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Safety and immunogenicity of a reformulated Vietnamese bivalent killed, whole-cell, oral cholera vaccine in adults☆,☆☆

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Abstract

Vietnam currently produces an orally administered, bivalent (O1 and O139) killed whole-cell vaccine and is the only country in the world with endemic cholera to use an oral cholera vaccine in public health practice. In order to allow international use, the vaccine had to be reformulated to meet World Health Organization (WHO) requirements. We performed a randomized, placebo controlled, safety and immunogenicity studies of this reformulated vaccine among Vietnamese adults. One hundred and forty-four subjects received the two-dose regimen and 143 had two blood samples obtained for analysis. We found that this reformulated oral killed whole-cell cholera vaccine was safe, well tolerated and highly immunogenic.

Keywords: Cholera vaccine; Safety; Immunogenicity

1. Introduction

Since the mid-1980s, when Vietnamese scientists developed a killed oral vaccine containing *Vibrio cholerae* following technology transfer from Sweden, the vaccine has been used extensively in Vietnam and it is the only country in the world with endemic cholera to use an oral cholera vaccine in public health practice. This vaccine has been found to be safe and protective. In a field trial in Hue, the vaccine conferred 66% protection against *V. cholerae* O1 El Tor Ogawa in an outbreak that occurred 8–10 months after vaccination [1]. Following the emergence of *V. cholerae* O139 in 1992, the Vietnamese government decided to modify the vaccine to include killed *V. cholerae* O139 cells. The subsequent bivalent vaccine produced have been found to be safe and elicited five-fold rises in serum anti-O1 vibriocidal antibodies when administered to Vietnamese adults [2]. It conferred significant protection against El Tor cholera in both children and adults with 50% over-all effectiveness against clinically significant El Tor cholera, 3–5 years following immunization [3].

More than 9 million doses have been administered since the licensure of the bivalent oral cholera vaccine in Vietnam in 1997, where it is used as part of the Expanded Programmed

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of Immunization (EPI) in high risk areas of the Mekong Delta, central coastal areas and some provinces in Northern Vietnam. Although no available data exist regarding the public health impact of the cholera vaccine in Vietnam, no cholera case has been reported to World Health Organization (WHO) since 1999. The vaccine is safe and there has been no report of serious adverse reaction [4]. It is easily administered since it does not require ingestion of buffer. Because of these features, and the relative inexpensive and easily transferable production technology for the vaccine, the Diseases of the Most Impoverished (DOMI) Program, funded by the Bill and Melinda Gates Foundation, targeted this vaccine for transfer to additional emerging vaccine producers in Asia and for potential use for the global control of endemic cholera. However, DOMI's evaluation of this vaccine found that cholera toxin could not be reliably removed by additional washing using diafiltration, or increased centrifugation/re-suspension steps, which still resulted in small amounts of toxin detected.

It was established that the most reliable way to guarantee undetectable toxin levels in the final vaccine was to replace the toxin hyper-producing classical Inaba strain 569B with another classical Inaba strain Cairo 48 which was low toxin producing. In addition to the replacement of 569B, a formalin-killed *V. cholerae* O1 classical Ogawa (Cairo 50) component was added to increase the proportion of Ogawa serotype in the vaccine.

Although the current vaccine is safe and is used widely in Vietnam's public health programs [1–4], WHO guidelines for the production and control of killed oral cholera vaccines specify that residual levels of clinical active cholera toxin in the final formulated vaccine should be insignificant [5]. To fulfill World Health Organization requirements, and thus to facilitate international use of this vaccine in programs for the poor in cholera-endemic countries, DOMI and the Company for Vaccine and Biological Production No. 1 (VABIOTECH) in Hanoi, Vietnam's vaccine producer, reformulated the vaccine so that it will meet WHO guidelines. This study reports on the safety and immunogenicity of the reformulated vaccine in Vietnamese adults.

2. Methods

We conducted a double-blind placebo-controlled randomized trial among adults residing in SonLa Province, Northwest Vietnam from May to June 2005. The trial protocol was approved by the Institutional Review Boards of the National Institute of Hygiene and Epidemiology in Hanoi, Vietnam and the International Vaccine Institute in Seoul.

