Effectiveness of Mass Oral Cholera Vaccination in Beira, Mozambique

Marcelino E.S. Lucas, M.Sc., Jacqueline L. Deen, M.D., M.Sc., Lorenz von Seidlein, M.D., Ph.D.,
Xuan-Yi Wang, M.D., Ph.D., Julia Ampuero, M.D., M.Sc., Mahesh Puri, M.S., Mohammad Ali, Ph.D.,
M. Ansaruzzaman, M.Sc., Juvenaldo Amos, M.D., M.P.H., Arminda Macuamule, M.S.,
Philippe Cavailler, M.D., M.Sc., Philippe J. Guerin, M.D., M.P.H., Claude Mahoudeau,
Pierre Kahozi-Sangwa, M.D., M.P.H., Claire-Lise Chaignat, M.D., M.P.H., Avertino Barreto, M.D., M.P.H.,
Francisco F. Songane, M.D., M.P.H., M.Sc., and John D. Clemens, M.D.

ABSTRACT

BACKGROUND

New-generation, orally administered cholera vaccines offer the promise of improved control of cholera in sub-Saharan Africa. However, the high prevalence of human immunodeficiency virus (HIV) infection in many cholera-affected African populations has raised doubts about the level of protection possible with vaccination. We evaluated a mass immunization program with recombinant cholera-toxin B subunit, killed whole-cell (rBS-WC) oral cholera vaccine in Beira, Mozambique, a city where the seroprevalence of HIV is 20 to 30 percent.

METHODS

From December 2003 to January 2004, we undertook mass immunization of nonpregnant persons at least two years of age, using a two-dose regimen of rBS-WC vaccine in Esturro, Beira (population 21,818). We then assessed vaccine protection in a case–control study during an outbreak of El Tor Ogawa cholera in Beira between January and May 2004. To estimate the level of vaccine protection, antecedent rates of vaccination were compared between persons with culture-confirmed cholera severe enough to have prompted them to seek treatment and age- and sex-matched neighborhood controls without treated diarrhea.

RESULTS

We assessed the effectiveness of the vaccine in 43 persons with cholera and 172 controls. Receipt of one or more doses of rBS-WC vaccine was associated with 78 percent protection (95 percent confidence interval, 39 to 92 percent; P=0.004). The vaccine was equally effective in children younger than five years of age and in older persons. A concurrently conducted case–control study designed to detect bias compared persons with treated, noncholeraic diarrhea and controls without diarrhea in the same population and found no protection associated with receipt of the rBS-WC vaccine.

CONCLUSIONS

The rBS-WC vaccine was highly effective against clinically significant cholera in an urban sub-Saharan African population with a high prevalence of HIV infection.

CHOLERA IS ENDEMIC IN MOZAMBIQUE, with cases usually detected from January to June during the rainy season. The cities of Maputo and Beira are the worst affected. Despite control strategies, including improved case management, water-chlorination campaigns, and dissemination of health-education messages, the burden of cholera has remained high.[1] As a result, there is increasing interest in the use of cholera vaccines.

New-generation, orally administered cholera vaccines are promising tools for the control of cholera in these settings. One such vaccine, consisting of recombinant cholera-toxin B subunit and killed whole cells (rBS-WC), has been shown to confer protection against endemic cholera and is internationally licensed.[2-5] The World Health Organization recently recommended that new-generation cholera vaccines be considered in certain endemic and epidemic situations but indicated that demonstration projects are needed to provide more information about the costs, feasibility, and effects of using these vaccines.[6] Several uncertainties about these vaccines, including their protective effect in settings in which the prevalence of human immunodeficiency virus (HIV) infection is high, must be addressed before they are introduced into public health programs.

To address the policy question of the feasibility and effects of mass immunization with the rBS-WC vaccine in Mozambique, we conducted a demonstration project in Beira, a city where cholera is endemic and the seroprevalence of HIV is high. We report the results of a case–control study designed to evaluate the protective effect of vaccination in this setting.

METHODS

STUDY APPROVAL AND INFORMED CONSENT

This project was approved by the government of Mozambique; the institutional review board of the International Vaccine Institute, Seoul, Korea; and the Secretariat Committee on Research Involving Human Subjects of the World Health Organization, Geneva. Informed consent was obtained orally at the community level through meetings with community leaders of Beira. Written informed consent was obtained from all participants during the mass vaccination and from all case subjects and controls before their participation in the study.

VACCINE

Each dose of the rBS-WC vaccine (Dukoral, SBL Vaccines) consists of 1 mg of recombinant cholera-toxin B subunit and approximately 1×10^{11} inactivated whole cells of the classic and El Tor biotypes of *Vibrio cholerae* O1, serotypes Inaba and Ogawa.[5] This vaccine and its predecessor (BS-WC), which contained chemically extracted rather than recombinant cholera-toxin B subunit, have been shown to be safe and protective in several trials conducted in settings where cholera is endemic,[2-5] although none of the trials were conducted in a setting with a high prevalence of HIV infection.

The rBS-WC vaccine was supplied as 3-ml single-dose vials, each with a sachet of sodium bicarbonate buffer. Buffer solution was prepared by dissolving the sachet in drinking water (150 ml of water per sachet). The full dose of vaccine was mixed with 40, 75, or 150 ml of buffer solution for persons 2 to 4, 5 to 11, and more than 11 years of age, respectively. The vaccine was refrigerated during shipping and was stored centrally in a commercial cold-room facility in Beira. Cold boxes were used for transport to the field.

