Effectiveness of an oral cholera vaccine campaign to prevent clinically-significant cholera in Odisha State, India

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A B S T R A C T

Background: A clinical trial conducted in India suggests that the oral cholera vaccine, Shanchol, provides 65% protection over five years against clinically-significant cholera. Although the vaccine is efficacious when tested in an experimental setting, policymakers are more likely to use this vaccine after receiving evidence demonstrating protection when delivered to communities using local health department staff, cold chain equipment, and logistics.

Methods: We used a test-negative, case-control design to evaluate the effectiveness of a vaccination campaign using Shanchol and validated the results using a cohort approach that addressed disparities in healthcare seeking behavior. The campaign was conducted by the local health department using existing resources in a cholera-endemic area of Puri District, Odisha State, India. All non-pregnant residents one year of age and older were offered vaccine. Over the next two years, residents seeking care for diarrhea at one of five health facilities were asked to enroll following informed consent. Cases were patients seeking treatment for laboratory-confirmed V. cholera-associated diarrhea. Controls were patients seeking treatment for V. cholerae negative diarrhea.

Results: Of 51,488 eligible residents, 31,552 individuals received one dose and 23,751 residents received two vaccine doses. We identified 44 V. cholerae O1-associated cases and 366 non V. cholerae diarrhea controls. The adjusted protective effectiveness for persons receiving two doses was 69.0% (95% CI: 14.5% to 88.8%), which is similar to the adjusted estimates obtained from the cohort approach. A statistical trend test suggested a single dose provided a modicum of protection (33%, test for trend, p = 0.0091).

Conclusion: This vaccine was found to be as efficacious as the results reported from a clinical trial when administered to a rural population using local health personnel and resources. This study provides evidence that this vaccine should be widely deployed by public health departments in cholera endemic areas.

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1. Introduction

Vaccine efficacy is measured using randomized, placebo-controlled clinical trials. Such studies are usually completed under ideal conditions where, among other criteria, healthy volunteers are enrolled, age restrictions and the cold chain are finely maintained, and the vast majority of subjects receive all scheduled doses. For these experimental studies, the estimated protective effect may be superior to that obtained when vaccine is administered under real-life conditions where vaccinees may suffer from undiagnosed diseases or a controlled temperature chain may be difficult to maintain.
Given the above situation, policymakers may request a study of vaccine effectiveness when the vaccine is delivered to populations using locally-available personnel and resources in rural areas or urban slums. To provide this information, we previously explored and reported on the operational feasibility of an oral cholera vaccine campaign using Shanchol, a licensed and World Health Organization (WHO) prequalified vaccine [1]. The campaign was conducted using local staff, cold chain equipment, and logistics in Satyabadi Block, Odisha State, India, which is a cholera endemic area. The aim of this study was to assess the effectiveness of this vaccine under these circumstances to prevent clinically-significant cholera.

2. Methods

2.1. Ethical review

The study protocol was approved by the Department of Health and Family Welfare, State Government of Odisha; the Human Ethical Committee of the Regional Medical Research Center (RMRC) in Bhubaneswar, Odisha; the Health Ministry Screening Committee, Government of India; and the Institutional Review Board of the International Vaccine Institute in Seoul, Korea. Trained and supervised interviewers obtained verbal informed consent using a scripted and pretested message from diarrhea patients 18 years and older and from guardians of patients aged 1 to 17 years of age. Assent was also obtained from patients 12 to 17 years old. Participation or refusal to participate was recorded for each patient.

2.2. Study site

As described in detail earlier [1], this study was conducted in Satyabadi Block in Puri District, near the eastern coastal area of Odisha State. Satyabadi Block is a cholera endemic area [1–3]. The state’s infant mortality rate was 61 per 1000 live births in 2010 [4] and literacy was 91% and 78% for males and females, respectively, in 2011. The study site in Satyabadi Block includes 145 villages and hamlets encompassing approximately 50,000 residents (Fig. 1). Temperatures in summer months, March to June, average 37 °C [5]. To enumerate the study site, a de jure census was carried out from 9 February to 2 April 2011 to enumerate the regular residents of the study area, map households, assign unique identification numbers to each individual, assess household socioeconomic, water-use, sanitation, and hygiene characteristics.