2.1. Vaccine and placebo

The previous formulation of the Vietnamese vaccine contained: 5.0×10^{10} formalin-killed *V. cholerae* Inaba, El Tor (strain Phil 6973); 2.5×10^{10} heat-killed *V. cholerae* Ogawa, classical (strain Cairo 50); 2.5×10^{10} formalin-killed *V. cholerae* Inaba, classical (strain 569B); and 5.0×10^{10} formalin-killed *V. cholerae* O139 (strain 4260B). Because the vaccine containing the 569B strain, which is a hyperproducer of cholera toxin, would not be produced effectively in a manner that reliably removed cholera toxin, DOMI and VABIOTECH reformulated the vaccine by removing the 569B strain and replacing it with two other strains, a formalin-killed *V. cholerae* O1 classical Ogawa (Cairo 50) and a heat-killed *V. cholerae* O1 classical Inaba (Cairo 48) (see Table 1). To comply with WHO requirements, the vaccine's antigen content was standardized using an ELISA assay specific for *V. cholerae* O1 and *V. cholerae* O139 LPS antigens (the earlier version of the vaccine had used optical density to standardize antigen content). The use of the ELISA assay for quantification of the LPS plus the additional O1 serogroups component effectively resulted in an increase in the amount of LPS antigen present in the vaccine. The reformulated vaccine contains no detectable cholera toxin.

Each dose of the reformulated Vietnamese vaccine contained 600 ELISA Units (EU) LPS of the formalin-killed *V. cholerae* Inaba, El Tor biotype cells (strain Phil 6973); 300 EU LPS of the heat-killed *V. cholerae* Ogawa classical biotype cells (Cairo 50); 300 EU LPS of the formalin-killed *V. cholerae* Ogawa classical biotype cells (Cairo 50); 300 EU LPS of the heat-killed *V. cholerae* Inaba, classical biotype cells (Cairo 48); and 600 EU LPS of the formalin-killed *V. cholerae* O139 (strain 4260B). The vaccine was also tested for toxin content and found to contain no detectable toxin (limit of detection 1 ng/ml). The LPS and toxin assays were performed at the University of Gothenburg. All other lot release assays were performed at VABIOTECH.

The locally produced placebo consisted of a heat-killed *Escherichia coli* K12 strain and had identical appearance as the reformulated vaccine. The reformulated vaccine and the placebo were packaged as liquid formulations in identical vials containing five 1.5-ml doses. Each study agent was

Table 1
Composition of the previous and reformulated bivalent Vietnamese killed oral cholera vaccines

Vaccine strain	Previous version	Reformulated version
V. cholerae O1 Inaba El Tor strain Phil 6973 formalin killed	5×10^{10} cells	600 Elisa units (EU) LPS
V. cholerae O1 Ogawa classical strain Cairo 50 heat killed	2.5×10^{10} cells	300 EU LPS
V. cholerae O1 Inaba classical strain 569B formalin killed	2.5×10^{10} cells	–
V. cholerae O1 Ogawa classical strain Cairo 50 formalin killed	–	300 EU LPS
V. cholerae O1 Inaba classical strain Cairo 48 heat killed	–	300 EU LPS
V. cholerae O139 strain 4260B formalin killed	5×10^{10} cells	600 EU LPS

stored at 4–8 °C before administration, and was given in two doses separated by an interval of 2 weeks.

2.2. Study participants

Healthy male and non-pregnant female residents of SonLa Province aged 18–40 years were recruited for the study. Written informed consent was obtained prior to enrolment. Subjects with history of diarrhea, anti-diarrheal and antibiotic use during the past week, or a history of diarrhea and abdominal pain lasting for 2 weeks during the 6 months prior to the start of the study were excluded from the study. We initially randomized 153 volunteers to receive either two doses of the reformulated vaccine or placebo. A randomization list was prepared by a statistician who otherwise was not involvement in the study. Randomization numbers were generated in blocks of four, which included two of each type of study agent. Doses were given 14 days apart and subject participation lasted for 28 days (see Fig. 1).