STUDY SITE AND MASS-IMMUNIZATION CAMPAIGN

The port city of Beira, built on swampy ground at the mouth of the Pungwe River, has a population of approximately 450,000. Every year since 1998, Beira has reported cases of cholera, and the disease is considered to be endemic in the area.[1] Periodic flooding, difficult access to safe water, the common practice of defecation in the open, the presence of nonsealed latrines, and drainage of municipal waste into the embankments increase the risk of cholera.[1] The government of Mozambique established the Cholera Treatment Center in Beira to improve case management and decrease case fatality rates.[1] Other government treatment facilities in Beira (health centers and the Central Hospital) routinely refer patients with acute, nonbloody diarrhea to the Cholera Treatment Center. There are few private physicians, and previous surveys have shown that traditional healers are unlikely to be consulted for diarrheal diseases. Thus, surveillance at the Cholera Treatment Center detects nearly all of the city's cases of acute, nonbloody diarrhea requiring medical care. Other than cholera, important public health problems in Beira are malaria, tuberculosis, HIV infection, and the acquired immunodeficiency syn-

drome. Sentinel surveillance for HIV infection among pregnant women in Beira has shown a seropositivity rate of 20 to 30 percent.[7]

Residents of Esturro, a neighborhood in the center of Beira with predominantly impoverished residents, were selected as the target population for immunization. A formal census in which the household address and the age and sex of each household member were recorded was conducted from September to October 2003 and enumerated a total population of 21,818 persons. All healthy, nonpregnant residents of Esturro who were two years of age or older were invited to participate in the mass-vaccination campaign. During the census, 1177 residents (5.4 percent) were less than two years of age, and an estimated 5.0 percent (or 1091 residents) were excluded because of potential pregnancy, leaving a target population of 19,550 persons. Before and throughout the mass immunization, meetings with community leaders and a general information campaign were conducted. Since there was substantial demand for vaccination, persons from outside Esturro who requested the vaccine were also immunized.

The mass vaccination was timed to take place before the anticipated yearly cholera outbreak, which usually coincides with the rainy season. The vaccine was administered in vaccination outposts set up in churches and schools within Esturro. The first round of immunization was conducted from December 11 to 20, 2003, and the second round from January 5 to 12, 2004, thus ensuring a minimum of 15 days between doses.

To administer the vaccine, health care workers shook the vial, opened it, and poured its contents into a cup with buffer solution and stirred. The recipient drank the mixture under direct observation, and the completeness of ingestion was recorded. No additional dose was given unless the contents spilled before any amount had been ingested. Because the accuracy of information about the receipt of vaccine was deemed to be crucial for the case–control study of the effect of the vaccine, considerable effort was devoted to recording information about dosing. During the first round, a card was issued to each vaccine recipient to record his or her name, age, and address; the name of the head of his or her household; the date of vaccination; and the completeness of ingestion of the dose. At the time of dosing, this information was also recorded in a vaccination registry. Only those persons who

had received a first dose (as documented on the vaccination card or in the registry) were given a second dose of the vaccine during the second round of immunization.

POSTVACCINATION SURVEILLANCE FOR CHOLERA

Surveillance for acute, nonbloody diarrhea at the Cholera Treatment Center began on January 1, 2004. An episode of acute diarrhea was defined as the passage of three or more loose or liquid stools over a 24-hour period, with an onset 14 or fewer days before presentation. The date of onset of an episode was defined as the day on which the diarrhea was reported to have begun. Clinical information was obtained for all patients and recorded on case-report forms; dehydration was classified as described elsewhere.[8]

For surveillance, rectal swabs were collected from all Esturro residents presenting to the Cholera Treatment Center with acute, nonbloody diarrhea. In addition, a rectal swab was collected daily from a resident of a neighborhood other than Esturro who was admitted to the Cholera Treatment Center with acute, nonbloody diarrhea associated with severe dehydration and whose illness was considered the sentinel case in the Beira community. The rectal swabs were transported every 12 hours in Cary-Blair medium at room temperature to the study laboratory. From the Cary-Blair medium, the swabs were plated directly onto thiosulfate citrate bile salts sucrose agar and tellurite taurocholate gelatin agar.[9] The swabs were also plated after enrichment in alkaline peptone water (pH 8.6) for six hours at 37°C. After overnight incubation at 37°C, suspected colonies on the agar plates were tested biochemically and agglutinated with polyvalent Ogawa and Inaba antiserum (Difco Laboratories). Nonagglutinating strains were tested with antiserum to *V. cholerae* O139. *V. cholerae* strains were transported to the Centre for Health and Population Research, in Dhaka, Bangladesh, where identification of the isolates was confirmed.[10]

CASE–CONTROL STUDIES

Two case–control studies were conducted concurrently: the first was designed to estimate the protective effect of the killed whole-cell oral cholera vaccine,[11] and the second (a "bias-indicator study") was designed to assess whether the results with respect to effectiveness could be attributed to bias.[12] The primary research question was as follows: Does

receipt of one or more doses of the rBS-WC vaccine provide protection against treated, culture-confirmed cholera? The case subjects and controls in each of the two studies were the residents of Esturro. In the first study, case subjects with cholera were compared with controls who did not have diarrhea; in the second, case subjects with noncholeraic diarrhea were compared with controls who did not have diarrhea. Study staff who enrolled the case subjects and controls and who obtained information on vaccination status and other exposure variables were unaware that a separate, bias-indicator study was being conducted and were also unaware of whether *V. cholerae* O1 was cultured from the case subject and of how the information on vaccination status was to be used in the analysis. An absence of vaccine protection in the second study was to be interpreted as suggesting an absence of bias in the first study.