2.3. Study design

To measure cholera vaccine effectiveness, we conducted a case-control study using a test-negative design [6–10]. Under this design, study participants were residents who sought care for acute diarrhea. All of these patients were tested for the infection of interest and assigned as pathogen positive cases or pathogen negative controls. Vaccine effectiveness was estimated from the ratio of the odds of vaccination among subjects testing positive to the odds of vaccination among subjects testing negative.

To validate the results obtained from the test-negative design, we also calculated vaccine effectiveness using a cohort approach. Using all residents in the study area, the protective effectiveness was estimated as the ratio of the relative risk of cholera between vaccinated and unvaccinated subjects. Since effectiveness in this approach may be influenced by differential health seeking behavior between vaccinated and un-vaccinated subjects (e.g., if vaccinated subjects were more likely to seek care), we measured the effectiveness after adjusting for the differential health seeking behavior between the two groups.

To assess whether effectiveness estimates could be attributed to study design bias, cases that are positive for a disease that should not be protected by the vaccine (e.g., Shigella infection) were compared to pathogen negatives. This design is also called a bias-indicator study [11,12]. An absence of vaccine protection from Shanchol in this analysis would provide additional evidence of an absence of bias in the study design and analysis.

2.4. Vaccine and vaccination

Single dose vials were provided by the manufacturer (Lot no: SCN006A11, Shantha Biotechnics, Hyderabad, India). Each dose of the modified killed bivalent whole-cell vaccine (Shanchol) contained 600 ELISA units of lipopolysaccharide of formalin-killed V. cholerae O1 El Tor Inaba (strain Phil 6973), 300 ELISA units of lipopolysaccharide of heat-killed V. cholerae O1 classical Ogawa (strain Cairo 50), 300 ELISA units of lipopolysaccharide of formalin-killed V. cholerae O1 classical Ogawa (strain Cairo 50), 300 ELISA units of lipopolysaccharide of heat-killed V. cholerae O1 classical Inaba (strain Cairo 48), and 600 ELISA units of lipopolysaccharide of formalin-killed V. cholerae O139 (strain 42608). Two doses were given at least 14 days apart. Individual vaccine doses were offered as per directions of the manufacturer. The vaccine cold chain was maintained as described earlier [1].

The vaccine was administered during a mass campaign held from 5 May to 25 June 2011 [1]. Ambient temperature during the campaign reached up to 42 °C on vaccination days. Vaccines were transported in temperature controlled vehicles from Hyderabad, the capital city of Andhra Pradesh to Bhubaneswar, the capital city of Odisha State and stored in a walk-in-cold room at 2 to 8 °C. Vaccines were distributed from the cold room to the vaccination sites on a daily basis throughout the campaign. Sixty-two vaccination booths, staffed by 395 trained health workers and volunteers provided vaccine. All healthy, non-pregnant women, one year of age and older were invited to participate. Pregnancy was ascertained by verbal screening using dates of last menstrual period.

A vaccination registry contained pre-printed information for each participant. The information was obtained during the census survey. This registry was also used to record doses. A vaccination card was issued to each participant at the time of administration of the first dose. Each participant was requested to bring the vaccination card at the time of second dose administration. The information from the vaccination record book was doubly entered into a password-protected data system (Microsoft Visual FoxPro 7.0).

2.5. Post vaccination surveillance

We implemented surveillance for study site residents who presented to public treatment facilities with diarrhea. The facilities included two primary health care centers (i.e., Alagum and Sukula PHC) and Sakhigopal Area Hospital in Satyabadi Block and the Infectious Disease Hospital and pediatric ward of District Headquarter Hospital located adjacent to Satyabadi Block in Puri District. The three local health facilities are marked in Fig. 1.

A diarrhea episode was defined as passage of three or more loose or liquid stools in any 24-h period within three days before presentation or one or two loose/liquid stools with any signs of dehydration according to WHO guidelines [13]. This definition was the same as that used in the five-year efficacy trial of Shanchol conducted in Kolkata, India [14]. Patients’ information was recorded in a structured questionnaire on a personnel digital assistant (PDA). Subsequent to the interview, a rectal swab was collected. Treatment was provided in accordance with national guidelines.
2.6. Laboratory confirmation