2.3. Surveillance for adverse events

Participants were asked to return for follow-up for 3 consecutive days after each dose. A physician who was unaware of the study agent received by the subject conducted a structured interview regarding the subjects' over-all level of activity and bowel movements as well as occurrence of symptoms such as diarrhea, abdominal pain, loss of appetite, nausea, general ill feeling, fever, headache or vomiting. Diarrhea was defined as three or more loose or liquid stools in a 24 h period. Two weeks after each dose was given, subjects were asked for any illness that may have occurred during the interval period.

2.4. Serological surveillance

Venipuncture was performed to obtain blood samples prior to administration of the study agents and 14 days after the second dose. Sera were separated, shipped frozen to the laboratory at the International Vaccine Institute (IVI) and stored at −70 °C until paired testing was performed. Serum vibriocidal antibodies to *V. cholerae* O1 El Tor Inaba strain (T19479) [6] were performed in IVI using the microtiter technique as previously described. The initial dilution of sera was 1:2.5. Sera were shipped frozen to University of Gothenburg where serum vibriocidal antibodies to *V. cholerae* O139 strain (A361) were evaluated. At Gothenburg, the initial dilution of sera used for the *V. cholerae* O139 assay was 1:20 as previously described [8]. Two-fold serial dilutions of pre- and post-vaccination sera were performed in duplicates, and the titre ascribed was the mean of the two determinations. If more than a two-fold difference was noted between the duplicate results, the assay was repeated. The titres were adjusted relative to a reference serum specimen included in each test to compensate for variations between analyses on different occasions. Testing was performed by technicians blinded to the study agent received by the subjects. Vibriocidal titres <2.5 for *V. cholerae* O1 or <20 for *V. cholerae* O139 were considered as 1.25 and 10, respectively, for statistical analyses. A four-fold or greater increase in titre between pre- and post-vaccination sera was used to indicate seroconversion.

2.5. Sample size

The sample size was calculated to evaluate diarrhoeal adverse events after either dose and seroconversion to *V. cholerae* O1 Inaba (defined as ≥4-fold increase in vibriocidal antibody titre between baseline and bleed 2). It was assumed that the background rate of diarrhoea after either dose was the same in both placebo and vaccine recipients at 10%. Using the method of Dunnett and Gent for precision-based sample size calculation [7] in order to exclude an upper boundary of a one-tailed 95% CI for the vaccine–placebo difference in the rate of diarrhoea of greater than 20% with a power of 0.95, the minimum number of subjects required for each group was 49. Similarly, for serum vibriocidal responses, it was assumed that the background rate of seroconversion among placebo recipients was 5% after the second dose and that the true vibriocidal response in the vaccine group was 60%; in order to exclude a lower-boundary of a one-tailed 95% CI of less than 33% for the vaccine–placebo difference with power of 0.95, a minimum of 64 subjects were required for each group. To adjust for the number of persons expected to drop out of the study after the first dose, we estimated that at least 70 persons per group would need to be enrolled in the study.

2.6. Data management and analysis

Data was entered in Visual Fox Pro™ V 7.0 (Microsoft Corp., USA) and analyses were performed using Stata™ V 8.0 (Stata Corp., USA). For safety data, an intention-to-vaccinate analysis was performed wherein all subjects who were randomized in the study and received one dose or more of any study agent were included. For immunogenicity data, a per-protocol analysis was performed wherein only those eligible and randomized subjects who received two doses of the correct study agent and were available until the last follow-up were included. The Chi-square test was used to compare two groups of dichotomous outcomes except for sparse date for which Fischer's exact test was used. Student's *t*-test was performed for continuous outcomes. Geometric mean titres and fold rises were determined and serum vibriocidal titres and fold rises were logarithmically transformed prior to statistical analyses. Analysis of covariance was used to adjust for imbalances in baseline titres for comparison of fold rise in titres.