Definition and Selection of Case Subjects

All patients with acute, nonbloody diarrhea severe enough to cause them to seek care at the Cholera Treatment Center between January 1 and May 31, 2004, were eligible to be included as case subjects with cholera if they fulfilled the following criteria: they gave written informed consent, or in the case of minors, a parent or guardian gave written informed consent for participation in the study; they had resided in Esturro since December 11, 2003; they were at least two years of age and were not pregnant during the time of the mass vaccination; they submitted a fecal specimen that yielded *V. cholerae* O1; and their residence could be located after discharge for acquisition of information about vaccination and other data. Repeated episodes meeting the criteria during the selection interval were excluded. The interval from January 1 to May 31, 2004, was chosen because all cases of cholera in 2004 occurred during this period.

The bias-indicator study included all patients presenting to the Cholera Treatment Center between April 1 and December 31, 2004, who met the same selection criteria as those used for the case subjects with cholera, except that their fecal cultures were negative for *V. cholerae* O1. Since the rBS-WC vaccine has been shown to provide cross-protection against diarrhea due to heat-labile-toxin–producing *Escherichia coli* during a period of several months after vaccination[13,14] and since the goal of the bias-indicator study was to assess whether there was an expected abs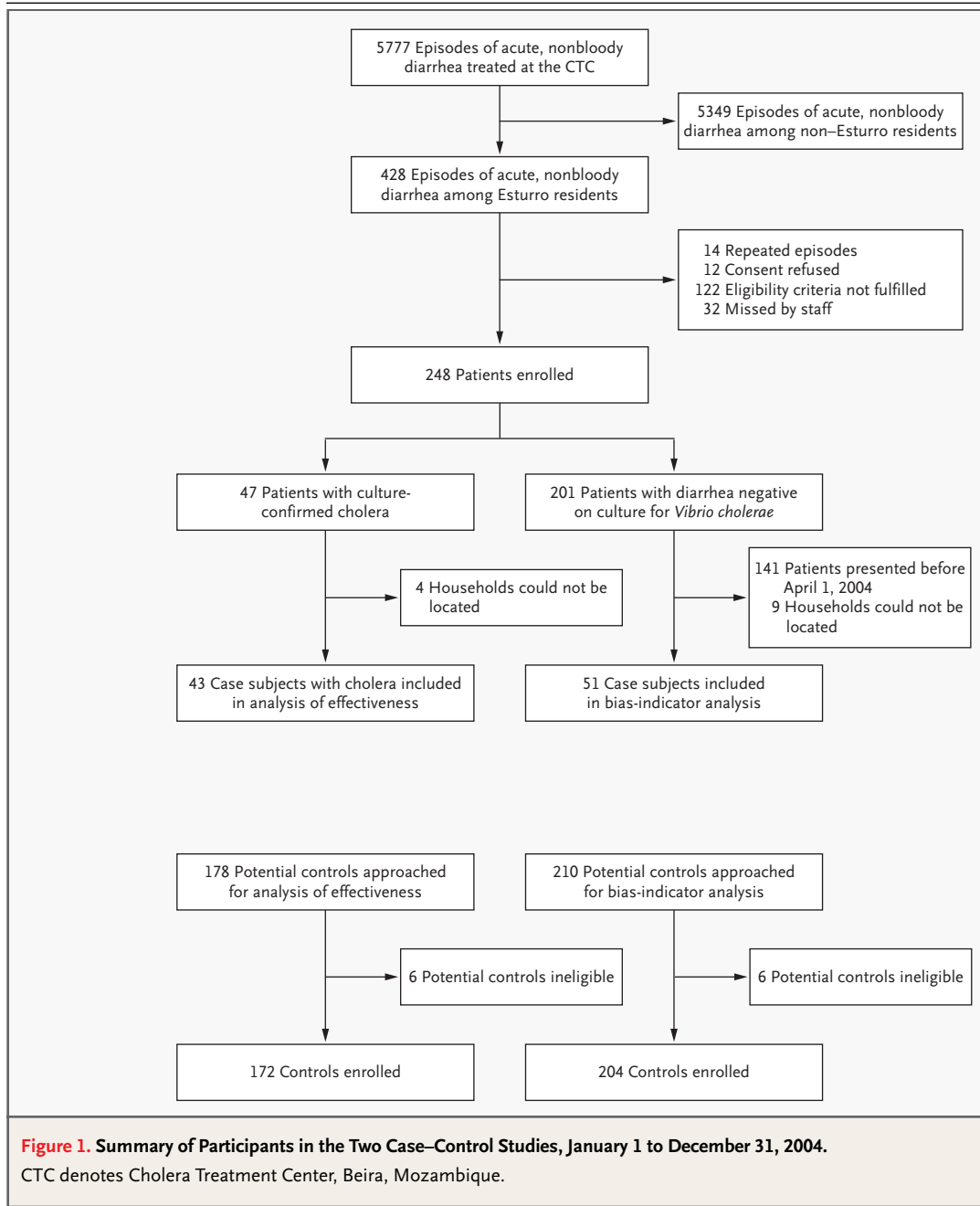ence of vaccine protection, we restrict-ed case subjects in the bias-indicator study to those presenting after April 1, 2004. A summary of the participation of the two groups of case subjects is shown in Figure 1.