A rectal swab was obtained from each enrolled diarrhea patient, placed in capped tube containing Cary-Blair transport media, and transported at room temperature to the laboratory of the Regional Medical Research Center in Bhubaneswar. The sample reached the laboratory within 6 to 8 h. Standard methods were used for isolation of *V. cholerae* and *Shigella* [15]. For *V. cholerae*, the swab was plated onto Eiken thiosulfate citrate bile salt sucrose (TCBS) agar directly as well as after enrichment in alkaline peptone water (APW) for 4 to 6 h at 37 °C and pH 8.6. After overnight incubation, morphologically suspected colonies were tested biochemically and agglutinated with O1 polyvalent Ogawa and Inaba antisera. Non-agglutinating strains were tested with antiserum to *V. cholerae* O139 strain. The O1 isolates were further biotyped with chicken erythrocyte agglutination tests, Voges-Proskauer test and with determination of polymyxin susceptibility [16]. The genes for ctxB Classical or El Tor were determined using the mismatch amplification mutation assay (MAMA) polymerase chain reaction [17,18]. For *Shigella* isolation, the rectal swab was plated onto MacConkey (MAC) agar and Hektoen enteric (HE) agar. After 18 to 24 h incubation at 37 °C, suspected colonies were tested biochemically for identification of Shigella spp. Once Shigella was confirmed, slide agglutination tests were performed to classify by serogroup: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*.

2.7. Cases and controls

Diarrhea patients were study site residents who sought treatment at one of five health care facilities, met the definition of diarrhea, gave informed consent, were listed in the census database, and submitted a fecal specimen. Cases were patients found positive for *V. cholerae* infection. Controls were diarrhea patients negative for *V. cholerae* infection. As cholera infection does not occur throughout the year, controls were selected only during the length of the cholera season as determined by monthly case distribution, that is, from 22 April to 19 October 2013 and 31 March to 10 April 2014.

For the bias-indicator study, cases were diarrhea patients positive for *Shigella* spp. and controls were diarrhea patients negative for *Shigella* spp. In the same way that cholera cases were chosen according to the monthly distribution, controls for the *Shigella* analysis were selected from 14 April to 16 December 2013 and 29 January to 22 April 2014.

2.8. Ascertainment of vaccination history

Vaccination history was ascertained by reviewing the electronic vaccination registry to identify each diarrhea patient within the study site who was dosed. Each patient was then classified into an ordinal variable for the number of doses received (i.e., 0, 1, or 2 vaccine doses).

2.9. Variable definitions

Demographic, environmental, and economic variables and disease severity were measured and compared between cases and controls. As data suggests that Shanchol is less efficacious in young children [14], a participant’s age was calculated as age at vaccination and categorized as <15 or ≥15 years old. As years of education is thought to be inversely associated with cholera risk, we categorized heads of household as having <6 or ≥6 six years of education. Six years was the median years of education for household heads as determined during the study census. As we have done in previous studies [11,19], socioeconomic status was defined as persons owning none vs. one or more of the following luxury items: bicycle, television or motorbike. For socioeconomic status, we also categorized households as having only one room vs. those with two or more rooms. Higher cholera risk is also found among persons having inadequate sanitation. Sanitation was defined as persons who used a constructed latrine compared to those who used open field for defecation. Access to clean water was defined as those who had...
tap, well, or hand pump water vs. those who used other resources such as pond or surface water. As we had noted previously, cholera cases were more likely to seek care at a distant health care facility [14], thus we evaluated the proportion of cases and controls traveling >2.5 vs ≤ 2.5 km to obtain treatment for diarrhea. The median distance from a household to the health care facility was 2.5 km as determined during the baseline census. Disease severity was measured as none vs. some or severe based on WHO criteria [13].

3. Analysis

To identify independent variables that were predictive of V. cholerae-associated diarrhea, we calculated the prevalence of each variable among cases and controls. We then obtained a p-value for the statistical difference, if any, between cases and controls by fitting the variable into a regression model using a logit function and employing generalized estimating equations (GEE) [20]. GEE were used to adjust for potential nonindependence of observations obtained from diarrhea patients seeking medical care at the same health care facility.

