Feasibility and effectiveness of oral cholera vaccine in an urban endemic setting in Bangladesh: a cluster randomised open-label trial


Summary

Background Cholera is endemic in Bangladesh with epidemics occurring each year. The decision to use a cheap oral killed whole-cell cholera vaccine to control the disease depends on the feasibility and effectiveness of vaccination when delivered in a public health setting. We therefore assessed the feasibility and protective effect of delivering such a vaccine through routine government services in urban Bangladesh and evaluated the benefit of adding behavioural interventions to encourage safe drinking water and hand washing to vaccination in this setting.

Methods We did this cluster-randomised open-label trial in Dhaka, Bangladesh. We randomly assigned 90 clusters (1:1:1) to vaccination only, vaccination and behavioural change, or no intervention. The primary outcome was overall protective effectiveness, assessed as the risk of severely dehydrating cholera during 2 years after vaccination for all individuals present at time of the second dose. This study is registered with ClinicalTrials.gov, number NCT01339845.

Findings Of 268 896 people present at baseline, we analysed 267 270: 94 675 assigned to vaccination only, 92 539 assigned to vaccination and behavioural change, and 80 056 assigned to non-intervention. Vaccine coverage was 65% in the vaccination only group and 66% in the vaccination and behavioural change group. Overall protective effectiveness was 37% (95% CI lower bound 18%; p=0.002) in the vaccination group and 45% (95% CI lower bound 24%; p=0.001) in the vaccination and behavioural change group. We recorded no vaccine-related serious adverse events.

Interpretation Our findings provide the first indication of the effect of delivering an oral killed whole-cell cholera vaccine to poor urban populations with endemic cholera using routine government services and will help policy makers to formulate vaccination strategies to reduce the burden of severely dehydrating cholera in such populations.

Funding Bill & Melinda Gates Foundation.

Introduction Cholera is a major global public health problem with no evidence of decline in recent years. It is also a major cause of morbidity and mortality in low-income countries, including Bangladesh, which has an estimated 300 000 cases and 4500 deaths each year.1–4 30–40% patients with cholera have severe dehydration, which can be fatal if not promptly treated with intravenous fluids.5 The financial cost of cholera to patients in Bangladesh can be very high.6

In 2001, WHO prequalified the licensed killed oral cholera vaccine Dukoral (Valneva; Stockholm, Sweden) for purchase by UN organisations.7 However, its use has been limited, partly because of its cost and the logistical challenges of its administration. The vaccine is mainly used by travellers from high-income countries who visit low-income countries.8 A killed whole-cell oral cholera vaccine was transferred from VaBiotech in Vietnam to Shantha Biotechnics in India, where it was licensed in 2009 as Shanchol. It was prequalified by WHO in 2011, on the basis of a large-scale field trial9 in Kolkata, which showed that the vaccine was safe and conferred 67% protection at 3 years after vaccination. The investigators later reported sustained 65% cumulative efficacy at 5 years after vaccination.10 The question remained of whether this vaccine would work equally well when delivered under realistic programme conditions in other populations at high risk for cholera.11 Cholera is endemic in Bangladesh, and the entire population is at risk. Outbreaks of cholera in Dhaka, Bangladesh spike in spring and autumn,12 with additional outbreaks during floods.13 Controlling cholera is therefore, a high priority for the Government of Bangladesh, and inclusion of an oral cholera vaccine in its public health programme is being considered.14 The decision to do so depends on the evidence of its feasibility, effectiveness, and cost-effectiveness when delivered in a public health setting. For this reason, we did the Introduction of Cholera Vaccine in Bangladesh study to assess the acceptability, programmatic feasibility, and protective effectiveness of Shanchol against severely dehydrating cholera in an urban setting with high rates of cholera. We also assessed whether an intervention to promote handwashing and home treatment of drinking water added to the effect of Shanchol.
**Methods**

**Study design and participants**

We did this cluster-randomised controlled trial to assess overall protection\(^9\) conferred by a two-dose regimen of Shanchol vaccine (Shantha Biotechnics-Sanofi) against hospital admission for severely dehydrating cholera when given to non-pregnant individuals aged 1 year and older in a cholera-endemic, urban population in Dhaka, Bangladesh. We targeted residents classified as high risk\(^10\) by virtue of socioeconomic status and sanitation and hygiene (appendix). In creating the clusters, we tried to ensure that the size of population in each cluster was balanced. The average cluster population size was 2988 (range 2288–4299). There was a buffer zone of at least 30 m between clusters to minimise spillover of the behavioural intervention to clusters not assigned to this intervention. The appendix contains descriptions of field sites and the geographically referenced census done for the study.

