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Author

Chowdhury, Fahima, Akter, Afroza, Bhuiyan, Taufiqur Rahman, Tauheed, Imam, Teshome, Samuel, Sil, Arijit, Park, Ju Yeon, Chon, Yun, Ferdous, Jannatul, Basher, Salima Raiyan, Ahmed, Faez, Karim, Mahbubul, Ahasan, Mohammad Mainul, Mia, Masudur Rahman, Masud, Mir Mohammad Ibna, et al.

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A non-inferiority trial comparing two killed, whole cell, oral cholera vaccines (Cholvax vs. Shanchol) in Dhaka, Bangladesh

Fahima Chowdhury [a,b], Afroza Akter [a], Taufiqur Rahman Bhuiyan [a], Imam Tauheed [a], Samuel Teshome [c], Arijit Sil [c], Ju Yeon Park [c], Yun Chon [c], Jannatul Ferdous [a], Salima Raiyan Basher [a], Faez Ahmed [d], Mahbubul Karim [d], Mohammad Mainul Ahasan [d], Masudur Rahman Mia [d], Mir Mohammad Ibna Masud [d], Abdul Wahab Khan [d], Masum Billah [d], Zebun Nahar [d], Imran Khan [d], Allen G. Ross [a,b], Deok Ryun Kim [c], Md. Muktadir Rahman Ashik [d], Laura Digilio [c], Julia Lynch [c], Jean-Louis Excler [c], John D. Clemens [a,e,f], Firdausi Qadri [a,*]

[a] International Centre for Diarrhoeal Disease Research Bangladesh (icddr,b), Dhaka, Bangladesh
[b] Menzies Health Institute Queensland, Gold Coast, Australia
[c] International Vaccine Institute (IVI), Seoul, Republic of Korea
[d] Incepta Vaccine Limited, Dhaka, Bangladesh
[e] UCLA Fielding School of Public Health, Los Angeles, CA, USA
[f] Korea University School of Medicine, Seoul, South Korea

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ABSTRACT

Bangladesh remains cholera endemic with biannual seasonal peaks causing epidemics. At least 300,000 severe cases and over 4,500 deaths occur each year. The available oral cholera vaccines have not yet been adopted for cholera control in Bangladesh due to insufficient number of doses available for endemic control. With a public private partnership, icddr,b initiated a collaboration between vaccine manufacturers in Bangladesh and abroad. A locally manufactured Oral Cholera Vaccine (OCV) named Cholvax became available for testing in Bangladesh. We evaluated the safety and immunogenicity of this locally produced Cholvax (Incepta Vaccine Ltd) inexpensive OCV comparatively to Shanchol (Shantha Biotechnics-Sanofi Pasteur) which is licensed in several countries. We conducted a randomized non-inferiority clinical trial of bivalent, killed oral whole-cell cholera vaccine Cholvax vs. Shanchol in the cholera-endemic area of Mirpur, Dhaka, among three different age cohorts (1–5, 6–17 and 18–45 years) between April 2016 and April 2017. Two vaccine doses were given at 14 days apart to 2,052 healthy participants. No vaccine-related serious adverse events were reported. There were no significant differences in the frequency of solicited (7.31% vs. 6.73%) and unsolicited (1.46% vs. 1.07%) adverse events reported between the Cholvax and Shanchol groups. Vibriocidal antibody responses among the overall population for O1 Ogawa (81% vs. 77%) and O1 Inaba (83% vs. 84%) serotypes showed that Cholvax was non-inferior to Shanchol, with the non-inferiority margin of −10%. For O1 Inaba, GMT was 462.60 (Test group), 450.84 (Comparator group) with GMR 1.02(95% CI: 0.92, 1.13). For O1 Ogawa, GMT was 419.64 (Test group), 387.22 (Comparator group) with GMR 1.12 (95% CI: 1.02, 1.23). Cholvax was safe and non-inferior to Shanchol in terms of immunogenicity in the different age groups. These results support public use of Cholvax to contribute for reduction of the cholera burden in Bangladesh. ClinicalTrials.gov number: NCT027425581.

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1. Introduction

Diarrheal diseases continue to be a major cause of morbidity and mortality in developing countries, *Vibrio cholerae* O1 being one of the major responsible bacterial pathogens. In Bangladesh an estimated 300,000 cholera cases and over 4,500 deaths occur annually [1]. Bangladesh remains endemic for cholera, which peaks

* Corresponding author at: International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), 68, Shaheed Tajuddin Ahmed Sarani, Mohakhali, Dhaka 1212, Bangladesh.
E-mail address: fqadri@icddrb.org (F. Qadri).

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biannually with further increases seen during floods and cyclones [2,3]. It affects all age groups, although the majority of fatal cases occur in children [4–6]. Therefore, vaccination against cholera remains an important public health tool for preventing and controlling the disease according to WHO [7]. Based on the Global Task Force on Cholera Control (GTFCC), and WHO guideline, the Government of Bangladesh (GoB) developed and approved a National Cholera Control Plan (NCCP) includes Cholvax as a part of vaccination plan [8]. Oral rehydration therapy has beee a key component of therapy for dehydrating diarrhoea, but such therapy does not reduce the incidence of disease or eliminate pathogens. And antimicrobials are rapidly diminishing in effectiveness due to spreading resistance among enteric pathogens. The World Health Organization (WHO) recommends oral cholera vaccines (OCV) for the control of both endemic and epidemic cholera [9]. For cholera control OCV has been imported from India and The Republic of Korea for quite a long time in Bangladesg and different countries around the world. Imported cholera vaccines are still not an economically feasible option for countries like Bangladesh to incorporate in the health care system. In Bangladesh, only Dukoral is licenced and available for commercial purchase. It is expensive and not affordable for the common people and not even feasible to be used in national programs. Even the WHO OCV stockpile at present does not include Dukoral, but the WHO stockpile does include the other WHO prequalified OCVs, Shanchol and Euvichol [10]. These OCVs are short in supply. These vaccines are not available in Bangladesh in the private sector because the country of production is not within the approval of the Directorate General of Drug Administration (DGDA). Moreover, the Government of Bangladesh spends only 42 dollars per person per year on health [11], whereas the Shanchol costs around $ 4 [12] and Dukoral cost $42 [13] which are not be sufficiently cost effective for the Government of Bangladesh to implement.

Nationwide as well as globally, OCV is in short supply and is not able to meet the demands for vaccine supply [14]. In order to increase supply of OCV, more manufacturers are required and initiatives are being planned. A leading pharmaceutical company, Incepta Vaccine Ltd, in Bangladesh is now producing an OCV Cholvax™ following a technology transfer from the International Vaccine Institute (IVI), Seoul, Republic of Korea which is legally licensed and authorized [15]. Availability of an affordable OCV in Bangladesh will be a critical step in setting policy through the country.

