *Bol Oficina Sanit Panam* 1985;98(3):251–260.
13. McLean M, Brennan R, Hughes JM, Korzeniowski OM, de Souza MA, Arujo J, et al. Etiology of childhood diarrhea and oral rehydration therapy in northeastern Brazil. *Bull Pan Am Health Organ* 1981;15(4):318–325.
14. Svennerholm AM, Jertborn M, Gothefors L, Karim AM, Sack DA, Holmgren J. Mucosal antitoxic and antibacterial immunity after clinical cholera and after cholera disease and after immunization with a combined B subunit-whole cell vaccine. *J Infect Dis* 1984;149(6):884–893.

♦♦♦

Dengue Epidemic in the Americas

As of 15 November, there had been 203 464 reported cases of dengue in the Region in 1995. That number represents an increase of 64 398 cases in under two months (since 24 September). The affected area extends from Mexico to Brazil and includes the Caribbean.

Of the total number of cases to 15 November, 5 548 were dengue hemorrhagic fever; this dangerous form of the disease was responsible for 77 deaths. As of the same date, the English-speaking Caribbean countries, Ecuador, and Peru had not reported hemorrhagic dengue cases or associated deaths.

Dengue viruses have been isolated in 11 Central and South American countries, with two or three serotypes isolated in 9 countries and all four found in El Salvador and Honduras.

Source: Country reports compiled by PAHO (HCT/HCP and CAREC), published in *Wkly Epidemiol Rec* 1995;70(44):313-314 and 70(47):333-334.