Definition and Selection of Controls

A systematic selection procedure was used to recruit four neighborhood controls for each case subject in the two studies.[15] Starting from every third house to the right of the case subject's house, up to six consecutive houses were visited until two eligible controls were enrolled. The procedure was then repeated starting from every third house to the left of the case subject's house. Only one control was recruited per household. A neighbor of the same sex and within the same age group (2 to 4, 5 to 15, or more than 15 years of age) as the case subject was eligible to be a control if he or she had not sought treatment for diarrhea at the Cholera Treatment Center between December 11, 2003, and the date of onset of the matched case subject's diarrheal illness and if he or she would have sought treatment at the Cholera Treatment Center if severe, watery diarrhea had developed. Eligibility for selection also required the same informed-consent, residency, age, and pregnancy criteria as those applied to the case subjects. A neighbor who had been recruited to be a control for one case subject could not subsequently be recruited to serve as a control for another case subject. In the absence of a sex-matched control for five case subjects in the two-to-four-year-old age group, a control of the opposite sex as the case subject was recruited. A summary of the participation of controls is shown in Figure 1.

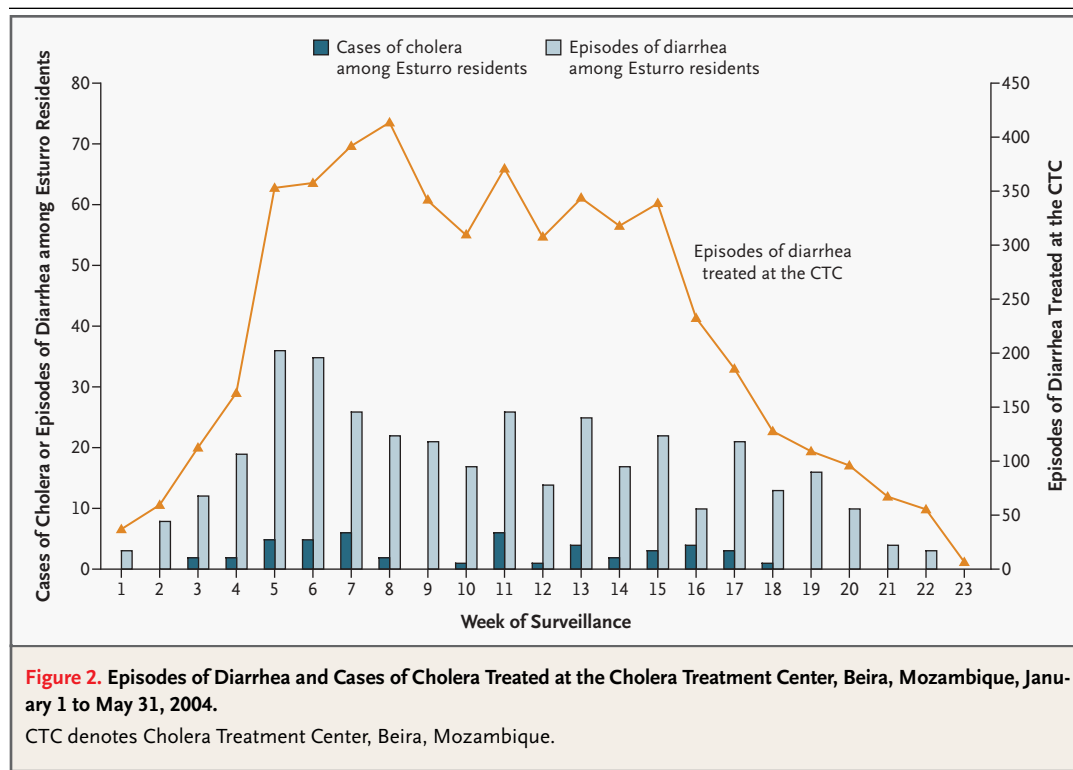### Ascertainment of Vaccination and Potentially Confounding Variables

For the purpose of obtaining preselection information about vaccination and other relevant variables, the date of selection was defined as the date of enrollment of the case subject at the Cholera Treatment Center, both for the case subject and his or her matched controls. Receipt of the cholera vaccine during the mass immunization program was ascertained in face-to-face, home interviews of the case subject and controls. Participants were asked whether they had been vaccinated and, if so, to show the vaccination cards distributed during the campaign. For those who reported that they had been vaccinated but were not in possession of a card, vaccination status and the completeness of dose ingestion

Figure 1. Summary of Participants in the Two Case–Control Studies, January 1 to December 31, 2004.
CTC denotes Cholera Treatment Center, Beira, Mozambique.

were ascertained by searching the vaccination registry. Decisions about linkage to the vaccination registry were made without knowledge of case–control status and were based on the participants' name, sex, age, and name of the head of the household. Demographic, socioeconomic, and environmental variables were ascertained through special questionnaires administered to case subjects and controls and their families.