Subsequently, we calculated the crude odds ratio for cholera-associated diarrhea among cases and controls by adding indicator variables for vaccine doses (2 doses, 1 dose, vs. 0 doses) to a logistic regression model using GEE as above. To obtain an adjusted odds ratio for cholera risk, we simultaneously added all independent variables with p-value less than 0.10 as well as vaccine doses to a single multivariate model. The adjusted odds ratio was obtained from these models by exponentiating the coefficients for cholera vaccination. Ninety-five percent confidence intervals (95% CIs) were calculated using model coefficients and empirical standard errors and exponentiating those results. The percent adjusted vaccine effectiveness was then calculated as one minus the odds ratio times 100. We also tested for a simple linear trend by ordered vaccine doses by adding statistically significant covariates and a single ordinal variable for doses to a logistic regression model using GEE.

To validate results of the case-control analysis, we assembled a cohort of all area residents and followed them post vaccination through the end of study year two. In the cohort analysis, we calculated the incidence rate for cholera (cases/baseline population) and non-cholera diarrhea by vaccine dose (i.e., two, one, no dose). We assumed that the incidence rate for non-cholera diarrhea would be the same for persons receiving two, one or no vaccine doses, if the vaccine had no impact on non-cholera diarrhea (as expected) and there was no difference in health care use by vaccine dose. If we did find a difference in incidence of non-cholera diarrhea by vaccine dose, then that difference would suggest that there was an association between vaccine dose and health seeking behavior and we needed to adjusted vaccine effectiveness for health seeking behavior. The factor for adjustment (f), was calculated by

\[ f = \frac{nIR_e}{nIR_0} \]

\( nIR_e = \) incidence rate of non-cholera diarrhea among vaccinees (two-dose recipients); \( nIR_0 = \) incidence rate of non-cholera diarrhea among non-dose recipients.

Thus, the adjusted protective efficacy (aPE) in cohort approach was calculated by

\[ aPE = 1 - \left( \frac{cIR_e}{cIR_0} \times f \right) \times 100\% \]

\( cIR_e = \) incidence rate of cholera among vaccinees; \( cIR_0 = \) incidence rate of cholera among non-dose recipients.

In the bias indicator analysis, differences between cases and controls as well as crude and adjusted odds ratio were calculated in the same manner as for the statistical evaluation of anti-V. Cholerae vaccine.

All tests were two-tailed, and statistical significance was set at \( p < 0.05 \). SAS version 9.03 (SAS Institute Inc., North Carolina, USA) was used for all analyses.

4. Results

In the study area, of 51,488 eligible residents, 31,552 individuals received at least one dose and 23,751 residents received two doses of the vaccine. During the first study year, there were only four diarrhea patients infected with V. cholerae and they were excluded from the study. In the second year, we identified 44 V. cholerae-associated cases and 366 V. cholerae-negative diarrhea controls. Of the 44 cases, 18 received no vaccine, nine received one dose, and 17 received two doses. For controls, 73, 59, and 234 received no, one, and two vaccine doses, respectively. None of the cases or controls were reported to have received a partial dose or spit out vaccine. There were no repeat treatment visits for the same illness. Of the 44 V. cholerae cases, all were O1 Ogawa of which 34 were El Tor Variant and 10 were Hybrid (El Tor/Classical) biotypes. Forty patients among cholera cases and 312 patients among controls were either some or severely dehydrated yielding no significant difference of the dehydration status between these two groups (\( p = 0.24 \)). In contrast, we observed a significantly higher dehydration (\( p < 0.0001 \)) for the bias indicator study (Shigella versus non-Shigella).

The cholera cases and controls were comparable for most population characteristics (Table 1). Still, cases were less likely to be under 15 years of age than controls (15.9% vs. 27.0%, \( p = 0.0011 \)) and cases were more likely to have traveled further than 2.5 km to seek health care than controls (47.7 vs. 27.0, \( p = 0.0012 \)). The adjusted vaccine effectiveness for residents who received two doses compared to those who received no vaccine was 69.0% (95% CI: 14.5% to 88.8%) (Table 2). Residents who received only one dose had 32.5% (95% CI: −318.0% to 89.1%) protection but this was not statistically significant. However, a trend test for effectiveness by dose was statistically significant (\( p = 0.0091 \)).

For the bias indicator analysis, there were 19 laboratory-confirmed Shigella-associated cases and 606 non-Shigella cases (Tables 1 and 2). Among the Shigella positive cases, 16 were S. flexneri, two were S. boydii, and one S. dysenteriae. Shigella-associated cases were more likely to be male than female, (73.7% vs. 57.7%, \( p < 0.0001 \)) and to reside further from a health care facility (57.9% vs. 31.0%, \( p = 0.0012 \)). There was no statistically significant association between Shigella risk and cholera vaccine doses. The trend test by dose was not statistically significant (\( p = 0.98 \)).