The study protocol was approved by the research review committee and the ethics review committee of the icddr,b, Dhaka, Bangladesh and the institutional review board of the International Vaccine Institute. Written informed consent was obtained from residents aged 18 years or older and from the parents or guardians of residents aged 1–17 years. Additional assent was obtained from residents aged 12–17 years. An independent data and safety monitoring board reviewed the study protocol, assessed adverse events, and approved freezing of data and the analysis plan before the analysis.

**Randomisation and masking**

We randomly assigned (with a computer-generated randomisation sequence) 90 geographical clusters to one of three groups (1:1:1): vaccination only, vaccination and a behaviour change intervention to encourage hand-washing and treatment of drinking water with chlorine (appendix), or non-intervention.

Before randomisation, we stratified clusters blocked into two categories: those with lower than median distance (in a straight line) to the nearest icddr,b hospital (Dhaka Hospital or Mirpur Treatment Centre) and those with median or higher distance to the hospital. All trial participants and investigators were aware of group assignment.

**Procedures**

Patients who were assigned to vaccination received two doses of the bivalent whole-cell inactivated vaccine Shanchol at an interval of at least 14 days.\(^6\) The first dose was given between Feb 17, and April 16, 2011, and the second dose was given between March 15, and April 16, 2011 (appendix). Zero time was defined as the date of the first dose for vaccine recipients, and as the median date of the first dose for non-vaccinated participants (appendix).

A non-governmental organisation with experience in community interventions delivered the behaviour change intervention. Community health workers offered a hand-washing station free of charge to household compounds (groups of homes sharing a common open space) and located it in a convenient place for compound residents to access.\(^11\) Each housing compound was given a bottle of soapy water and an initial sachet of soap (also free of charge) to demonstrate its use. Handwashing promotion began 2 months after vaccination. 4 months after

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**Figure 1:** Study site showing the 90 clusters allocated to the three groups of the trial
vaccination, trained community health workers returned to each compound to promote the use of a liquid chlorine-based treatment for household drinking water. Each drinking water station included a chlorine dispenser. These interventions were continued up to August, 2013 (appendix).

Passive surveillance for diarrhoeal disease was done at the two icddr,b hospitals and ten other hospitals serving the study population (appendix). Patients from the study area were identified by household identification cards and an on-site computer database. Physicians examined and assessed the patients. Surveillance staff entered data onto structured surveillance forms and obtained faecal specimens, which were transported to the central laboratory in Cary-Blair media. The sensitivity of the surveillance system was maximised by including all known sources of medical care for severe diarrhoea in the study catchment area. Specificity was maximised by both culture and identity-confirmation of all cases via home checks after discharge from hospital.

![Figure 2: Trial profile](image)

*Median date of second dose for recipients of one dose or no doses.