The two primary objectives of the study were to assess whether the vaccine induced serum vibriocidal antibody responses to vaccine that exceeded those to placebo by a specified threshold, and to evaluate whether the diarrhoea risk after any dose among vaccinees exceeded that after placebo by no more than a specified threshold. Accord-

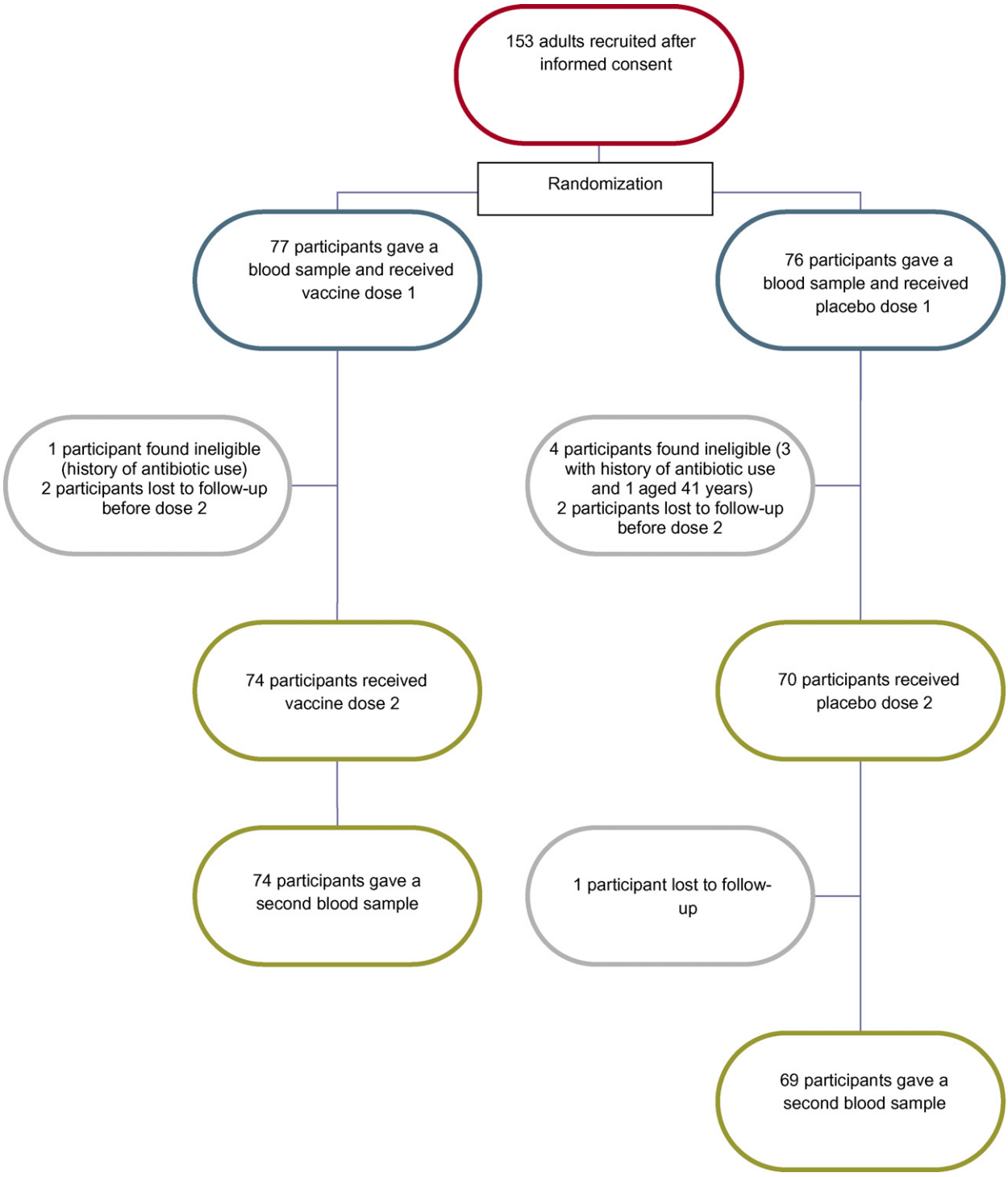

Fig. 1. Flowchart of participants in the study.

ingly, differences between vaccine and placebo groups in regard to the occurrence of diarrhoea and seroconversions were evaluated with one-tailed 95% confidence intervals. All other contrasts were evaluated using the $p < 0.05$ (two-tailed). The 95% confidence intervals for these differences when the numbers were small were calculated using an exact method.