We conducted a clinical trial to evaluate the safety and immunogenicity of a locally produced OCV Cholvax™ and compared with the WHO-prequalified OCV Shanchol™ in an urban setting in Bangladesh. The objective of the study was to evaluate and compare the safety and immunogenicity of test vaccine Cholvax™ with Shanchol™.

2. Methods

2.1. Field site, study participants and ethical considerations

We conducted a randomized, observer blinded, age-de-escalation, non-inferiority clinical trial in Mirpur, located in north-east part of Dhaka city. The urban poor community mainly resides in Mirpur and around 2.1 million people live in a 39-km^2 area. icddr,b has conducted many epidemiological and vaccine-related studies on cholera, ETEC, rotavirus and typhoid in Mirpur over the last 20 years [1,2,16]. Study participants were recruited between April 2016 and April 2017.

This comparative study was conducted in a total of 2,052 healthy participants. Of them, 1,026 received Cholvax™ (Test Group) and 1,026 received Shanchol™ (Comparator Group) and included adults (n = 868), children (n = 746), and younger children (n = 438). Participants received two doses of either Cholvax™ or Shanchol™ 14 days apart. Participants were enrolled in the study in an age descending order. The 28-day safety surveillance data of each age cohort (adult onwards) were presented to the Data Safety Monitoring Board (DSMB) before proceeding to the enrollment of the next study age group cohorts (children followed by younger children). After completion of all age cohorts, the final results were submitted to the DSMB. The DSMB unblinded the study at the end of the trial before the analysis.

Healthy male or female participant considered as per medical judgement by the study physician aged 1–45 years of age and who agreed to participate in the study and be in the study for at least for the next 6 months were recruited. At screening (day −7), before enrollment the participants were explained the study purpose and procedures. The participants provided informed written consent and stool for culture for *V. cholerae*. Culture negative participants were invited for recruitment in the study at the field site clinic (day 0).

Participants with diarrhea, abdominal pain or vomiting in the past 24 h or diarrhea lasting for more than 2 weeks in the past 6 months were excluded. At screening potential participants with a history of chronic illness or severe malnutrition, history of anaphylaxis or serious vaccine reaction, those currently taking immunosuppressive or antimicrobial therapy; or had any febrile illness ($\geq$38 °C) prior to vaccination; receipt of blood products or parental immunoglobulin in last 3 months, and pregnant women (identified by the urine dipstick test in married women) were excluded. Moreover, participants who had ever received OCV or any other live or killed enteric vaccine in the last 8 weeks or had any history of confirmed cholera were also excluded.

The study protocol was approved by the research and the ethical review committees of the International Centre for Diarrhoeal Disease Research (icddr,b) and the institutional review board of IVI. The DSMB approved the protocol and monitored the progress of the study. Written informed consent was obtained from all participants or from parents or guardians of participants less than 18 years of age and assent was also obtained from those 11 to 17 years of age. The study was registered in 2016 with ClinicalTrials.gov (NCT02742558).

2.2. Vaccines and randomization process

Shanchol™ vaccine (Shantha Biotechnics-Sanofi Pasteur; lot SCN027A15) contained approximately 1.5×10^{11} CFU of inactivated *Vibrio cholerae* O1 and 5×10^{10} CFU of inactivated *V. cholerae* O139 , as described previously [16]. Cholvax™ vaccine (Incepta Vaccine Limited, Dhaka) also contained 1.5×10^{11} CFU of inactivated *Vibrio cholerae* O1 and 5×10^{10} CFU of inactivated *V. cholerae* O139. Each dose of Shanchol™ and Cholvax™ vaccine contains 1500 lipopolysaccharide (LPS) ELISA units of *Vibrio cholerae* O1and 600 of O139 LPS (ratio of 5:2). Shanchol™ has been licensed in India since 2009 and WHO-prequalified since 2011. Both vaccines were available as single-dose vial containing 1.5 ml of study agent. Vaccines were stored at 2–8 °C before administration.

Since the test and comparator vaccines used in this clinical study were vialed differently, we designed an observer blinded study. To prevent bias of the investigator's assessment of adverse events the study physicians, study investigators, field staff, data and safety management team and laboratory personnel remained blinded. Unblinded vaccine administrators and pharmacist were appointed separately and only gave investigational products to participants, they were not involved in the evaluation of vaccine safety as well as in the serological analysis. The study participants were not informed about which vaccine they would receive;

however, as the vials were differently labelled there were chances of unblinding which cannot be ruled out.

Three individual randomization lists for adult, children and younger children were generated by the statistician from IVI. The study agents (Shanchol™ or Cholvax™) were labelled by Incepta as per the randomization list and each eligible participant was assigned to receive 'Cholvax™' or 'Shanchol™' at a 1:1 ratio across the age cohorts. Study agents were labelled from 1 to 2052 which was unique to each participant in a block randomization process (block sizes of "four and six") was employed to ensure effective balance between the interventions. Participants who were eligible and enrolled in the study by a study physician referred to the person (randomizer) who was solely responsible for determination of the randomization number for the participants. Every enrolled participant was provided a randomization number from the list and was selected for vaccination. The unblinded study pharmacist checked the study ID number and matched the randomization number from the master list and the participant was sent to the vaccinator for vaccination. The vaccinator allocated the study agents to the participants according to their entry into the trial (e.g., first participant entering into the trial received the study agent labelled 1 and the second participant got study agent labelled 2, and so on).

2.3. Safety assessments

Participants were observed for 30 min at the vaccination site to monitor for any immediate adverse events after intake of each dose of vaccine. After enrollment on day 0 (1st dose of vaccine and 1st phlebotomy), the participants visited the field clinic on day 7 (2nd phlebotomy), day 14 (2nd dose of vaccine), day 21 (3rd phlebotomy), day 28, day 42 and day 180 for clinical evaluation. Post-vaccination all participants were visited daily for seven consecutive days to ascertain solicited adverse events (AE) (diarrhea, abdominal pain, nausea/vomiting, weakness, fever, itching, rash, cough, vertigo, dryness of mouth) with any other unsolicited AEs, using a structured questionnaire after each dose of vaccine. The occurance of any AE was monitored until 42 days after the first dose and serious adverse events (SAE) were monitored from the beginning of the study up to the end (180 days after 1st dose vaccination). A biweekly phone call was scheduled with every participant followed up till study day 42. An independent safety monitoring committee assessed all AEs of the study at regular intervals (after completion of day 28 of each age cohort). Regular study monitoring was performed by the IVI staff throughout the study period.

2.4. Immune response assessment

Blood was collected at three time points; on day 0 before the first dose of vaccination, at day 7 post first dose and on day 21 (7 days post 2nd dose). Participants were given a + 3-day window for day 7 and day 21 blood draws. Sera were stored at (-15 °C to −25 °C) until analysis.