STATISTICAL ANALYSIS

The primary intention-to-vaccinate analysis, formulated a priori, addressed the protection conferred by one or more doses of vaccine against culture-confirmed cholera that was severe enough to have prompted the participant to present for care at the Cholera Treatment Center. This analysis included case subjects with cholera and their matched controls whose dates of selection were between Janu-

Figure 2. Episodes of Diarrhea and Cases of Cholera Treated at the Cholera Treatment Center, Beira, Mozambique, January 1 to May 31, 2004.

CTC denotes Cholera Treatment Center, Beira, Mozambique.

ary 1 and May 31, 2004, with vaccination defined as receipt of at least one dose, documented either by a vaccination card or information in the vaccination registry, regardless of the volume of vaccine successfully ingested. For comparison, intention-to-vaccinate analysis in the bias-indicator study addressed the protection conferred by one or more doses of vaccine against noncholeraic diarrhea that was severe enough to have prompted the participant to present for care at the Cholera Treatment Center. This analysis included case subjects with noncholeraic diarrhea and their controls, with dates of selection between April 1 and December 31, 2004.

In contrast, the per-protocol analysis addressed the protection conferred by the receipt of two completely ingested doses of vaccine. Vaccinated case subjects and controls were retained for this analysis if their dates of selection were at least 14 days after receipt of the second dose; nonvaccinated case subjects and controls were retained if they had been selected anytime between January 1 and May 31, 2004. A per-protocol analysis was performed in the bias-indicator study in an analogous fashion.

Demographic, environmental, and socioeconomic variables were compared between case subjects and their matched controls in bivariate analy-

ses with the use of conditional logistic regression. Matched P values for associations between exposure variables and case–control status were assessed in these models, with case–control status as the dependent variable and the exposure variable of interest as the independent variable. For both case–control studies, all variables associated (at a significance level of P<0.05) with case–control status in either analysis were then adjusted for in conditional logistic-regression models that also included vaccination status as an independent variable and case–control status as the dependent variable. The exponential of the coefficient for the vaccination variable in these models was computed to estimate the adjusted odds ratio, and the standard error of the coefficient was used to estimate the P value and 95 percent confidence interval for this adjusted odds ratio. To estimate the adjusted level of vaccine protection, the following value for the vaccination variable[11,15] was computed: (1−adjusted odds ratio)×100 percent.

A slightly different strategy was used in subgroup analyses of vaccine protection. To avoid fitting the conditional logistic-regression models with too many independent variables for the number of outcome events observed,[16] small strata were analyzed without control for potential confounding variables,

Table 1. Characteristics of Case Subjects with Cholera and Case Subjects with Noncholeraic Diarrhea and Their Respective Controls.*

Characteristic†	Cholera			Noncholeraic Diarrhea		
	Case Subjects (N=43)	Controls (N=172)	P Value‡	Case Subjects (N=51)	Controls (N=204)	P Value‡
Age — yr	19±13	22±17	0.16	28±16	28±16	0.90
Female sex — no./total no. (%)	23/43 (53)	97/172 (56)	0.34	27/51 (53)	108/204 (53)	1.00
Food consumed in previous 5 days — no./total no. (%)						
Dairy products	8/42 (19)	39/172 (23)	0.61	3/51 (6)	32/203 (16)	0.07
Uncooked food	25/43 (58)	64/172 (37)	0.01	30/50 (60)	115/203 (57)	0.71
Dried fish	14/43 (33)	93/172 (54)	0.01	16/51 (31)	99/203 (49)	0.69
Street food	0/42	3/172 (2)	0.10	1/51 (2)	2/204 (1)	0.57
Food away from home	37/42 (88)	165/171 (96)	0.04	42/51 (82)	200/204 (98)	<0.001
No. of household members	8±15	6±3	0.21	8±13	6±3	0.27
Multiple kitchen implements owned — no./total no. (%)	42/43 (98)	126/172 (73)	0.003	50/51 (98)	140/204 (69)	0.001
Access to a water tap — no./total no. (%)	34/43 (79)	152/172 (88)	0.10	38/51 (75)	189/204 (93)	<0.001
Use of a communal toilet — no./total no. (%)	19/43 (44)	63/169 (37)	0.39	18/50 (36)	63/199 (32)	0.29
Electricity in household — no./total no. (%)	7/43 (16)	32/172 (19)	0.64	6/51 (12)	45/204 (22)	0.04
A *machamba* (small farm) owned — no./total no. (%)	16/43 (37)	59/171 (35)	0.71	17/51 (33)	80/203 (39)	0.41

* Plus–minus values are means ±SD.
† Other characteristics, including the number and percentage of participants with a personal history of cholera, a family member with cholera in past years, and a family member with diarrhea in the previous week, were not significantly different between case subjects and their matched controls.
‡ P values are for comparisons between case subjects and their matched controls.

and in larger strata, variables found to most affect the magnitude of the odds ratio relating vaccination to case–control status were included. On the basis of previously published studies showing differences in protection according to the severity of disease and age strata,[2,3] case subjects were divided into prespecified subgroups. All P values and 95 percent confidence intervals were interpreted in a two-tailed fashion. Statistical significance was designated as a P value less than 0.05. Stata/SE 8 software was used for the statistical analysis.

