Vibriocidal Antibody Responses to a Bivalent Killed Whole-Cell Oral Cholera Vaccine in a Phase III Trial in Kolkata, India

Suman Kanungo1*, Anna Lena Lopez2, Mohammad Ali3, Byomkesh Manna1, Deok Ryon Kim3, Tanmay Mahapatra1, Jan Holmgren4, Mandeep S. Dhwira5, Thomas F. Weirzba3, G. Balakrish Nair6, Sujit K. Bhattacharya7, John D. Clemens8, Dipika Sur1

1 Department of Epidemiology, National Institute of Cholera and Enteric Diseases, Kolkata, West Bengal, India, 2 University of the Philippines, National Institutes of Health, Manila, Philippines, 3 International Vaccine Institute, SNU Research Park, Nakseongdae-dong, Gwanak-gu, Seoul, Korea, 4 University of Gothenburg, Gothenburg, Sweden, 5 Shantha Biotechnics Limited, Hyderabad, Andhra Pradesh, India, 6 Executive Director, Translational Health Science and Technology Institute, Gurgaon, Haryana, India, 7 Senior Scientist Platinum Jubilee Fellow, The National Academy of Sciences, Allahabad, India, 8 Executive Director, International Centre for Diarrheal Disease Research, Dhaka, Bangladesh

Abstract

Background: During the development of a vaccine, identification of the correlates of protection is of paramount importance for establishing an objective criterion for the protective performance of any vaccine. However, the ascertainment of correlates of immunity conferred by any vaccine is a difficult task.

Methods: While conducting a phase three double-blind, cluster-randomized, placebo-controlled trial of a bivalent killed whole-cell oral cholera vaccine in Kolkata, we evaluated the immunogenicity of the vaccine in a subset of participants. Randomly chosen participants (recipients of vaccine or placebo) were invited to provide blood samples at baseline, 14 days after the second dose and one year after the first dose. At these time points, serum geometric mean titers (GMT) of vibriocidal antibodies and seroconversion rates for vaccine and placebo arms were calculated and compared across the age strata (1 to 5 years, 5 to 15 years and more than 15 years) as well as for all age groups.

Results: Out of 137 subjects included in analysis, 69 were vaccinees and 68 received placebo. There were 5±7 and 5±8 geometric mean fold (GMF) rises in titers to Vibrio cholerae Inaba and Ogawa, respectively at 14 days after the second dose, with 57% and 61% of vaccinees showing a four-fold or greater titer rise, respectively. After one year, the titers to Inaba and Ogawa remained 1±7 and 2±8 fold higher, respectively, compared to baseline. Serum vibriocidal antibody response to V. cholerae O139 was much lower than that to Inaba or Ogawa. No significant differences in the GMF-rises were observed among the age groups.

Conclusions: The reformulated oral cholera vaccine induced a statistically significant anti-O1 Inaba and O1 Ogawa vibriocidal antibody response 14 days after vaccination, which although declined after one year remained significantly higher than baseline. Despite this decline, the vaccine remained protective five years after vaccination.

Citation: Kanungo S, Lopez AL, Ali M, Manna B, Kim DR, et al. (2014) Vibriocidal Antibody Responses to a Bivalent Killed Whole-Cell Oral Cholera Vaccine in a Phase III Trial in Kolkata, India. PLoS ONE 9(5): e96499. doi:10.1371/journal.pone.0096499

Editor: Stephen J. Turner, University of Melbourne, Australia

Received November 30, 2013; Accepted April 8, 2014; Published May 6, 2014

Copyright: © 2014 Kanungo et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This study was supported by the International Vaccine Institute, Seoul, Korea through the "Diseases of the Most Impoverished" Program and the "Cholera Vaccine Initiative" of Bill & Melinda Gates Foundation. Additional funding was provided by the Swedish International Development Cooperation Agency and the Governments of South Korea, Sweden, and Kuwait. Shantha Biotechnics Limited donated the vaccine and placebo for the study. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have read the journal's policy and have the following conflicts: Shantha Biotechnics donated the vaccine and placebo used in the study. Dr. Mandeep S. Dhwira is an employee of Shantha Biotechnics Inc., Hyderabad, India. Neither Shantha nor Dr. Dhwira was involved in the randomization, analyses or interpretation of the study results. This does not alter the authors' adherence to PLOS ONE policies on sharing data and materials. The authors have declared that no other competing interest exists except the ones mentioned above.