In cohort analysis, we observed the incidence rate of non-cholera diarrhea among two-dose recipients was 2.7 times higher than that among non-dose recipients (Table 3), which indicates heterogeneity in healthcare seeking behavior between vaccinees and non-vaccinees in that setting. This had led to a very low protective efficacy when performing a classical analysis (21%; 95% CI: −54–59; \( p = 0.49 \)). We, therefore, calculated the adjusted incidence rate of cholera after controlling for the health seeking behavior, which yielded 2.42/100,000 population, and provided 70% protective effectiveness (95% CI: 48–83; \( p < 0.0001 \)).

5. Discussion

A controlled clinical trial conducted in Kolkata, India suggests that Shanchol, a locally-licensed, WHO-prequalified oral cholera vaccine, provides 65% protection against clinically-significant cholera over five years. To evaluate the feasibility of deploying this vaccine in a non-experimental setting, we conducted and previously reported on a vaccine campaign with Shanchol using local government health personnel and infrastructure in a rural cholera
Table 1
Socio-demographic characteristics and disease severity of Cholera cases, Shigella cases, and controls.

<table>
<thead>
<tr>
<th></th>
<th>Cholera (n=44)</th>
<th>Controls (n=366)</th>
<th>p Value</th>
<th>Shigella (n=19)</th>
<th>Controls (n=606)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Male</td>
<td>21</td>
<td>47.7</td>
<td>187</td>
<td>51.1</td>
<td>0.129</td>
<td>14</td>
</tr>
<tr>
<td>Age at vaccination (May 5, 2011) &lt; 15 years</td>
<td>7</td>
<td>15.9</td>
<td>99</td>
<td>27.0</td>
<td>0.001</td>
<td>6</td>
</tr>
<tr>
<td>Household head had six years of education or less</td>
<td>32</td>
<td>72.7</td>
<td>279</td>
<td>76.2</td>
<td>0.646</td>
<td>17</td>
</tr>
<tr>
<td>Only one room in house</td>
<td>14</td>
<td>31.8</td>
<td>103</td>
<td>28.0</td>
<td>0.510</td>
<td>7</td>
</tr>
<tr>
<td>Own bicycle, television, or motorbike</td>
<td>34</td>
<td>77.3</td>
<td>281</td>
<td>76.8</td>
<td>0.953</td>
<td>15</td>
</tr>
<tr>
<td>Use open field as toilet</td>
<td>40</td>
<td>90.9</td>
<td>325</td>
<td>88.8</td>
<td>0.543</td>
<td>19</td>
</tr>
<tr>
<td>Use own tap, well, or hand pump water</td>
<td>8</td>
<td>18.2</td>
<td>42</td>
<td>11.5</td>
<td>0.082</td>
<td>1</td>
</tr>
<tr>
<td>Longer than median distance from the household to the nearest health facility (&gt;2.5 km)</td>
<td>21</td>
<td>47.7</td>
<td>99</td>
<td>27.0</td>
<td>0.001</td>
<td>11</td>
</tr>
<tr>
<td>Some to severe dehydration</td>
<td>40</td>
<td>90.9</td>
<td>312</td>
<td>85.2</td>
<td>0.239</td>
<td>18</td>
</tr>
</tbody>
</table>

Note: The simple linear trend of cholera vaccine dose and vaccine effectiveness is inversely associated (p=0.0091), but vaccine dose is not associated (p=0.9833) with Shigella infection.

* For cholera, results are adjusted for age at vaccination, sex, and distance to the nearest health facility and for Shigella, adjusted for the same variables plus disease severity.

-endemic area of India [1]. The campaign included almost 400 government health workers (with one day training) and volunteers working at 62 vaccination posts placed throughout the area of almost 52,000 residents. From May to June 2011, the hottest season of the year, non-pregnant residents one year of age and above were offered two vaccine doses, 14 days apart. Sixty-one percent of the population received one or two vaccine doses. Despite the difficult circumstances, the investigators from that study concluded that the vaccine campaign was realistic and affordable.