RESULTS

Of the estimated 19,550 persons in the target population in Esturro, 14,164 (72 percent) received a complete first dose and 11,070 (57 percent) received two complete doses of the rBS-WC cholera vaccine. From January 1 to December 31, 2004, 5777 episodes of acute, nonbloody diarrhea were treated at the Cholera Treatment Center (Fig. 1); 2599 patients (45 percent) were admitted, and 20 of them died (12 adults and 8 children younger than 15 years of age), for in an in-hospital case fatality rate of 1 percent. None of the case subjects enrolled in the study

died. The first case of laboratory-confirmed cholera occurred in a resident of a neighborhood other than Esturro, who presented to the Cholera Treatment Center on January 12, 2004. Thereafter, cases of cholera occurred among Esturro residents from mid-January to mid-April (Fig. 2). Cholera continued to be detected in the sentinel case subjects from outside Esturro until May 26, 2004. All cholera isolates were *V. cholerae* O1, El Tor Ogawa.

We compared several baseline characteristics between the 43 selected case subjects with cholera and their 172 matched controls and, in the bias-indicator study, between the 51 case subjects with noncholeraic diarrhea and their 204 matched controls (Table 1). Case subjects with diarrhea (both choleraic and noncholeraic) were more likely than their controls to own multiple kitchen implements and were less likely to have recently eaten outside their home. Case subjects with cholera were more likely than their controls to have recently eaten uncooked food and less likely to have recently eaten dried fish. Case subjects with noncholeraic diarrhea were less likely than their controls to have electricity and access to a water tap.

In the primary intention-to-vaccinate analysis,

Table 2. Effectiveness of the Oral Cholera Vaccine in Beira, Mozambique.*

Study and Analysis	Vacinees		Crude Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)†	Vaccine Effectiveness (95% CI)	P Value
	Case Subjects	Controls				
	no./total no. (%)				%	
Case–control study of vaccine effectiveness						
Intention-to-vaccinate analysis‡	10/43 (23)	94/172 (55)	0.19 (0.08 to 0.54)	0.22 (0.08 to 0.61)	78 (39 to 92)	0.004
Per-protocol analysis§	8/39 (21)	80/156 (51)	0.17 (0.06 to 0.49)	0.16 (0.05 to 0.57)	84 (43 to 95)	0.005
Bias-indicator case–control study						
Intention-to-vaccinate analysis¶	21/51 (41)	107/204 (52)	0.65 (0.35 to 1.18)	1.00 (0.33 to 3.03)	0 (–203 to 67)	1.00
Per-protocol analysis‖	19/51 (37)	101/204 (50)	0.62 (0.33 to 1.15)	1.53 (0.52 to 4.53)	–53 (–353 to 48)	0.44

* In the case–control study of vaccine effectiveness, case subjects with cholera were compared with controls who did not have diarrhea; in the bias-indicator case–control study, case subjects with noncholeraic diarrhea were compared with controls who did not have diarrhea. CI denotes confidence interval.
† Odds ratios were adjusted for statistically significant baseline characteristics: consumption of uncooked food, consumption of dried fish, consumption of food away from home, ownership of multiple kitchen implements, access to a water tap, and residence in a household with electricity.
‡ This analysis entailed an assessment of the protection conferred by one or more doses of vaccine against cholera among participants selected between January 1 and May 31, 2004.
§ This analysis entailed an assessment of the protection conferred by two complete doses of vaccine against cholera among vaccinated participants selected at least 14 days after receipt of the second dose and nonvaccinated participants selected anytime between January 1 and May 31, 2004.
¶ This analysis entailed an assessment of the protection conferred by one or more doses of vaccine against noncholeraic diarrhea among participants enrolled between April 1 and December 31, 2004.
‖ This analysis entailed an assessment of the protection conferred by two complete doses of vaccine against noncholeraic diarrhea among participants enrolled between April 1 and December 31, 2004.

vaccination was associated with significant protection (78 percent; 95 percent confidence interval, 39 to 92 percent; P=0.004) against cholera, after adjustment for potentially confounding variables (Table 2). Because most vaccinees in the intention-to-vaccinate analysis had received complete two-dose regimens, the per-protocol analysis indicated a similar level of protection (84 percent; 95 percent confidence interval, 43 to 95 percent; P=0.005). In contrast, there was no evidence that the vaccine conferred protection against noncholeraic diarrhea in either the intention-to-vaccinate or per-protocol analysis.

We performed an intention-to-vaccinate subgroup analysis of vaccine protection, according to age at selection and severity of cholera (Table 3). We found vaccine-associated protection both among children two to four years of age and among those five years of age or older. The effectiveness of the vaccine among those more than 15 years of age — an age group in which rates of HIV coinfection may be as high as those among pregnant women in Beira — was 72 percent (95 percent confidence interval, 24 to 91 percent; P=0.03). Although there was considerable overlap of the confidence limits, the point

estimates were higher for vaccine-associated protection against cholera accompanied by severe dehydration than for protection against disease of lesser severity (89 percent vs. 73 percent) and were higher for protection against cholera requiring intravenous rehydration than for protection against that treated only with oral rehydration (82 percent vs. 75 percent).