2.5. Vibriocidal antibody assay

We assessed vibriocidal antibody responses in serum as previously described, using guinea pig complement (Sigma-Aldrich Chemie GmbH) and *V. cholerae* O1 Ogawa (X-25049) and Inaba (T-19479) as the target organism [17]. We defined the vibriocidal titer as the reciprocal of the highest dilution resulting in $\geq$ 50% reduction of the optical density compared to that of control wells without serum. We considered responders individuals with a $\geq$ 4-fold increase in vibriocidal titer above baseline for both convalescent patients and vaccinees. To control for variations, test plates containing pooled convalescent serum samples from patients with cholera were used as a positive control (pooled O1 Ogawa and O1 Inaba sera from our collection of specimens from cholera patients) [18,19]. Geometric Mean Titer (GMT) and ratio of Geometric Mean Rate (GMR) by anti-*V. cholerae* O1 antibody titer were calculated at Day 7 and Day 21 after the first dose of each vaccine and compared to baseline (Day 0).

2.6. Sample size

A non-inferiority study on immune response was designed to show that the proportion of subjects responding to Test Vaccine was not less than the proportion of subjects responding to the Comparator Vaccine by the pre-defined margin. The difference in immunogenic response between the Cholvax and Shanchol vaccines of less than 10% was considered of no clinical importance. Since Cholvax is manufactured with the same technology transfer from IVI and have similar composition as Shanchol, we accepted the non-inferiority margin of 10% in the current study based on a previous OCV non-inferiority trial [20]. This study concluded a strong comparable vibriocidal response between the Euvichol and Shanchol vaccine groups at a −10% (δ = -0.10) non-inferiority margin [20,21]. In Bangladesh, a 10% lower effectiveness would be acceptable given that the decrease in cost would enable greater access to the vaccine due to its affordability. We assumed the true response induced by Shanchol™ would be 62% for all ages combined, 50% for the age greater than 18 years, 69% for 6–17 years, and 86% for 1–5 years and with no difference in the true response of the two vaccines (i.e., ϵ=0), based on the magnitude of the vibriocidal response rate of the participants[22–24]. The study was powered for immunogenicity but not for safety. With a one-tail test at 2.5% level of significance (α = 0.025), 80% power (1-β = 0.80), and 10% attrition, the sample size for each stratum, consisted of 219 participants for age group 1–5 years, 373 participants for age 6–17 years and 434 for age 18–45 years which were equally distributed between intervention group and total sample size was 2,052 for all age cohort.

2.7. Statistical analysis

Data were directly entered into the electronic database, via Tablet computer. Data were verified and checked by a clinical monitor from IVI. We had a statistical analysis plan (SAP) in the protocol at the start of the trial and the analysis was conducted following procedures outlined in the trial according to the SAP.

After data base lock, the study vaccine groups were unblinded following which statistical analyses were performed using SAS 9.4 (SAS Institute, Cary NC). For comparison of demographic data between vaccine groups, Pearson's Chi-square analysis was carried out for categorical data, and Wilcoxon's rank sum test for continuous data were used. Analyses for comparisons of AEs and seroconversion rates were performed with the chi-square test or Fisher's exact test. Data for immunogenicity analysis for all age cohorts as well as for each age cohort (adult, children and younger children) was conducted with Modified intention-to-treat (mITT) set and per protocol (PP) set. The mITT set analysis included all participants who received a dose of investigational vaccines and who had at least one post-baseline immunogenicity data available. The PP set was a subset of the mITT set that were compliant with study procedures, completed visits for investigational product dosing, blood collection for immunogenicity as scheduled and received the correct vaccinations. The mITT set was used for the primary analysis of interest for the safety endpoint and PP set was used for the primary analysis of the immunogenicity endpoints.

There were two primary endpoints in this study; the safety and the immunogenicity endpoints. The safety endpoints were to

measure the proportion of reactogenicity (immediate reactions within 30 min after each dose), solicited AE within 7 days after each dose vaccination, unsolicited AE occurring 28 days following each dose of vaccination and SAE 6 months post first dose between the vaccinees. The primary immunogenicity endpoints were to evaluate the proportion of participants showing sero-conversion against *V. cholerae* O1, El Tor Inaba after vaccination with Cholvax in comparison with Shanchol™ and to assess proportion of participants showing sero-conversion against *V. cholerae* O1, El Tor Ogawa after vaccination with Cholvax being tested in comparison with Shanchol™.

For primary safety endpoint, safety was analyzed for all randomized participants who received at least one dose of study vaccines including incorrect or incomplete doses. The proportions of participants who experienced solicited AEs and unsolicited AEs after each vaccination and the 95% CI were presented for each vaccine group.

For primary immunogenicity endpoint, non-inferiority was evaluated by calculating the difference in seroconversion rates between Cholvax™ and Shanchol™ from baseline to 7 days post second dose. The lower limit of one-tailed 97.5% confidence interval of seroconversion rate difference (Cholvax™-Shanchol™) for O1 Inaba and O1 Ogawa was evaluated using a non-inferior margin of −10%. The number and percentage of adults, children and younger children (with 95% CI) of seroconversion who exhibit at least a fourfold rise in serum vibriocidal titer after vaccination to the *V. cholerae* O1 Ogawa and Inaba serogroups was compared between the study groups. Non-inferiority was confirmed if the lower limit of one-tailed 97.5% CI (equivalent to the lower limit of two-tailed 95% CI) of the difference of seroconversion rate between Test vaccine and Comparator vaccine was equal to or greater than the non-inferiority margin of −10%. GMT was also analyzed after log transformation and compared for different age strata including adult (18–45 years), children (6–17 years) and younger children (1–5 years) using and analysis of model adjusting for baseline titers. The GMT of vibriocidal antibodies against *V. cholerae* O1 Inaba and Ogawa were measured at Day 21 for the overall population. The non-inferiority of the Test group in comparison to Comparator group was confirmed if the lower limit of the 95% CI of the GMT ratio (GMR) was equal to or greater than the pre-defined non-inferiority margin of 0.5 for serotypes O1 Inaba and O1 Ogawa according to the study SAP.

3. Results

3.1. Study participants

The study cohort consisted of 2,052 participants. The participants' flow is illustrated in Fig. 1. No significant difference was found in the demographic characteristics between Test and Comparator groups (Table 1).

This study was performed in 1–45-year-old healthy participants stratified into three age cohorts: Adults aged 18–45 years 868 participants (median age 30 years; 434 in each vaccine group); children aged 6–17 years 746 participants (median age 11 years; 373 in each vaccine group) and younger children 1–5 years 438 participants (median age 3 years; 219 in each vaccine group).

3.2. Safety endpoint analysis

The safety cohort included 2,052 participants. There was no significant difference in the proportion of solicited AEs between Test and Comparator groups within 7 days after first, within 7 days after second, and within 7 days after any dose of vaccine (95% CI) (Table 2) as values overlapped in CI.