3. Results

One hundred and fifty-three subjects were initially enrolled in the study and received one dose of either vaccine or placebo. However, upon review after the first dose, five randomized subjects were later found to be ineligible, four of whom received antibiotics within 1 week prior to vaccina-

Table 2
Demographic characteristics of vaccine and placebo recipients

Characteristics	Vaccine recipients, $N=77$	Placebo recipients, $N=76$	p-Value
Age (in years)[a]			
Mean (S.D.)	23 (4)	23 (5)	0.70
Median	21	21	
Sex[a]			
Male	51 (67%)	43 (57%)	0.22

[a] No statistically significant differences were found between vaccine and placebo recipients.

tion and one was 41 years of age. Of the 148 eligible subjects, 144 (74 vaccinees and 70 placebo recipients), received the full two-dose regimen and 143 two-dose recipients were followed up with a second blood collection (Fig. 1). In the intention-to-vaccinate analysis, there were no significant differences in the age and sex distribution of the two groups (Table 2). No adverse event occurred more frequently in the vaccine group (Table 3). The majority of the reported events were mild. Two subjects developed diarrhoea, both after the first dose, one subject received vaccine and the other received placebo. Neither required treatment. The 95% one-sided confidence interval for this comparison excluded a more than 9% greater occurrence of diarrhoea among vaccinees than placebo recipients. Headache was the most commonly reported adverse event among both placebo recipients and vaccinees. There were no serious adverse events reported during the study.

The baseline vibriocidal antibody titre to *V. cholerae* O1 of the subjects ranged from <2.5 to 2560. The geometric mean vibriocidal antibody titres to *V. cholerae* O1 for vaccinees were 26.8-fold higher than for the placebo recipients 14 days after intake of the second dose ($p<0.001$). Sixty-seven (90.5%) of the vaccine recipients had a ≥4-fold rise in the vibriocidal antibody titres, with 35 of them having a 32-fold increase from baseline. In contrast, none of the placebo recipients seroconverted ($p<0.001$; Table 3). Six of 11 vaccinees with baseline titres ≥320 seroconverted (Table 5). The 95% confidence interval of differences between seroconversion among vaccine and placebo recipients excluded an 81% lower response rate among vaccinees.

Table 3
Comparison of solicited adverse events among participants following receipt of dose 1 and dose 2 of vaccine or placebo

	Vaccine, N (%)	Placebo, N (%)	p-Value
Within 3 days after dose 1	$N=77$	$N=76$	
Diarrhoea	1 (1)	1 (1)	1.0
Abdominal pain	7 (9)	5 (7)	0.56
Loss of appetite	1(1)	2 (3)	0.55
Nausea	7 (9)	7 (9)	0.98
Vomiting	1 (1)	1 (1)	0.99
Fever	3 (4)	1(1)	0.32
Headache	11 (14)	14 (18)	0.49
General ill feeling	4 (5)	3 (4)	0.71
Within 3 days after dose 2	$N=74$	$N=70$	
Diarrhoea	0	0	–
Abdominal pain	5 (6)	3 (4)	0.72
Loss of appetite	0	0	-
Nausea	2 (2)	0	0.50
Vomiting	1 (1)	0	1.0
Fever	1 (1)	2 (2)	0.61
Headache	7 (9)	4 (6)	0.40
General ill feeling	1 (1)	0	1.0
No (%) with ≥ one adverse event within 3 days of dose 1	25 (32)	25 (33)	0.95
No (%) with ≥ one adverse event within 3 days of dose 2	14 (20)	9 (13)	0.32
No (%) with ≥ one adverse event within 28 days	29 (38)	29 (38)	0.95
No (%) with a serious adverse events	0	0	