DISCUSSION

Our estimates of the 78 to 84 percent protection against cholera conferred by the rBS-WC vaccine in this sub-Saharan setting are nearly identical to the 85 percent protection reported four to six months after vaccination in previous trials of BS-WC or rBS-WC vaccines in Bangladesh[2,3] and Peru.[4] In agreement with the previous trials, our data suggest that the rBS-WC vaccine offers better protection against life-threatening cholera than against cholera of lesser severity. However, in contrast to the previous trials, the current study was conducted in a population with a high prevalence of coexisting HIV infection. Moreover, in contrast to the previous trials, which represented idealized evaluations of vac-

Table 3. Intention-to-Vaccinate Subgroup Analysis of the Effectiveness of the Oral Cholera Vaccine.*

Variable	Vaccinees		Odds Ratio (95% CI)	Vaccine Effectiveness (95% CI)	P Value
	Case Subjects	Controls			
	no./total no. (%)			%	
Age					
2–4 yr †	2/9 (22)	22/36 (61)	0.18 (0.02 to 1.19)	82 (–19 to 98)	0.04
≥5 yr	8/34 (24)	72/136 (53)	0.33 (0.14 to 0.84)	67 (16 to 86)	0.02
Severity of illness					
Severe dehydration	2/10 (20)	24/40 (60)	0.11 (0.01 to 0.93)	89 (7 to 99)	0.04
Some or no dehydration‡	8/33 (24)	70/132 (53)	0.27 (0.09 to 0.77)	73 (33 to 91)	0.01
Treatment§					
Intravenous rehydration‡	6/29 (21)	63/115 (55)	0.18 (0.05 to 0.65)	82 (35 to 95)	0.009
Oral rehydration	4/13 (31)	28/53 (53)	0.25 (0.05 to 1.37)	75 (–37 to 95)	0.11

* This analysis entailed an assessment of protection conferred by one or more doses of vaccine against cholera among participants enrolled between January 1 and May 31, 2004. CI denotes confidence interval.

† For this age group, there were no discordant case–control sets, and therefore the stratum was analyzed unmatched and exact P values (calculated by Fisher's exact test) and 95 percent confidence intervals are presented.

‡ The analysis was adjusted for recent consumption of food away from home and for ownership of multiple kitchen implements.

§ Information about treatment was missing for one case subject, whose controls were therefore also excluded from the analysis.

cine efficacy, the current study was conducted under the realistic conditions of a public health program and thus measured the effectiveness of the vaccine.[17]

Previous data that support the use of the killed-whole-cell oral cholera vaccine in sub-Saharan African settings comes from a study in Ugandan refugee camps. A two-dose mass immunization campaign with the rBS-WC vaccine was found to be feasible in this setting.[18] A cholera outbreak occurred in the area the following year, resulting in cholera attack rates of 1 percent in the nonrefugee Ugandan villages, less than 1 percent in the 29 non-vaccinated refugee camps, and 0 percent in the six vaccinated refugee camps.[19] Although the attack rates suggest that vaccination was protective, the differences may have been due to varying conditions in the villages and camps and the geographically circumscribed nature of cholera outbreaks.

Our study, though not a randomized trial, incorporated several features to help ensure the validity of the results. Prospective surveillance was conducted in the only treatment facility providing care for clinically severe, acute, nonbloody diarrhea, and it is therefore likely that the detection of cases that required medical care was nearly complete. Patients underwent systematic microbiologic evaluation, the results of which were independently validated in an external laboratory. Histories of vaccination were prospectively documented, and all decisions about vaccination status that required linkage to an independent registry, rather than inspection of a vaccination card, were made without knowledge of case–control status. Controls were selected in a matched fashion, and extensive information about potentially confounding variables was collected and controlled for in the analyses. The analyses, which were formulated in an a priori fashion, assumed a conservative, intention-to-vaccinate perspective. Finally, a bias-indicator case–control study was performed by workers who used procedures identical to those for the study of vaccine effectiveness and who were unaware that a separate, bias-indictor study was being conducted. As expected, this second study showed that the vaccine did not provide protection against noncholeraic diarrhea.

Although our results suggest that the vaccine offers protection among HIV-infected persons, the study did not include HIV testing and thus could not evaluate vaccine protection in that group directly. For the same reason, the study could not directly assess the safety of the vaccine among HIV-infected persons, although no clinically significant adverse reactions to the vaccine were reported during the

vaccination campaign. In trials of the rBS-WC vaccine in Sweden, Brazil, the United Kingdom, and Kenya, the vaccine was not associated with adverse reactions among HIV-infected persons or with progression of HIV disease, although a transient increase in HIV viremia was observed in one study.[20-22] Additional research is needed to evaluate directly the effectiveness of the rBS-WC vaccine among HIV-infected persons and to clarify the interaction, if any, between HIV infection and cholera.

The results of this study are very promising and should encourage policymakers to consider the use of the rBS-WC oral cholera vaccine as a public health tool in similar settings, particularly when short-term protection is crucial, as in the prevention of cholera outbreaks in refugee settings. More evidence is still needed with respect to long-term protection before use of this vaccine can be recommended for the control of endemic cholera. It is uncertain whether the vaccine induces indirect ("herd") protection, in addition to direct protection of vaccinees.[23] It will be important for future studies to evaluate more comprehensively the combined direct and indirect protective effects of the vaccine when it is deployed in mass-immunization programs. Finally, the question remains as to how the costs of acquisition and delivery of the vaccine can be borne by international donors and by developing countries with areas at risk for cholera outbreaks.