Weakness was the most common solicited AEs following any dose of Test (n = 17, 1.66%) and Comparator vaccine (n = 16, 1.56%). Weakness was also the most common solicited symptoms following any dose of Test (n = 15, 3.46%) and Comparator (15, 3.46%) in the adult cohort. In the children and younger children cohort, diarrhea and cough were the most common solicited AEs. The number of participants with unsolicited AEs in all age cohorts within 42 days after the first dose was 15 (1.46%); in Test group (95% CI: 0.89, 2.40) and 11 (1.07%) in the Comparator group (95% CI: 0.60, 1.91).

Two SAEs were reported throughout the study period, neither being judged related to the investigational vaccine. One participant had a surgical intervention due to fibroadenoma of both breasts (in Shanchol™ group, day 104) and another died in a road traffic accident (in Cholvax™ group, day 106).

3.3. Immunogenicity endpoint analysis

In the overall population, as per the per-protocol analysis set, the seroconversion rates to *V. cholerae* O1 Inaba one week after the second vaccine dose were 82.92% (95% CI: 80.47, 85.12) in the Test group and 83.93% (95% CI: 81.53, 86.08) in the Comparator group, showing a non-inferiority difference between Test group and Comparator group of −0.87 (-4.11, 2.36) (as the lower limit of the two-tailed 95% CI of the difference is −4.11, which is greater than the predefined non-inferiority margin of −10%). The seroconversion rates to *V. cholerae* O1 Ogawa one week after the second vaccine dose were 80.64% (95% CI: 78.08, 82.96) in the Test group and 77.35% (95% CI: 74.65, 79.83) in the Comparator group, showing a non-inferiority difference between Test group and Comparator group of 3.13 (-0.38, 6.64) (as the lower limit of the two-tailed 95% CI of the difference was −0.38, which is greater than the predefined non-inferiority margin of −10%) (Table 3).

Vibriocidal antibody responses to *V. cholerae* O1 Inaba in the three age cohorts following two doses of Cholvax™ were non-inferior to those of Shanchol™ in adults by tests of non-inferiority of −3.02 (-8.31, 2.26) (80% vs. 83%; lower limit of the 95% CI of the difference was −8.31), in children of 0.73 (-4.48, 5.93) (85% vs. 84%; lower limit of the 95% CI of the difference was −4.48) and in younger children of −0.00 (-6.58, −6.58) (86% vs. 86%; lower limit of the 95% CI of the difference is −6.58). Similar findings were observed for O1 Ogawa in adults demonstrating non-inferior differences of 3.99 (-1.86, 9.84) (77% vs. 73%; lower limit of the 95% CI of the difference was −1.86), in children of 4.21 (-1.54, 9.95) (82% vs. 78%; lower limit of the 95% CI of the difference was −1.54) and in younger children of 0.47 (-6.33, 7.27) (85% vs. 84%; lower limit of the 95% CI of the difference was −6.33) (Table 4, Fig. 2).

Cholvax™ was non-inferior to Shanchol™ in terms of GMT of vibriocidal antibodies for *V. cholerae* O1 Inaba and O1 Ogawa; based on the non-inferiority margin of the ratio of GMT of 0.5 in the overall population as well as by age cohort (Table 5, Table 6).

Cholvax™ was safe and immunogenic in the participants and non-inferior to Shanchol™ among the overall population as well as by age strata.

4. Discussion

This is the first study testing the locally produced oral cholera vaccine, Cholvax™ with the WHO-prequalified vaccine Shanchol™ among Bangladeshi participants living in a high-risk cholera endemic setting. Our data suggest that the killed bivalent whole cell OCV, Cholvax™ is safe and induces strong and comparable vibriocidal immune responses in comparison to Shanchol™.

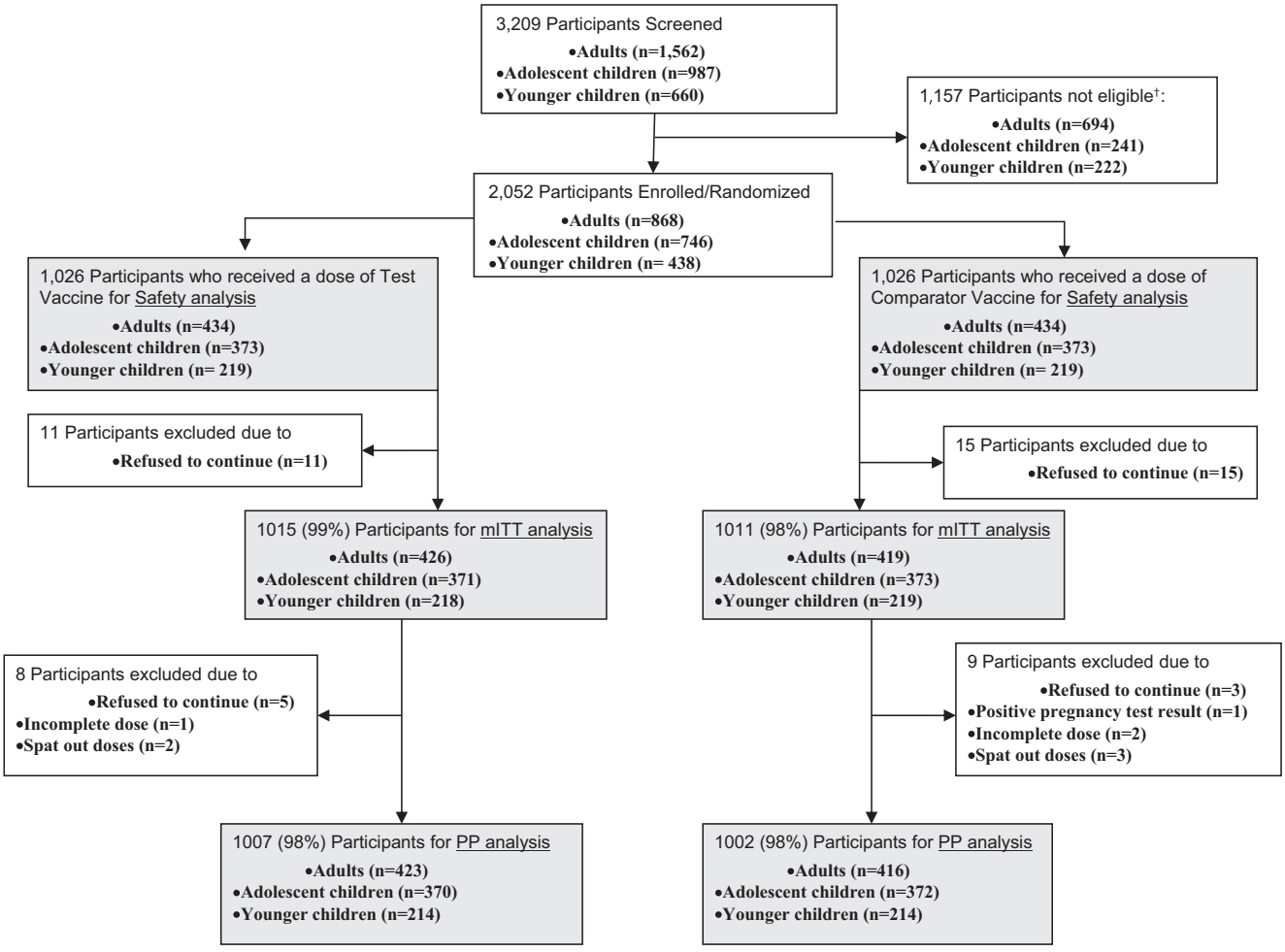

Fig. 1. Flow diagram of Participant Disposition (CONSORT flow diagram).