One hundred and forty-one paired sera were available for testing of vibriocidal antibodies to *V. cholerae* O139. Baseline vibriocidal antibodies to *V. cholerae* O139 ranged from <5 to 1280. None of the placebo recipients had a four-fold or greater increase from baseline whereas 8 (11%) vaccine recipients did ($p=0.006$). A two-fold increase from baseline was noted among 9 (13%) placebo recipients and 15 (20%) vaccine recipients. The geometric mean fold increase from baseline of vibriocidal antibodies to O139 among vaccine recipients was statistically significantly higher than the placebo recipients 14 days after intake of the second dose ($p=0.001$) (Table 4). This difference remained

Table 4
Serum vibriocidal antibody titres to *V. cholerae* O1 and *V. cholerae* O139 serogroups at baseline and 14 days after the second dose among vaccine and placebo recipients

	V. cholerae O1			*V. cholerae* O139		
	Vaccine recipients, $N=74$	Placebo recipients, $N=69$	p-Value	Vaccine recipients, $N=72$	Placebo recipients, $N=69$	p-Value
GMT[a]						
Bleed 1	18.6	17.2	0.8	36.3	48.4	0.3
Bleed 2	497.0	17.2	<0.001	52.4	46.0	0.6
GMF-rise[b]	26.8	1	<0.001	1.4	1	0.001
No. of subjects who seroconverted[c]	67 (91%)	0	<0.001	8 (11%)	0	0.006

[a] Geometric mean reciprocal titre for the cited bleed.
[b] Geometric mean fold rise between first and second bleed.
[c] Number of subjects with ≥4-fold rise in titres between first and second bleed.

Table 5
Frequency table of fold rise of serum vibriocidal antibodies to *V. cholerae* O1 and O139 from baseline and 14 days post-immunization according to pre-immunization titres among vaccine recipients

Baseline titres	Fold increase[a]																	
	V. cholerae O1										*V. cholerae* O139							
	≤1	2	4	8	16	32	64	128	256	512	≤1	2	4	8	16	32	64	≥128
≤5				2	1	4	6	5	3	5	19	1		3	2	2		
10							2	3										
20			3	3	3		1					1						
40			1			6					4	7						
80			3	1	6						9	2	1					
160	1	1	1	2							9	1						
320	1		3	3							3	2						
≥640		4									4	2						

[a] From baseline to 14 days after receipt of second dose among vaccine recipients.

statistically significant after controlling for baseline titres ($p < 0.05$).

4. Discussion

The two-dose regimen of this reformulated oral killed whole-cell cholera vaccine was safe, well tolerated and immunogenic in this trial, though the immune responses to the O1 serogroup were substantially greater than the responses to the O139 serogroup. No serious adverse events occurred during the study period. No adverse events were more frequently detected in the vaccine group than in the placebo group.

Ninety percent of the vaccine recipients developed ≥4-fold rises in vibriocidal antibodies to *V. cholerae* O1, whereas none of the placebo recipients did. Among vaccinees there was a 27-fold rise in the geometric mean titres. All vaccine recipients in our study with baseline titre of ≤80 seroconverted, in contrast only 9 (12%) vaccine recipients with baseline titres of 160 or higher seroconverted and none of the participants with baseline titres of 640 or greater did (Table 5). In previous studies in Vietnam with the older Vietnamese vaccine formulation and the internationally licensed rBS-WC vaccine using the same vaccination schedule, 60% of adult vaccine recipients seroconverted and the GMF rise in titres was only five-fold with either vaccine [2]. Attenuated immune responses in individuals with pre-existing high vibriocidal antibody titre had been reported in studies with other cholera vaccines [9–11]. However, the baseline GMT in our study was even higher than the baseline GMT in the previous study in Vietnam.