* E-mail: sumankanungo@gmail.com

Introduction

The past decade has seen an increase in the number of cholera outbreaks worldwide [1]. An inexpensive, killed oral cholera vaccine (OCV) was produced in Vietnam in 1997 following technology transfer from Sweden. Various generations of the vaccine were found to be safe and protective [2,3]. The vaccine was reformulated to comply with WHO recommendations [4] and current Good Manufacturing Practices while the vaccine production technology was transferred to a manufacturer in India (Shantha Biotechnics Limited), where the national regulatory authority was WHO recognized.

Prior to the transfer of the technology to India, immunogenicity studies were first conducted in adults in SonLa, Vietnam (a cholera non-endemic area) [5] and in children and adults in Kolkata, India.
(a cholera endemic region) [5,6]. In SonLa, 90% of the vaccine recipients, aged 18–49 years, developed ≥ four-fold rise in vibriocidal antibodies to V. cholerae O1 Inaba, and there was a 26.8-fold rise in the geometric mean titers (GMT) 14 days after the second dose suggesting the reformulated vaccine was highly immunogenic. Overall geometric mean-fold (GMF) rises in serum antibodies were lower in Kolkata (4.5-fold in adults and 12.6-fold in children) than that in SonLa (26.8-fold) where only adults participated. The lower GMF rises seen in Kolkata were explained by higher levels of pre-existing vibriocidal antibody titers in Kolkata compared to that seen in SonLa [5–8].

Oral cholera vaccines stimulate anti-LPS secretory IgA responses similar to infection itself, but it is impractical to obtain intestinal immune responses in large-scale clinical trials [9]. Currently, no correlate of protection exists for oral cholera vaccines, however serum vibriocidal antibody responses that appear following the ingestion of antigens have been used as indicators for development of potential intestinal immunity that endures long after the serum vibriocidal antibody titres have returned to baseline levels [4]. The results of the studies conducted in SonLa and Kolkata indicated that the vaccine was immunogenic and likely protective against cholera. During the Phase III cluster-randomized, double blind, placebo-controlled trial of the reformulated OCV conducted in Kolkata to evaluate the efficacy of the vaccine [10], immunogenicity was assessed in a small subset of individuals at 14 days and 1 year after vaccination.

Methods

Ethics statement

The study protocol was approved by the Drugs Controller General of India, the Ethics Committee of the National Institute of Cholera and Enteric Diseases, the Health Ministry Screening Committee of India and the International Vaccine Institute Institutional Review Board.

Written informed consent was obtained from residents older than 18 years and from the guardians of residents aged 1 to 17 years. Written assent was obtained from residents aged 12 to 17 years. Additional consent and assent forms were obtained from participants included in the immunogenicity subset. An independent data and safety monitoring board reviewed the study protocol, assessed serious adverse events, and approved freezing of data and the analytical plan prior to starting the analysis.

The trial was registered at ClinicalTrials.gov number, NCT00289224.

The vaccine

Each dose of the modified killed whole cell vaccine contained 600 ELISA units (EU) of lipopolysaccharide (LPS) of formalin-killed Vibrio cholerae O1 El Tor Inaba (strain Phil 6573), 300 EU of LPS of heat-killed V. cholerae O1 classical Ogawa (strain Cairo 50), 300 EU of LPS of formalin-killed V. cholerae O1 classical Ogawa (strain Cairo 50), 300 EU of LPS of heat-killed V. cholerae O1 classical Inaba (strain Cairo 50) and 600 EU of LPS of formalin-killed V. cholerae O139 (strain 4260B). Identical vials containing heat-killed Escherichia coli K12 were used as placebo. Vaccine and placebo were stored at temperature between 2 to 8°C until dosing. Vaccine was presented in single-dose vials labeled with one of four letter codes, two for vaccine and two for placebo.

The trial

The Phase III trial was conducted in a cholera-endemic area in Kolkata encompassing a population of about 109,000. Details of the study site and study procedures were previously reported [11,12]. Briefly, residents aged one year and older who were not pregnant were invited to participate. Eligible residents (107,774) were cluster-randomized (3,933 clusters), using dwellings as clusters and pre-assigned to receive two-dose regimens of either the oral cholera vaccine (OCV), or oral placebo so that subjects residing in the same dwelling received identical intervention. Enrollment and administration of the pre-assigned agents were performed after acquisition of written informed consent by dosing teams in vaccination centers serving the population.