Table 1
Demographic characteristics of the study participants.

Characteristics Adults cohort		Test Group N = 434	Comparator Group N = 434	Total N = 868	p-value
Gender	Male (%)	110 (25.35)	119 (27.42)	229 (26.38)	0.488
	Female (%)	324 (74.65)	315 (72.58)	639 (73.62)	
Age (years)	Mean (SD)	31.14 (6.83)	30.24 (6.80)	30.69 (6.82)	0.038
	Median (min, max)	31.00 (18.00, 45.00)	30.00 (18.00, 45.00)	30.00 (18.00, 45.00)	
Children cohort		**N = 373**	**N = 373**	**N = 746**	
Gender	Male (%)	176 (47.18)	196 (52.55)	372 (49.87)	0.143
	Female (%)	197 (52.82)	177 (47.45)	374 (50.13)	
Age (years)	Mean (SD)	10.92 (3.12)	10.93 (3.13)	10.92 (3.12)	0.946
	Median (min, max)	11.00 (6.00, 17.00)	11.00 (6.00, 17.00)	11.00 (6.00, 17.00)	
YoungerChildren cohort		**N = 219**	**N = 218**	**N = 438**	
Gender	Male (%)	106 (48.40)	126 (57.53)	232 (52.97)	0.056
	Female (%)	113 (51.60)	93 (42.47)	206 (47.03)	
Age (years)	Mean (SD)	3.11 (1.40)	3.05 (1.21)	3.08 (1.31)	0.382
	Median (min, max)	3.00 (1.00, 5.00)	3.00 (1.00, 5.00)	3.00 (1.00, 5.00)	

No SAE related to the Test and Comparator vaccines was observed during the study period. The safety profile of this new OCV Cholvax™ was similar compared to Shanchol™ which was also previously tested in endemic settings [23–26]. Shanchol™ was lower in price [27] than first generation vaccine Dukoral® [10] while Cholvax™ price is much lower (0.5$ /dose) than Shanchol™. Dukoral was previously the only registered cholera vaccine in Bangladesh but since 2019 Cholvax™ became licensed and available for use in public health programs in Bangladesh [10]. The company (Incepta Vaccine Ltd.) has obtained licensure from the Bangladesh Drug Administration. The dossier will be submitted for WHO prequalification once the National Regulatory Authority of the DGDA attains Level 3 functionality which is expected in June 2022.

Table 2
Proportion of participants with solicited AEs overall and per age cohort in Test and Comparator groups.

	Test Group			Comparator Group		
Within 7 days after first dose	N	Number of participants (%)	95% CI†	N	Number of participants (%)	95% CI†
All age cohorts	1026	44 (4.29%)	(3.21, 5.71)	1026	44 (4.29%)	(3.21, 5.71)
Adults cohort	434	22 (5.07%)	(3.37, 7.56)	434	19 (4.38%)	(2.82, 6.74)
Children cohort	373	2 (0.54%)	(0.15, 1.93)	373	4 (1.07%)	(0.42, 2.72)
Younger Children cohort	219	20 (9.13%)	(5.99, 13.68)	219	21 (9.59%)	(6.36, 14.21)
Within 7 days aftersecond dose	**N**	**Number of participants (%)**	**95% CI†**	**N**	**Number of participants (%)**	**95% CI†**
All age cohorts	1012	35 (3.46%)	(2.50, 4.77)	1007	28 (2.78%)	(1.93, 3.99)
Adults cohort	424	11 (2.59%)	(1.45, 4.59)	418	10 (2.39%)	(1.30, 4.35)
Children cohort	370	7 (1.89%)	(0.92, 3.85)	373	5 (1.34%)	(0.57, 3.10)
Younger Children cohort	218	17 (7.80%)	(4.93, 12.13)	216	13 (6.02%)	(3.55, 10.02)
Within 7 days after any dose	**N**	**Number of participants (%)**	**95% CI†**	**N**	**Number of participants (%)**	**95% CI†**
All age cohorts	1026	75 (7.31%)	(5.87, 9.07)	1026	69 (6.73%)	(5.35, 8.42)
Adults cohort	434	31 (7.14%)	(5.08, 9.96)	434	29 (6.68%)	(4.69, 9.43)
Children cohort	373	9 (2.41%)	(1.27, 4.52)	373	9 (2.41%)	(1.27, 4.52)
Younger Children cohort	219	35 (15.98%)	(11.72, 21.41)	219	31 (14.16%)	(10.15, 19.39)

† Calculation for all ages was derived using stratified Chi-square (Cochran-Mantel-Haenszel) test stratified by age. Calculations for each age cohort were derived using Pearson's Chi-square test or Fisher's exact test.

Table 3
Vibriocidal antibody seroconversion rates to *V. cholerae* O1 Inaba and O1 Ogawa for all age groups.

All age groups	Post first vaccine dose					Post second vaccine dose				
	Test Group (N = 1,007)		Comparator Group (N = 1,002)		Test – Comparator (95% CI) †	Test Group (N = 1,007)		Comparator Group (N = 1,002)		Test – Comparator (95% CI) †
	n*	Seroconversion rate (95% CI)	n*	Seroconversion rate (95% CI)		n*	Seroconversion rate (95% CI)	n*	Seroconversion rate (95% CI)	
O1 Inaba	1007	83.12 (80.68, 85.30)	1002	83.33 (80.90, 85.51)	−0.22 (-3.49, 3.05)	1007	82.92 (80.47, 85.12)	1002	83.93 (81.53, 86.08)	−0.87 (-4.11, 2.36)
O1 Ogawa	1007	79.94 (77.36, 82.30)	1002	78.04 (75.38, 80.50)	1.60 (-1.96, 5.16)	1007	80.64 (78.08, 82.96)	1002	77.35 (74.65, 79.83)	3.13 (-0.38, 6.64)

* Number of participants analyzed.
† Seroconversion rate difference and their lower limit of two-tailed 95% CI (equivalent to the lower limit of one-tailed 97.5% CI) post first dose or post second dose were calculated by subtracting Comparator vaccine from Test vaccine. The Test vaccine is non-inferior to the Comparator vaccine if the lower limit of the proportion difference is equal to or greater than pre-defined non-inferior margin of −10%.