In this study, we present findings assessing the effectiveness of this oral cholera vaccine campaign to prevent clinically-significant cholera. Our results suggest that the vaccination campaign was successful. More than two in three vaccinees receiving two vaccine doses were protected from clinically significant cholera. The results of our study also hinted that one dose reduces severe cholera cases by nearly one-third. A study conducted in Zanzibar using Dukoral, a product similar to Shanchol [11], and an effectiveness study in Guinea of persons who received incomplete vaccination (i.e., one dose or if one or both dose spat out or vomited [21]) demonstrated similar levels of protection for a single dose. Combined, these data suggest that there is a protective benefit from a single vaccine dose.

We believe these results to be robust. Since we considered all diarrheal patients coming from our study area to the treatment sites, the test-negative design minimized the effects of healthcare seeking behavior on vaccine effectiveness. This is in contrast to the results we observed using in the cohort analysis. Our analysis also indicates that classical way of evaluating vaccine effectiveness using cohort or case-control models may seriously underestimate vaccine effectiveness unless healthcare seeking behavior of the study population are addressed analytically.

The test-negative design used in this study is predicated on the assumption that the intervention has no effect on other non-targeted etiologies resulting in similar symptomology and there is high laboratory test specificity [7]. To the best of our knowledge, there is no indication that this vaccine would provide protection against other diarrheal etiologies. And, as our data shows, we did not detect protection against Shigella-associated infections. With regards to test specificity, while bacterial culture is the gold standard for cholera diagnosis, there is an indication that the specificity is marginally lower when compared to diagnostics that include bacterial culture and polymerase chain reaction methods [22]. Given this, the estimated effectiveness in this study may vary from the true effectiveness. However, this would be the case for all Shanchol trials and effectiveness studies as PCR have not been widely used in trials to screen for V. cholerae infection.

Table 2

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Vaccination status</th>
<th>Cases</th>
<th>Odds</th>
<th>Crude odds ratio (95%CI)</th>
<th>Adjusted odds ratio* (95%CI)</th>
<th>% Vaccine effectiveness (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholera</td>
<td>0 dose</td>
<td>18</td>
<td>0.25</td>
<td>1</td>
<td>1</td>
<td>32.5 (‐318.0, 89.1)</td>
</tr>
<tr>
<td></td>
<td>1 dose</td>
<td>9</td>
<td>0.15</td>
<td>0.605 (0.116, 3.157)</td>
<td>0.675 (0.109, 4.180)</td>
<td>60.0 (14.5, 88.8)</td>
</tr>
<tr>
<td></td>
<td>2 doses</td>
<td>17</td>
<td>0.07</td>
<td>0.287 (0.110, 0.749)</td>
<td>0.310 (0.112, 0.855)</td>
<td>39.6 (‐273.3, 90.2)</td>
</tr>
<tr>
<td>Shigella</td>
<td>0 dose</td>
<td>5</td>
<td>0.04</td>
<td>1</td>
<td>1</td>
<td>–10.6 (‐218.3, 61.6)</td>
</tr>
<tr>
<td></td>
<td>1 dose</td>
<td>2</td>
<td>0.02</td>
<td>0.568 (0.092, 3.498)</td>
<td>0.604 (0.098, 3.733)</td>
<td>39.6 (‐273.3, 90.2)</td>
</tr>
<tr>
<td></td>
<td>2 doses</td>
<td>12</td>
<td>0.03</td>
<td>0.951 (0.328, 2.785)</td>
<td>1.106 (0.384, 3.183)</td>
<td>–10.6 (‐218.3, 61.6)</td>
</tr>
</tbody>
</table>

Note: The simple linear trend of cholera vaccine dose and vaccine effectiveness is inversely associated (p=0.0091), but vaccine dose is not associated (p=0.9833) with Shigella infection.

* For cholera, results are adjusted for age at vaccination, sex, and distance to the nearest health facility and for Shigella, adjusted for the same variables plus disease severity.