Subjects and sampling

For the immunogenicity subset, from the list of 107,774 eligible residents of the study area, by stratified random sampling, a list of residents was generated from which we planned to enrol 300 subjects based on: allocated agent (vaccine or placebo) and age group (less than 5 years, 5 to 15 years and over 15 years of age).

Assuming a 5% background rate of response in the placebo group after the second dose and the true rate of vibriocidal responses in the vaccine group being 25%, at p<0.05 (one tailed), to have 80% power with an 1:1 allocation of subjects in the vaccine and placebo, 46 subjects per arm per age group (1 to 5 years, 5 to 15 years and more than 15 years) were required. Thus approximately a total of 300 subjects were planned to be recruited for the age-stratified analyses, accounting for drop-outs.

Randomization and masking

A statistician who was otherwise not involved in the study prepared the randomization list. Study participants and the investigators were blinded to the study agent (whether vaccine or placebo) applied to each individual. Technicians blinded to the study agent received by the subjects, performed the assays.

Study procedures and definitions

5 ml of blood were obtained from the selected subjects at baseline (prior to administration of the study agent), 14 days after the second dose and one year after the first dose. The microtiter technique was used to detect serum vibriocidal antibodies to V. cholerae O1 El Tor Inaba strain (T19479) and El Tor Ogawa strain (X25049) [13]. For the serum vibriocidal antibodies to V. cholerae O139, a modified microtiter assay was performed at the University of Gothenburg [6,14]. Two-fold serial dilutions of pre- and post vaccination sera were performed in duplicates, and the mean of the two determinations was the final titer. The assay was repeated if a ≥ two-fold difference was noted between the results of the duplicate tests. Initial serum dilutions for testing were 1:2.5 for V. cholerae O1 and 1:10 for V. cholerae O139, respectively. Vibriocidal titers <2.5 for V. cholerae O1 and <10 for V. cholerae O139 were considered as 1:2.5 and 5, respectively, for statistical analyses. Seroconversion was defined as a ≥ four-fold increase in titer of serum vibriocidal antibodies between baseline and post-second dose blood collections. For samples with limited serum volume, the following testing priority was followed: O1 Inaba, O139 and O1 Ogawa.

Data analysis

The percentages of subjects in the vaccine and placebo groups who seroconverted from baseline to 14 days after the second dose and from baseline to one year after the first dose were calculated and compared among the two dose recipients. Serum vibriocidal titers and fold-rises were logarithmically transformed prior to statistical analyses. Comparison of the GMT and GMF rises between vaccine and placebo groups at baseline, 14 days after the second dose and at one year after the first dose were performed.
The Student’s t-test, Welch’s t-test or Wilcoxon rank sum test was used for continuous data (GMT) depending on whether the variance was equal or not and depending on the distribution of data. For categorical data, we used the chi-square or the Fisher’s exact test, if a cell count was sparse.

Comparison of the GMF rise among different age groups was performed using one-way ANOVA. We also derived simulated p-value using simanova implemented in STATA, which was more robust to violation of homogeneity of variance assumption. Bartlett’s test for equal variance was employed to evaluate homogeneity of the data among age groups. If the test yielded homogeneity in the data, then the nominal p-value of the one way ANOVA was accepted, otherwise the simulated p-value. Two tailed tests were conducted for all analyses.

**Results**

Among 300 selected subjects, 167 received the first dose of either vaccine or placebo. 19 of them either refused to provide required blood sample or migrated out of the study area before completion of blood collection, and two persons did not accept the second dose. Only one subject in a cluster was taken, thus nine subjects were excluded (Figure 1). Finally, 137 subjects (69 in the vaccine and 68 in the placebo arm) who received both doses and bled at least twice (at baseline and 14 days after the second dose) were included in the analysis. One year after the first dose, there were 19% drop-outs in the vaccine group, and 12% in the placebo group (Figure 1 and Table 1).

For responses against Inaba, a six-fold GMF rise from baseline was seen 14 days after the second dose, which declined to two-fold after one year. The GMF rises at both the time points were significantly different from baseline ($p < .01$). Approximately 56% and 27% of the vaccinees developed four-fold rise in titers 14 days after the second dose and one year after the first dose respectively. In contrast only 1% of the placebo recipients developed four-fold rise to Inaba at both the time points. The increase of GMT in the vaccine group compared to that in the placebo group was statistically significant at both time points ($p < .001$ and $p = .01$ for 14 days after the second dose and one year after the first dose, respectively). We used 42 pairs of samples for serum vibriocidal tests against the Ogawa serotype and 55 pairs of samples for tests for responses to O139 serogroup amongst vaccinees. The results of the vibriocidal test for Ogawa were similar to that for Inaba (Table 1). However, GMF rises to O139 were much lower than that to Inaba or Ogawa. Titers at one year were lower compared to 14 days after the second dose (Table 1). The seroconversion rate at one year after the first dose declined by $\sim$50% for all strains while the decline in the seroconversion rate against O139 at one year after the first dose was not statistically significant.