The immunogenicity of this new vaccine was determined using vibriocidal antibody titers, which is an indirect and surrogate measure for cholera protection [18]. The results generated in a cholera patient study using a vibriocidal assay suggested that the vibriocidal antibody response may be an indirect surrogate marker for protection, it is not the best correlate. The present study was only an immunogenicity study and was not designed to asses protection from cholera or prevention of the disease. This serum complement-fixing antibody likely to be active at the mucosal surface, playing an important role as protective mucosal immune response [28]. The baseline vibriocidal antibody responses for both Test and Comparator vaccine groups in different age cohorts were similar among themselves and also similar when compared to other studies conducted in an endemic setting [24].

In this study, serum O1 Inaba and Ogawa vibriocidal antibody responses in three age cohorts were comparable for both vaccines. The overall vibriocidal antibody responses induced by Cholvax™ to *V. cholerae* were O1 Inaba 83% and Ogawa 81%, similar to the results from another Shanchol™ immunogenicity study, 73% and 75%, respectively [24] after two doses of vaccination. The vibriocidal titers induced by Cholvax™ are also comparable to the Korean Euvichol® vaccine (82% in Inaba and 80% in Ogawa) [20] for which WHO recommends two doses for full protection against cholera [29].

Increased fold rise and seroconversion rates with Cholvax™ were comparable to those observed with other OCVs in other countries. Geometric fold rise (∼19, 11, 9, and 12) and seroconversion were elevated to (∼91%, 70%, 65%, and 80%) in Haiti, Ethiopia, Kolkata, and Bangladesh (in this study), respectively. All regions still considered as endemic settings for cholera [23,30–32]. The younger children (1–5 years of age) strata had lower vibriocidal immune responses to the test and comparator vaccine than adults and older children. This may due to lower intensity of environmental exposure to the organism or to the inability of young children to respond to T cell-independent carbohydrate antigens [33]. This finding is similar to other studies conducted in endemic settings [34].

The GMT rises were higher after intake of the first dose of vaccine compared to the second dose of vaccine from the baseline in both test and comparator vaccine. A single dose of Cholvax™ elicited a magnitude of vibriocidal response similar to that of Shanchol™ on day 7 in the adults, children and younger children. This may be explained by Shanchol™ as well as Cholvax™ vaccines having a high level of LPS content, which elicits higher serum vibriocidal responses after the first dose of vaccine and which does not further elicit an increase in immune responses after a short interval second dose [35]. Kanungo and colleagues have hypothesized in their study that immune response in the gut may neutralize the effects of the second dose of the vaccine, therefore resulting in decreased vibriocidal titers [23]. This finding is well-matched with previous studies that high vibriocidal titers lessen further immune responses [1,36].

In earlier studies, it has been shown the LPS-specific IgA response in adults was higher than that in young children with

Table 4
Vibriocidal antibody seroconversion rates to *V. cholerae* O1 Inaba and O1 Ogawa per age group.

Adults	**Post first vaccine dose**					**Post second vaccine dose**				
	Test Group (N = 423)		**Comparator Group (N = 416)**		Test – Comparator (95% CI) †	**Test Group (N = 423)**		**Comparator Group (N = 416)**		**Test – Comparator (95% CI)** †
	n*	**Seroconversion rate (95% CI)**	**n***	**Seroconversion rate (95% CI)**		**n***	**Seroconversion rate (95% CI)**	**n***	**Seroconversion rate (95% CI)**	
O1 Inaba	423	81.32 (77.33, 84.75)	416	81.97 (77.99, 85.37)	−0.65 (-5.89, 4.59)	423	79.67 (75.57, 83.23)	416	82.69 (78.76, 86.02)	−3.02 (-8.31, 2.26)
O1 Ogawa	423	77.54 (73.33, 81.26)	416	71.39 (66.87, 75.53)	6.15 (0.26, 12.04)	423	77.07 (72.83, 80.82)	416	73.08 (68.62, 77.11)	3.99 (-1.86, 9.84)
Children	**Post first vaccine dose**					**Post second vaccine dose**				
	Test Group (N = 370)		**Comparator Group (N = 372)**		Test – Comparator (95% CI) †	**Test Group (N = 370)**		**Comparator Group (N = 372)**		**Test – Comparator (95% CI)** †
	n*	**Seroconversion rate (95% CI)**	**n***	**Seroconversion rate (95% CI)**		**n***	**Seroconversion rate (95% CI)**	**n***	**Seroconversion rate (95% CI)**	
O1 Inaba	370	86.76 (82.92, 89.84)	372	85.22 (81.25, 88.46)	1.54 (-3.45, 6.54)	370	84.86 (80.86, 88.16)	372	84.14 (80.08, 87.50)	0.73 (-4.48, 5.93)
O1 Ogawa	370	85.14 (81.15, 88.40)	372	83.87 (79.79, 87.26)	1.26 (-3.94, 6.47)	370	82.16 (77.94, 85.73)	372	77.96 (73.47, 81.87)	4.21 (-1.54, 9.95)
Younger children	**Post first vaccine dose**					**Post second vaccine dose**				
	Test Group (N = 214)		**Comparator Group (N = 214)**		Test – Comparator (95%	**Test Group (N = 214)**		**Comparator Group (N = 214)**		**Test - Comparator (95% CI)** †
	n*	**Seroconversion rate (95% CI)**	**n***	**Seroconversion rate (95% CI)**		**n***	**Seroconversion rate (95% CI)**	**n***	**Seroconversion rate (95% CI)**	
O1 Inaba	214	80.37 (74.54, 85.14)	214	82.71 (77.08, 87.19)	−2.34 (-9.68, 5.01)	214	85.98 (80.69, 90.00)	214	85.98 (80.69, 90.00)	−0.00 (-6.58, 6.58)
O1 Ogawa	214	75.70 (69.53, 80.96)	214	82.71 (77.08, 87.19)	−5.14 (-12.9, 2.66)	214	85.05 (79.65, 89.20)	214	84.58 (79.14, 88.80)	0.47 (-6.33, 7.27)

* Number of participants analyzed.
† Seroconversion rate difference and lower limit of two-tailed 95% CI (equivalent to the lower limit of one-tailed 97.5% CI) post first dose or post second dose were calculated by subtracting Comparator vaccine from Test vaccine. The Test vaccine is non-inferior to the Comparator vaccine if the lower limit of the proportion difference is equal to or greater than pre-defined non-inferior margin of −10%