In summary, the rBS-WC oral cholera vaccine was highly effective in conferring short-term protection against severe cholera in an area of sub-Saharan Africa with a high prevalence of HIV infection. This is one step toward the goal of wider use of oral cholera vaccination where it is needed most. Many questions remain, including the duration of protection after mass immunization, financing schemes for sustainable supply and delivery of the vaccine, and the potential of the vaccine to control cholera through combined direct and indirect protective effects. To help provide answers to the first two questions, we are continuing the case–control study throughout the second year after the mass vaccination and will be assessing the privately and publicly borne cost of cholera in Beira.

REFERENCES

1. Report on cholera control programme in Mozambique. Geneva: World Health Organization Global Task Force on Cholera Control, June 2003.
2. Clemens JD, Sack DA, Harris JR, et al. Field trial of oral cholera vaccines in Bangladesh. Lancet 1986;2:124-7.
3. Clemens JD, Sack DA, Harris JR, et al. Field trial of oral cholera vaccines in Bangladesh: results from three-year follow-up. Lancet 1990;335:270-3.
4. Sanchez JL, Vasquez B, Begue RE, et al. Protective efficacy of oral whole-cell/recombinant-B-subunit cholera vaccine in Peruvian military recruits. Lancet 1994;344:1273-6.
5. Holmgren J, Berquist C. Oral B subunit–killed whole-cell cholera vaccine. In: Levine MM, Kaper JB, Rappuoli R, Liu MA, Good MF, eds. New generation vaccines. 3rd ed. New York: Marcel Dekker, 2004:499-509.
6. Cholera vaccines: a new public health tool? Report of a WHO meeting, 10-11 December 2002. Geneva: World Health Organization Global Task Force on Cholera Control, 2004. (WHO/CDS/CPE/ZFK/2004.5.)
7. World Health Organization. Mozambique: summary country profile for HIV/AIDS treatment scale-up, 2004: the 3 by 5 Initiative. (Accessed February 3, 2005, at http://www.who.int/3by5/en/Mozambique.pdf.)
8. A manual for the treatment of diarrhoea. 2nd ed. rev. Geneva: World Health Organization Programme for the Control of Diarrhoeal Diseases, 1990. (WHO/CDD/SER/80.2.)
9. Monsur KA. A highly selective gelatin-taurocholate-tellurite medium for the isolation of *Vibrio cholerae.* Trans R Soc Trop Med Hyg 1961;55:440-2.
10. Ansaruzzaman M, Bhuiyan NA, Nair GB, et al. Cholera in Mozambique, variant of *Vibrio cholerae.* Emerg Infect Dis 2004;10:2057-59.
11. Orenstein WA, Bernier RH, Dondero T, et al. Field evaluation of vaccine efficacy. Bull World Health Organ 1985;63:1055-68.
12. Shapiro ED. Case control studies of the effectiveness of vaccines: validity and assessment of potential bias. Pediatr Infect Dis J 2004;23:127-31.
13. Peltola H, Siitonen A, Kyronseppa H, et al. Prevention of travellers' diarrhoea by oral B-subunit/whole-cell cholera vaccine. Lancet 1991;338:1285-9.
14. Clemens JD, Sack DA, Harris JR, et al. Cross-protection by B subunit-whole cell cholera vaccine against diarrhea associated with heat-labile toxin-producing enterotoxigenic *Escherichia coli*: results of a large-scale field trial. J Infect Dis 1988;158:372-7.
15. Schlesselman JJ. Case-control studies: design, conduct, analysis. New York: Oxford University Press, 1982:69-103.
16. Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. J Clin Epidemiol 1996;49:1373-9.
17. Clemens J, Brenner R, Rao M, Tafari N, Lowe C. Evaluating new vaccines for devel-

oping countries: efficacy or effectiveness? JAMA 1996;275:390-7.

18. Legros D, Paquet C, Perea W, et al. Mass vaccination with a two-dose oral cholera vaccine in a refugee camp. Bull World Health Organ 1999;77:837-42.

19. Dorlencourt F, Legros D, Paquet C, Neira M, Ivanoff B, Le Saout E. Effectiveness of mass vaccination with WC/rBS cholera vaccine during an epidemic in Adjumani district, Uganda. Bull World Health Organ 1999;77: 949-50.

20. Eriksson K, Kilander A, Hagberg L, Norkrans G, Holmgren J, Czerkinsky C. Intestinal antibody responses to oral vaccination in HIV-infected individuals. AIDS 1993; 7:1087-91.

21. Lewis DJ, Gilks CF, Ojoo S, et al. Immune response following oral administration of cholera toxin B subunit to HIV-1-infected UK and Kenyan subjects. AIDS 1994;8:779-85.

22. Ortigao-de-Sampaio MB, Shattock RJ, Hayes P, et al. Increase in plasma viral load after oral cholera immunization of HIV-infected subjects. AIDS 1998;12:F145-F150.

23. Clemens JD, Sack DA, Harris JR, et al. Breast feeding and the risk of severe cholera in rural Bangladeshi children. Am J Epidemiol 1990;131:400-11.

Copyright © 2005 Massachusetts Medical Society.