The distribution of samples by age at dosing (1 to $< 5$ years, 5 to $< 15$ years, and $15$ years and older) for the vaccine recipients is shown in Table 2. Although the GMF rise to Inaba, Ogawa, and O139 was higher among younger subjects ($< 15$ years old) than that among older subjects at 14 days after the 2nd dose, the rise was not significantly different among the different age groups ($p$-values for Inaba, Ogawa, and O139 were 0.08, 0.30, and 0.33, respectively).

**Discussion**

The results of our study confirmed that the reformulated bivalent oral cholera vaccine was found to remain immunogenic in this current study, which was a part of a large Phase III trial conducted in an endemic setting in the city of Kolkata. The vaccine elicited significant levels of serum vibriocidal antibodies to Inaba, Ogawa and O139, 14 days after the second dose. These
for an anamnestic response persists for many years [18] the most
within approximately one year while the immunological memory
believed to mediate the protection after vaccination, also decline
levels, which differ from the serum vibriocidal antibodies are
are not correlates of protection [4]. Since intestinal IgA antibody
rating earlier evidence that serum vibriocidal antibody responses
efficacy for at least five years post-vaccination [10,11], corrobo-
in vibriocidal antibodies at one year, the vaccine sustained its
limitations pertaining to sample size. However, despite the decline
although it was not possible to do so due to study design and
measured in children stratified by age (1–2 years and 2–5 years)
protection. It would have been good to have vibriocidal responses
correlating serum vibriocidal antibody responses with vaccine
participants in this immunogenicity assessment prevented us from
obtained earlier than the14th day post second dose, a higher
titers on 10th post-immunization day. [17] Thus if sera were
CVD 103HgR, CVD 103-HgR2 and CVD 110 detected higher
cmpared to baseline. Earlier studies on live cholera vaccines:
dered substantially, these remained significantly higher when
year after vaccination. However, in our study, although the titers
decayed to near baseline levels, one
antibody titers is a rapid anamnestic response upon re-exposure
curtiling the infection before it causes illness [19].
Because there were no O139 cases seen in the field site since the
beginning of surveillance, even prior to the start of the Phase III
trial, the clinical significance of the lower vibriocidal titers to O139
remains unknown. The role of serum vibriocidal antibody
responses to O139 remains debatable [7,8].
Due to nonparticipation, the required number of subjects (46 in
each age group in each arm) for the age-stratified analysis was not
attained and is a major limitation of this assessment. Thus we
evaluated the differences in serum vibriocidal responses among
different age groups using one-way ANOVA. The results of the
tests could not identify any significant differences in GMF rises to
Inaba, Ogawa, or O139 across different age groups. However,
since we did not have adequate power to evaluate such differences,
we could not conclude that the age-related differences in serum
vibriocidal responses did not exist.

The absence of cholera cases among the subgroup of
participants in this immunogenicity assessment prevented us from
correlating serum vibriocidal antibody responses with vaccine
protection. It would have been good to have vibriocidal responses
measured in children stratified by age (1–2 years and 2–5 years)
although it was not possible to do so due to study design and
limitations pertaining to sample size. However, despite the decline
in vibriocidal antibodies at one year, the vaccine sustained its
efficacy for at least five years post-vaccination [10,11], corrobo-
ating earlier evidence that serum vibriocidal antibody responses
are not correlates of protection [4]. Since intestinal IgA antibody
levels, which differ from the serum vibriocidal antibodies are
believed to mediate the protection after vaccination, also decline
within approximately one year while the immunological memory
for an anamnestic response persists for many years [18] the most
plausible explanation for long-lasting protection despite waning
antibody titers is a rapid anamnestic response upon re-exposure
curtiling the infection before it causes illness [19].