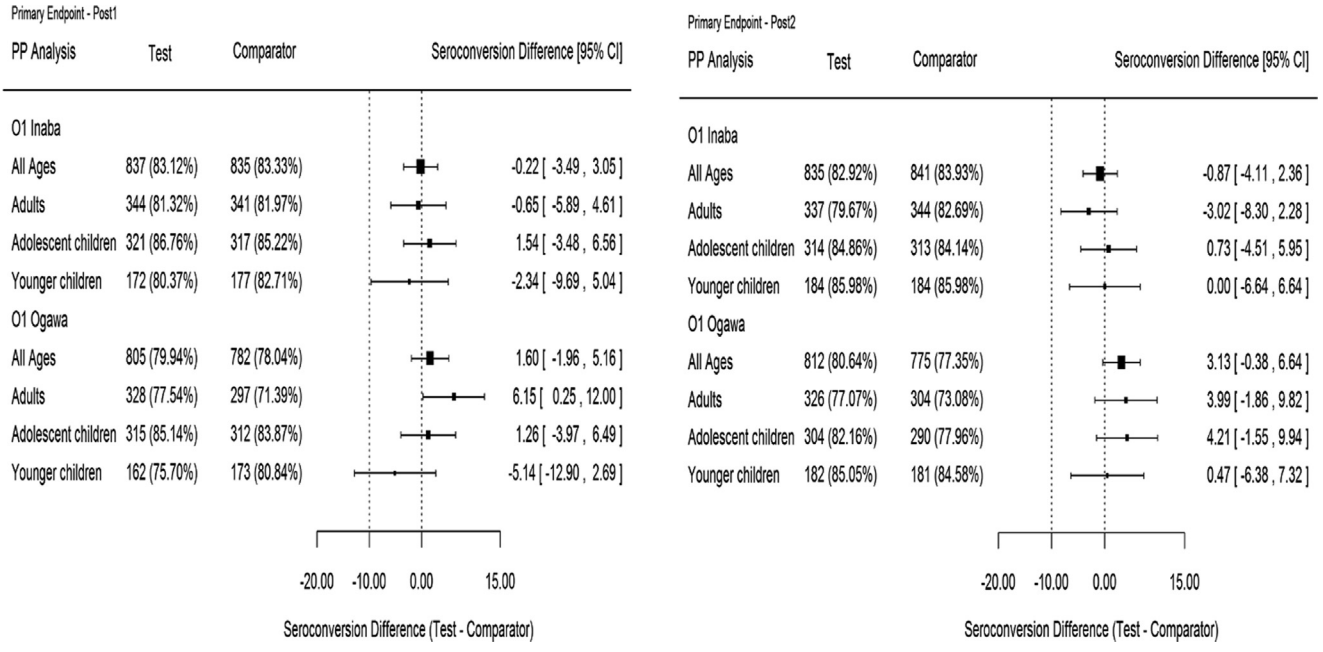

Fig. 2. Difference of seroconversion rates of vibriocidal antibodies against *V. cholerae* to O1 Inaba and O1 Ogawa.

natural cholera infection. This observation may reflect the ability of adults to mount more potent immune responses to T cell-independent antigens than young children [37,38]. Polysaccharides are T cell-independent antigens that elicit poor immune responses in young children due to lack of affinity maturation, poor antibody subclass switching and inability to generate memory especially in infants [39]. Poor immune responses inpediatric vaccinees may be due to lack of T-cell responses that mature with age as suggested by comparable responses observed in older children and adults [40].

In-depth analysis of adaptive responses to oral cholera vaccine at later time points is warranted to better understand antibody-secreting cell responses, memory B- and T-cell responses in different age groups.

Table 5
Geometric Mean Titers of vibriocidal antibodies to *V. cholerae* O1 Ogawa and Inaba for all age groups.

All Age groups	Baseline				Post first vaccine dose				
	Test Group (N = 1007)		Comparator Group (N = 1002)		Test Group (N = 1007)		Comparator Group (N = 1002)		T/C[#]†
	n*	GMT[a] (95% CI)	n*	GMT[a] (95% CI)	n*	GMT[a] (95% CI)	n*	GMT[a] (95% CI)	
Inaba	1007	34.28 (30.86, 38.09)	1002	33.93 (30.53, 37.70)	1007	575.23 (522.88, 632.82)	1002	549.65 (502.86, 600.80)	1.04 (0.93)
Ogawa	1007	37.57 (33.80, 41.76)	1002	40.95 (36.94, 45.40)	1007	466.62 (422.85, 514.93)	1002	450.67 (411.51, 493.56)	1.08 (0.96)
All Age groups	**Baseline**				**Post second vaccine dose**				
	Test Group (N = 1007)		**Comparator Group (N = 1002)**		**Test Group (N = 1007)**		**Comparator Group (N = 1002)**		**T/C[#]†**
	n*	GMT[a] (95% CI)	n*	GMT[a] (95% CI)	n*	GMT[a] (95% CI)	n*	GMT[a] (95% CI)	
Inaba	1007	34.28 (30.86, 38.09)	1002	33.93 (30.53, 37.70)	1007	462.79 (427.16, 501.39)	1002	455.06 (421.93, 490.80)	1.01 (0.92)
Ogawa	1007	37.57 (33.80, 41.76)	1002	40.95 (36.94, 45.40)	1007	419.98 (387.86, 454.76)	1002	390.55 (361.47, 421.96)	1.11 (1.01)

* Number of participants analyzed.
[a] Geometric mean reciprocal titers.
[#]Test /Comparator
† Adjusted for baseline titers in the model. Ratio of GMT of Test group to Comparator group. The Test group is non-inferior to the Comparator group as the lower limit of the ratio is equal to or greater than pre-defined non-inferior margin of 0.5.

Table 6
Geometric mean titers (GMTs) to *Vibrio cholerae* O1 Ogawa and Inaba in three age groups.