The higher vibriocidal antibody responses to *V. cholerae* O1 observed for the reformulated Vietnamese vaccine than for the previous version may have been due to differences in the quantities of LPS antigen in the two vaccines. The ELISA-based standardization methods used for the reformulated vaccine resulted in significantly higher levels of LPS antigen in this vaccine than in the previous vaccine, which was standardized with use of optical density measurements. Moreover, the addition of two other *V. cholerae* O1 strains further increased the antigenic content of the reformulated vaccine. These findings suggest that the reformulated vaccine is more immunogenic than the earlier formulations [2,8,10,11].

The baseline vibriocidal titres to O139 among our subjects were higher than the baseline titres to *V. cholerae* O1. Our study was performed in a non-cholera-endemic area in Vietnam and these pre-existing antibodies may reflect prior exposure to bacteria with cross-reacting O antigens to O139 [16,17]. Unlike *V. cholerae* O1, vibriocidal responses to *V. cholerae* O139 were modest. Only 11% of vaccine recipients seroconverted to *V. cholerae* O139 and a GMF-rise of 1.4 was achieved. All eight responders to *V. cholerae* O139 were vaccinees, seven of whom had baseline vibriocidal titres of <10. The lower vibriocidal response to *V. cholerae* O139 than to *V. cholerae* O1 has been reported in previous studies and is believed to be due to the presence of a capsular polysaccharide in *V. cholerae* O139 that may interfere with the immune response and detection of vibriocidal antibodies [12–14,16].

The vibriocidal responses to O139 were somewhat lower in our study than in a study that tested the previous formulation of the bivalent Vietnamese vaccine [2]. This difference could be due to methodological differences between the two studies. The earlier study used two different test strains for the assay and selected only the higher vibriocidal responses to the two strains for analysis [2]. Among adults, seroconversion ranged from 17 to 31% and GMF rise from baseline titres ranged from 1.4 to 2.4, depending on the test organism used [2]. In our study, only 11% of vaccine recipients seroconverted and a GMF-rise of anti-O139 serum vibriocidal antibodies of 1.4 was achieved. In contrast, the current study used only one test organism (*V. cholerae* O139 A361) for the O139 vibriocidal assay, which differed from the two test organisms used in the earlier study. Whether the lower vibriocidal titres to *V. cholerae* O139 in our study indicate a poorer immune response or differences in the sensitivity

of the vibriocidal assay used for O139 remains to be seen. Lastly, although serum vibriocidal antibodies to O1 have been regarded as immunological correlates of protection against cholera, their use for *V. cholerae* O139 remains contentious [12,14]. Earlier studies with *V. cholerae* O139 revealed protection against homologous rechallenge without concomitant detection of a vibriocidal antibody response [15]. Clarification of whether this bivalent vaccine protects against *V. cholerae* O139 therefore will likely require studies of clinical vaccine efficacy.

A phase III trial is currently planned for this vaccine in an endemic area in West Bengal, India. If found safe and effective, the reformulated vaccine will provide an inexpensive alternative for use in the control of cholera in outbreak settings, as well as endemic areas.

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Contributors: D.D. Anh, V.D. Thiem, A.L. Lopez, J.L. Deen, L. von Seidlein and J.D. Clemens contributed to the design, implementation and supervision of the study and the writing of the paper. V.D. Thiem and S.H. Shin created and implemented the data management system and supervised its use. V.D. Thiem, A.L. Lopez and S.H. Shin analyzed the data and take responsibility for the accuracy of the data analysis. D.G. Canh, P.T. Long and N.H. Son, contributed to the implementation and supervision of the study. S.H. Han was responsible for the *V. cholerae* O1 vibriocidal assay and S. Attridge was responsible for the *V. cholerae* O139 assay. R. Carbis and J. Holmgren assured quality control of the reformulated vaccine. All authors had had full access to all of the data in the study. *Conflict of interest statement*: None declared. *Funding*: This work was supported by the Bill and Melinda Gates Foundation through the Diseases of Most Impoverished Program (grant C-8) administered by the International Vaccine Institute; and the Swedish International Development Cooperation Agency.