Table 3
Incidence rate for cholera and non-cholera diarrhea and protective efficacy of the vaccine using cohort approach.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Vaccination status</th>
<th>Population</th>
<th>Cases</th>
<th>Incidence rate/100,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholera</td>
<td>0 dose</td>
<td>19,936</td>
<td>18</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>1 dose</td>
<td>7,801</td>
<td>9</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>2 doses</td>
<td>23,751</td>
<td>17</td>
<td>0.72</td>
</tr>
<tr>
<td>Non-cholera diarrhea</td>
<td>0 dose</td>
<td>19,936</td>
<td>73</td>
<td>3.66</td>
</tr>
<tr>
<td></td>
<td>1 dose</td>
<td>7,801</td>
<td>59</td>
<td>7.56</td>
</tr>
<tr>
<td></td>
<td>2 doses</td>
<td>23,751</td>
<td>234</td>
<td>9.85</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Protective efficacy (%)</th>
<th>Crude estimate (95 CI, p-value)</th>
<th>Adjusted estimate* (95 CI, p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholera</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Non-cholera diarrhea</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* The adjustment was made considering that vaccinees (2-dose recipients) and non-vaccinees would have similar rates for the non-cholera diarrhea, if the health seeking behavior had been same for both the groups. And, in that case, the incidence rate of cholera among non-vaccinees would have been 2.42/100,000. Thus, the adjusted protective efficacy is calculated by (1 – 0.72/2.42) x 100%.
There were several other issues to consider when evaluating the results of our study. Although we validated the test-negative design using a cohort analysis which addressed the healthcare seeking behavior of the study population, this design has not been validated against the results of randomized controlled trials of cholera vaccines. Such an analysis would hopefully add additional credence to our results. Our estimate of vaccine effectiveness could be biased if disease severity differed for the vaccinated and unvaccinated [23] and affected health-seeking behavior. For cholera, we did not find significant differences in disease severity between the vaccinated and unvaccinated. There is an opportunity for selection bias in studies of vaccine effectiveness if only a subgroup of all cholera patients are tested for infection. The test–negative design minimizes this possibility as all patients seeking care for diarrhea and meeting case definition are cultured for *V. cholerae* [8,23]. Like other observational study designs, difference in the risk of disease could bias our estimates if the risk is not the same for vaccinees and non-vaccinees. We have tried to ensure for comparability between groups by including variables for sanitation and water use into our statistical models and have limited our analysis to calendar time when residents were at risk of cholera. We were underpowered to detect a statistically significant effect from a single dose. However, as noted earlier, the trend test was significant. Still, the question concerning the efficacy of a single vaccine dose will be best answered by an individually randomized, controlled trial being conducted in Bangladesh by icddr,b and the International Vaccine Institute, Republic of Korea. We were unable to present age-specific estimates for protection as the study was not sufficiently powered for that analysis. However, age-specific effectiveness has been observed in other oral cholera vaccine studies, where protection in preschool children was less relative to older children and adults [14]. Finally, the analysis of the cohort approach suggest that the test–negative design is free from bias due to healthcare seeking behavior, as the adjusted rate of the cohort approach is similar to our results. Still, if there is any effect from differential care seeking behavior by vaccinated and unvaccinated, then our estimates would be considered as conservative as non-vaccinees were less likely to visit in our target hospitals than that by vaccinees.

In summary, cholera remains a serious public health problem in many developing nations. In endemic countries of Asia and Africa, an estimated 2.8 million cases and 91,000 deaths occur each year [24]. Cholera prevention has been based on provision of safe water, sanitation, and treatment with rehydration therapy and antibiotics. In 2012, researchers added to the anti-cholera armamentum by reporting that a clinical trial in Kolkata, India suggests that an oral cholera vaccine afforded 65% protection against severe disease over five years for non-pregnant residents, one year of age and older cholera [14]. That vaccine is now prequalified, that is, approved by the World Health Organization for purchase by United Nations agencies. In 2014, researchers also showed that this vaccine afforded substantial protection, 86.6%, when used at the onset of a cholera outbreak in Guinea [21]. In this study, we observed protection against clinically-significant *V. cholerae* infection in a rural, cholera endemic area of India. This study supports the use of Shanchol in government immunization programs and further suggests that the vaccine should be made widely available to health departments throughout India as well as other cholera endemic countries.

**Author contribution**

Conceived and designed the study: TFW, SKK, VAY, MA, BS. Performed the study: TFW, SKK, VVM, ASK, YAY, PB, SBR, AB, BS. Conducted the statistical analysis: TFW, VVM, YAY, MA. Conducted laboratory analysis: HKK. Contributed to writing the manuscript: TFW, SKK, VVM, YAY.

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**Conflict of interest statement**

None.

**References**