Adults	Baseline				Post second vaccine dose				
	Test Group (N = 423)		**Comparator Group (N = 416)**		**Test Group (N = 423)**		**Comparator Group (N = 416)**		**T/C[#]†**
	n*	**GMT[a](95% CI)**	**n***	**GMT[a](95% CI)**	**n***	**GMT[a] (95% CI)**	**n***	**GMT[a] (95% CI)**	
Inaba	423	47.90 (41.18, 55.72)	416	43.91 (37.73, 51.11)	423	583.88 (519.46, 656.30)	416	511.08 (456.09, 572.70)	1.11 (0.96)
Ogawa	423	61.45 (53.05, 71.18)	416	59.77 (51.56, 69.28)	423	527.48 (470.19, 591.75)	416	437.72 (391.03, 489.98)	1.19 (1.04)
Adults	**Baseline**				**Post second vaccine dose**				
	Test Group (N = 423)		**Comparator Group (N = 416)**		**Test Group (N = 423)**		**Comparator Group (N = 416)**		**T/C[#]†**
	n*	**GMT[a](95% CI)**	**n***	**GMT[a](95% CI)**	**n***	**GMT[a](95% CI)**	**n***	**GMT[a](95% CI)**	
Inaba	423	47.90 (41.18, 55.72)	416	43.91 (37.73, 51.11)	423	477.30 (429.20, 530.80)	416	434.81 (390.18, 484.54)	1.07 (0.93)
Ogawa	423	61.45 (53.05, 71.18)	416	59.77 (51.56, 69.28)	423	456.65 (410.25, 508.28)	416	394.75 (353.94, 440.28)	1.14 (1.01)
Children	**Baseline**				**Post first vaccine dose**				
	Test Group (N = 370)		**Comparator Group (N = 372)**		**Test Group (N = 370**		**Comparator Group (N = 372)**		**T/C[#]†**
	n*	**GMT[a](95% CI)**	**n***	**GMT[a](95% CI)**	**n***	**GMT[a](95% CI)**	**n***	**GMT[a](95% CI)**	
Inaba	370	34.63 (28.93, 41.45)	372	35.84 (29.94, 42.90)	370	782.05 (682.04, 896.73)	372	730.52 (640.02, 833.82)	1.08 (0.90)
Ogawa	370	39.78 (33.24, 47.60)	372	44.65 (37.67, 52.92)	370	673.20 (582.67, 777.81)	372	648.40 (571.72, 735.37)	1.08 (0.90)
Children	**Baseline**				**Post second vaccine dose**				
	Test Group (N = 370)		**Comparator Group (N = 372)**		**Test Group (N = 370)**		**Comparator Group (N = 372)**		**T/C[#]†**
	n*	**GMT[a](95% CI)**	**n***	**GMT[a](95% CI)**	**n***	**GMT[a](95% CI)**	**n***	**GMT[a](95% CI)**	
Inaba	370	34.63 (28.93, 41.45)	372	35.84 (29.94, 42.90)	370	562.40 (498.69, 634.24)	372	554.46 (494.97, 621.10)	1.02 (0.88)
Ogawa	370	39.78 (33.24, 47.60)	372	44.65 (37.67, 52.92)	370	539.69 (481.84, 604.48)	372	483.95 (430.75, 543.72)	1.15 (0.99)
Younger children	**Baseline**				**Post first vaccine dose**				
	Test Group (N = 214)		**Comparator Group (N = 214)**		**Test Group (N = 214)**		**Comparator Group (N = 214)**		**T/C[#]†**
	n*	**GMT[a](95% CI)**	**n***	**GMT[a](95% CI)**	**n***	**GMT[a](95% CI)**	**n***	**GMT[a](95% CI)**	
Inaba	214	17.40 (13.96, 21.69)	214	18.68 (14.88, 23.46)	214	328.40 (245.24, 439.76)	214	386.13 (297.41, 501.33)	0.89 (0.63)
Ogawa	214	12.87 (10.57, 15.69)	214	16.90 (13.66, 20.91)	214	194.32 (145.43, 259.65)	214	253.44 (192.17, 334.23)	0.93 (0.66)
Younger children	**Baseline**				**Post second vaccine dose**				
	Test Group (N = 214)		**Comparator Group (N = 214)**		**Test Group (N = 214)**		**Comparator Group (N = 214)**		**T/C[#]†**
	n*	**GMT[a](95% CI)**	**n***	**GMT[a](95% CI)**	**n***	**GMT[a](95% CI)**	**n***	**GMT[a](95% CI)**	
Inaba	214	17.40 (13.96, 21.69)	214	18.68 (14.88, 23.46)	214	310.81 (247.50, 390.31)	214	352.66 (288.34, 431.31)	0.91 (0.70)
Ogawa	214	12.87 (10.57, 15.69)	214	16.90 (13.66, 20.91)	214	230.71 (184.78, 288.06)	214	263.48 (214.66, 323.40)	1.00 (0.76)

* Number of participants analyzed.
[a]Geometric mean reciprocal titers.
[#]Test /Comparator
† Adjusted for baseline titers in the model. Ratio of GMT of Test group to Comparator group. The Test group is non-inferior to the Comparator group as the lower limit of the

The comparable safety and immunogenicity findings established for Cholvax™ in this trial is a key step towards pursuing WHO prequalification, which would make Cholvax™ OCV suitable for mass vaccination, in places where cholera still is a significant public health concern. Dukoral is the only imported licensed vaccine approved in Bangladesh and the composition is different from Shanchol and Cholvax. It requires buffer to dispense the vaccine and also requires more storage space. The cost of Dukoral is also very high. On the other hand, the vaccine formulation of Shanchol™, Euvichol® plus and Cholvax are basically the same and manufactured through transfer of technology from IVI to Korea and Bangladesh respectively. Neither of these vaccines requires a buffer for administration. A new plastic packaging of Euvichol-Plus® reduces the vial's volume which allows easier transportation, distribution and waste management. But while considering the cost, Euvichol-Plus® is currently available at $1.20 per dose [41] whereas the price of Cholvax™ is much lower. Therefore, the ultra low cost of Cholvax vaccine improves the programmatic benefits for supply and deployment in the national immunization system in Bangladesh, improves field ability and easy deliverability for other endemic countries for mass campaigns. This non-inferiority trial can serve as evidence for policy makers to support its use as a complement to the present OCVs.

We acknowledge some limitations in this study. We calculated the sample size based on the immune response to the vaccine and the study was not powered for a statistical comparison of safety. The statistician who performed the analyses in this trial was not blinded, this was another limitation. However, the data set was frozen and locked before being sent to the statistician for analysis. Furthermore, the study participants were not blinded since the two vaccines had different appearances and this might have effect on adverse events reporting. However, we found similar frequency of solicited and unsolicited safety events between the two vaccines.

In 2017, a WHO position paper recommended the use of oral cholera vaccines to control the disease particularly in outbreaks, epidemics even in endemic settings where rapid action is required [42]. In 2017, the Global Task Force on Cholera Control launched a strategy which aims to reduce cholera deaths by 90% and to eliminate cholera in as many as 20 countries by 2030 [43]. This ambitious plan may be difficult to achieve due to the high vaccine cost, low vaccine availability, logistical challenges in delivery and challenges in implementing rigorous interventions with WaSH controls efforts. From the initiation of the WHO OCV stockpile in 2013 through May 2018, around 25 million doses of OCV were deployed through campaigns in 19 different countries [10,41,44]. However, the global demand of OCV for both epidemic and endemic settings far exceeds the present supply [45]. In order to increase supply of OCV, more manufacturers are needed [46]. Based on this need, the locally manufactured killed bivalent whole-cell OCV, Cholvax™ was developed and has been found to be safe and immunogenically non-inferior to Shanchol™. Incepta Vaccine Ltd has the capacity to manufacture 12 million doses of Cholvax™ vaccine annually, which may contribute to meet the local demand of OCV, though 65 million people in Bangladesh need protection from cholera [47]. Cholvax™ can be deployed in Bangladesh and once WHO-prequalified be used to meet the growing demand of lower income countries where cholera remains a major cause of epidemics and outbreaks and has a significant public health impact.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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