findings were comparable to an earlier study conducted in Kolkata
[6]. Although serum vibriocidal responses rapidly declined one
year after the first dose for Inaba and Ogawa, these remained
significantly different from baseline titers. In earlier studies
conducted in Bangladesh [15] and Peru [16] with the B-subunit
containing whole cell OCV (Dukoral®, Crucell), the vibriocidal
titers to Inaba and Ogawa declined to near baseline levels, one
year after vaccination. However, in our study, although the titers
decayed substantially, these remained significantly higher when
compared to baseline. Earlier studies on live cholera vaccines:
CVD 103HgR, CVD 103-HgR2 and CVD 110 detected higher
titers on 10th post-immunization day. [17] Thus if sera were

| Vaccine          | Placebo          |          | 14 days after dose 2 | 1 year after dose 1 |          |
|------------------|------------------|----------|----------------------|---------------------|----------|
| Baseline         | Baseline         | Baseline | Baseline             | Baseline            | Baseline |
| (n = 69)         | (n = 69)         | (n = 56) | (n = 68)             | (n = 68)            | (n = 60) |
| Inaba            |                  |          |                      |                     |          |
| GMTa             | 90.2             | 518.3    | 226.3                | 48.5                | 55.4     |
| GMT-riseb        | 5.7              | 1.7      | 1.1                  | 1.2                 |          |
| p valuec         | <0.001           | 0.001    |                      |                     |          |
| No (%) seroconvertedd | 39 (56.5)  | 15 (26.8) | 3 (4.4)             | 5 (8.3)              |          |
| p valuee         | <0.001           | 0.01     |                      |                     |          |
| Ogawa            |                  |          |                      |                     |          |
| GMTa             | 115.0            | 672.5    | 320.0                | 98.8                | 94.4     |
| GMT-riseb        | 5.8              | 2.8      | 1.0                  | 1.1                 |          |
| p valuec         | <0.001           | 0.001    |                      |                     |          |
| No (%) seroconvertedd | 26 (61.9)  | 13 (31.0) | 1 (2.2)             | 3 (6.5)              |          |
| p valuee         | <0.001           | 0.005    |                      |                     |          |
| O139             |                  |          |                      |                     |          |
| GMTa             | 184.7            | 319.1    | 271.1                | 164.0               | 168.3    |
| GMT-riseb        | 1.7              | 1.5      | 1.0                  | 1.1                 |          |
| p valuec         | <0.001           | 0.02     |                      |                     |          |
| No (%) seroconvertedd | 8 (14.6)  | 5 (9.1)   | 1 (1.8)             | 3 (5.5)              |          |
| p valuee         | 0.03             | 0.72     |                      |                     |          |

GMT is Geometric Mean Titer.

GMF rise is Geometric Mean Fold rise from baseline to 14 days after dose 2 or from baseline to 1 year after dose 1.

p value for comparison between vaccine and placebo for GMF rise after controlling for the baseline titre.

No (%) seroconverted from baseline to baseline to 14 days after dose 2 or from baseline to 1 year after dose 1.

GMF rise is Geometric Mean Fold rise from baseline to 14 days after dose 2 or from baseline to 1 year after dose 1.

p value for comparison between vaccine and placebo for % seroconversion after controlling for the baseline titre.

doi:10.1371/journal.pone.0096499.t001

Table 1. Serum vibriocidal antibody titers to *V. cholerae* O1 Inaba among vaccine and placebo recipients.
Table 2. Comparison of geometric mean fold rises across age groups among vaccine recipients in Kolkata, India.

| Age* groups (years) | Median age (years) | Baseline GMT | 14 days after the second dose GMT | 1 year after the first dose GMT | GMF rise | P-value |
|---------------------|--------------------|--------------|----------------------------------|--------------------------------|----------|---------|
| Inaba               | 1                  | 3.5          | 7.4                              | 10.0                            | 39.5     | 0.081   |
| Ogawa               | 1                  | 3.0          | 10.0                             | 16.0                            | 6.0      | 0.391   |
| O139                | 1                  | 3.0          | 10.0                             | 16.0                            | 6.0      | 0.391   |

*Age at the date of 1st dose. GMT refers to Geometric mean titer.

The p-value is either nominal from one way ANOVA or simulated using somanova implemented in STATA depending on the results of the Bartlett’s test for equal variance (described in the text).