References

[1] Trach DD, Clemens JD, Ke NT, Thuy HT, Son ND, Canh DG, et al. Field trial of a locally produced, killed, oral cholera vaccine in Viet Nam. Lancet 1997;349:231–5.

[2] Trach DD, Cam PD, Ke NT, Rao MR, Dinh D, Hang PV, et al. Investigations into the safety and immunogenicity of a killed oral cholera vaccine developed in Viet Nam. Bull World Health Organ 2002;8: 2–8.

[3] Thiem VD, Deen JL, von Seidlein L, Canh DG, Anh DD, Park JK, et al. Long-term effectiveness against cholera of oral-killed whole cell vaccine produced in Vietnam. Vaccine 2006;24:4297–303.

[4] Thiem VD, Hossain MM, Son ND, Hoa NT, Rao MR, Canh DG, et al. Coverage and costs of mass immunization of an oral cholera vaccine in Vietnam. J Health Popul Nutr 2003;4:304–8.

[5] WHO Expert Committee on Biological Standardization: Fifty-second report. Geneva, Switzerland, 2001.

[6] Jertborn M, Svennerholm A-M, Holmgren J. Saliva, breast milk, and serum antibody responses as indirect measures of intestinal immunity after oral vaccination or natural disease. J Clin Microbiol 1986;24:203–9.

[7] Dunnett CW, Gent M. Significance testing to establish equivalence between treatments with special reference to data in the form of 2×2 tables. Biometrics 1997;33:593–602.

[8] Jertborn M, Svennerholm A-M, Holmgren J. Intestinal and systemic immune responses in humans after immunization with a bivalent B-subunit-O1/O-139 whole cell cholera vaccine. Vaccine 1996;14:1459–65.

[9] Gotuzzo E, Butron B, Seas C, Penny M, Ruiz R, Losonsky G, et al. Safety immunogenicity and excretion pattern of single-dose live oral cholera vaccine CVD 103-HgR in Peruvian adults of high and low socioeconomic levels. Infect Immun 1993;61:3994–7.

[10] Taylor DN, Cardenas V, Perez J, Puga R, Svennerholm AM. Safety, immunogenicity, and lot stability of the whole cell/recombinant B subunit (WC/rCTB) cholera vaccine in Peruvian adults and children. Am J Trop Med Hyg 1999;61:869–73.

[11] 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;155:79–85.

[12] Losonsky GA, Lim Y, Motamedi P, Comstock LE, Johnson JA, Morris Jr JG, et al. Vibriocidal antibody responses in North American volunteers exposed to wild-type or vaccine *Vibrio cholerae* O139: specificity and relevance to immunity. Clin Diagn Lab Immunol 1997;4: 264–9.

[13] Qadri F, Mohi G, Hossain J, Azim T, Khan AM, Salam MA, et al. Comparison of the vibriocidal antibody response in cholera due to *Vibrio cholerae* O139 Bengal with the response in cholera due to *Vibrio cholerae* O1. Clin Diagn Lab Immunol 1995;2:685–8.

[14] Saha D, LaRocque RC, Khan AI, Harris JB, Begum YA, Akramuzzaman SM, et al. Incomplete correlation of serum vibriocidal antibody titer with protection from *Vibrio cholerae* infection in urban Bangladesh. J Infect Dis 2004;189:2318–22.

[15] Morris Jr JG, Losonsky GE, Johnson JA, Tacket CO, Nataro JP, Panigrahi P, et al. Clinical and immunologic characteristics of *Vibrio cholerae* O139 Bengal infection in North American volunteers. J Infect Dis 1995;171:903–8.

[16] Attridge SR, Qadri F, Albert MJ, Manning PA. Susceptibility of *Vibrio cholerae* O139 to antibody-dependent, complement-mediated bacteriolysis. Clin Diagn Lab Immunol 2000;7:444–50.

[17] Attridge SR, Johansson C, Trach DD, Qadri F, Svennerholm AM. Sensitive microplate assay for detection of bactericidal antibodies to *Vibrio cholerae* O139. Clin Diagn Lab Immunol 2002;9:383–7.