References

1. Global Alert and Response (GAR): Cholera. WHO.
2. Thiem VD, Deen JL, Von Seideflein L, Canh DG, Anh DD, et al. (2006) Long-term effectiveness against cholera of oral killed whole-cell vaccine produced in Vietnam. Vaccine 24: 4927–4930.
3. Trach D, Clemens J, Ke N, Thuy H, Son N, et al. (1997) Field trial of a locally produced, killed, oral cholera vaccine in Vietnam. Lancet 349: 231–235.
4. (2004) WHO Expert Committee on Biological Standardization: fifty-second report. Technical report series (World Health Organization), 924.
5. Anh DD, Canh do G, Lopez AL, Thiem VD, Long PT, et al. (2007) Safety and immunogenicity of a reformulated Vietnamese bivalent killed, whole-cell, oral cholera vaccine in adults. Vaccine 25: 1149–1155.
6. Mahalanabis D, Lopez AL, Sur D, Deen J, Manna B, et al. (2008) A randomized, placebo-controlled trial of the bivalent killed, whole-cell, oral cholera vaccine in adults and children in a cholera endemic area in Kolkata, India. PLoS One 3: e2323.
7. Losonsky GA, Lin Y, Motamedi P, Comstock LE, Johnson JA, et al. (1997) Vibriocidal antibody responses in North American volunteers exposed to wild-type or vaccine Vibrio cholerae O139: specificity and relevance to immunity. Clin Diag Lab Immunol 4: 264–269.
8. Saha D, LaRocque RC, Khan AI, Harris JR, Begum YA, et al. (2004) Incomplete correlation of serum vibriocidal antibody titer with protection from Vibrio cholerae infection in urban Bangladesh. J Infect Dis 189: 2318–2322.
9. Plotkin SA (2010) Correlates of protection induced by vaccination. Clin Vaccine Immunol 17: 1055–1065.
10. Bhattacharya S, Sur D, Ali M, Kanungo S, You Y, et al. Five-year efficacy of a bivalent killed whole-cell oral cholera vaccine against cholera among residents of Kolkata, India. N Engl J Med (submitted).
11. Sur D, Kanungo S, Sah B, Manna B, Ali M, et al. (2011) Efficacy of a low-cost, inactivated whole-cell oral cholera vaccine: results from 3 years of follow-up of a randomized, controlled trial. PLoS Negl Trop Dis 5: e1289.
12. Sur D, Lopez AL, Kanungo S, Paisley A, Manna B, et al. (2009) Efficacy and safety of a modified killed-whole-cell oral cholera vaccine in India: an interim analysis of a cluster-randomised, double-blind, placebo-controlled trial. The Lancet 374: 1694–1702.
13. Jethborn M, Svennerholm A-M, Holmgren J (1994) Immunological memory after immunization with oral cholera B subunit-whole-cell vaccine in Swedish volunteers. Vaccine 12: 1078–1082.
14. Attridge SR, Johansson C, Trach DD, Qadri F, Svennerholm A-M (2002) Sensitive microplate assay for detection of bactericidal antibodies to Vibrio cholerae O139. Clin Diag Lab Immunol 9: 383–387.
15. Sack D, Clemens J, Huda S, Harris J, Khan M, et al. (1991) Antibody responses after immunization with killed oral cholera vaccines during the 1985 vaccine field trial in Bangladesh. J Infect Dis 164: 407–411.
16. Begue RE, Castellares G, Cabezas C, Sanchez J, Meza R, et al. (1995) Immunogenicity in Peruvian volunteers of a booster dose of oral cholera vaccine consisting of whole cells plus recombinant B subunit. Infect Immun 63: 3726–3728.
17. Wasserman SS, Losonsky GA, Noriega F, Tacket CO, Cantaneda E, et al. (1994) Kinetics of the vibriocidal antibody response to live oral cholera vaccines. Vaccine 12: 1000–1003.
18. Jerborn M, Svennerholm AM, Holmgren J (1988) Five-year immunologic memory in Swedish volunteers after oral cholera vaccination. J Infect Dis 157: 374–377.
19. Lycke N, Hellstrom U, Holmgren J (1987) Circulating cholera antitoxin memory cells in the blood one year after oral cholera vaccination in humans. Scand J Immunol 26: 207–211.

20. Saha A, Chowdhury MI, Khanam F, Bhanayan MS, Chowdhury F, et al. (2011) Safety and immunogenicity study of a killed bivalent (O1 and O139) whole-cell oral cholera vaccine Shanchol, in Bangladeshi adults and children as young as 1 year of age. Vaccine 29: 8285–8292.
21. (2010) Cholera vaccines: WHO position paper. Wkly Epidemiol Rec 85: 117–128.