<table>
<thead>
<tr>
<th>Vaccination only group (n=94 675)</th>
<th>Vaccination and behaviour change group (n=92 539)</th>
<th>Non-intervention group (n=80 056)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (SD; years)</td>
<td>23.9 (15.8)</td>
<td>23.9 (15.7)</td>
</tr>
<tr>
<td>Male participants</td>
<td>45 677 (48.2%)</td>
<td>45 164 (48.8%)</td>
</tr>
<tr>
<td>Diarrhoea within previous 6 months</td>
<td>12 657 (13.4%)</td>
<td>12 143 (13.1%)</td>
</tr>
<tr>
<td>Diarrhoea within previous 48 h</td>
<td>11 771 (12.1%)</td>
<td>11 75 (12.1%)</td>
</tr>
<tr>
<td>Mean time living in the area (SD; months)</td>
<td>67.9 (116.9)</td>
<td>61.8 (110.5)</td>
</tr>
<tr>
<td>Lived in study area for less than 1 year</td>
<td>43 174 (45.6%)</td>
<td>42 104 (45.5%)</td>
</tr>
<tr>
<td>Live in own house</td>
<td>19 892 (21.0%)</td>
<td>18 945 (20.5%)</td>
</tr>
<tr>
<td>Households using safe water source (household tap)</td>
<td>4493 (4.7%)</td>
<td>5083 (5.5%)</td>
</tr>
<tr>
<td>Live in a household with a specific place for waste disposal</td>
<td>76 146 (80.4%)</td>
<td>77 634 (81.9%)</td>
</tr>
<tr>
<td>Live in a household with a flushing toilet</td>
<td>65 499 (69.2%)</td>
<td>74 260 (80.2%)</td>
</tr>
<tr>
<td>Live in a household with a concrete roof</td>
<td>82 263 (87.9%)</td>
<td>78 233 (84.5%)</td>
</tr>
<tr>
<td>Live in a household with only one room</td>
<td>78 173 (82.6%)</td>
<td>74 522 (80.5%)</td>
</tr>
<tr>
<td>Sharing kitchen with other households</td>
<td>82 207 (86.8%)</td>
<td>81 486 (90.2%)</td>
</tr>
<tr>
<td>Live in a household sharing water source with others</td>
<td>61 378 (64.8%)</td>
<td>65 514 (70.8%)</td>
</tr>
<tr>
<td>Live in a household using treated water (boiled, filtered, or chemical treatment)</td>
<td>48 512 (51.2%)</td>
<td>53 525 (57.8%)</td>
</tr>
<tr>
<td>Live in a household that knows about cholera vaccine</td>
<td>5944 (6.3%)</td>
<td>7895 (8.9%)</td>
</tr>
<tr>
<td>Live in household close (less than the median distance) to the nearest icddr,b hospital</td>
<td>44 246 (46.7%)</td>
<td>45 708 (49.4%)</td>
</tr>
<tr>
<td>Mean number of individuals per household (SD)</td>
<td>4.7 (2.0)</td>
<td>4.7 (2.9)</td>
</tr>
<tr>
<td>Median distance to the nearest icddr,b hospital (IQR)</td>
<td>1792 (1121-2266)</td>
<td>1792 (1307-2306)</td>
</tr>
<tr>
<td>Mean percentage of children younger than age 5 years in the cluster (SD)</td>
<td>10.0% (1.1)</td>
<td>10.0% (0.9)</td>
</tr>
<tr>
<td>Mean percentage of male participants in the cluster (SD)</td>
<td>48.3% (1.3)</td>
<td>48.8% (1.3)</td>
</tr>
<tr>
<td>Mean percentage of individuals using safe water source in the cluster (SD)</td>
<td>4.7% (4.4)</td>
<td>5.5% (4.7)</td>
</tr>
<tr>
<td>Mean percentage of individuals living in their own house in the cluster (SD)</td>
<td>21.0% (17.8)</td>
<td>20.4% (18.0)</td>
</tr>
<tr>
<td>Mean percentage of individuals using specific place for waste disposal in the cluster (SD)</td>
<td>80.4% (25.6)</td>
<td>81.9% (20.9)</td>
</tr>
<tr>
<td>Mean percentage of individuals using flushing toilet in the cluster (SD)</td>
<td>69.2% (28.2)</td>
<td>80.2% (15.3)</td>
</tr>
</tbody>
</table>

Table 1: Baseline characteristics for the analysis of overall protection.
A diarrhoeal visit was defined as having, in the 24 h before presentation, three or more loose stools or, one to two or indeterminate number of loose stools with evidence of dehydration according to WHO criteria.\textsuperscript{12} The onset of a diarrhoeal visit was the day on which the patient first reported loose or liquid stools. Diarrhoeal visits for which the date of onset was within 7 days of the date of discharge for the previous visit were grouped into the same diarrhoeal episode, with the onset of the episode corresponding to the onset of the initial constituent diarrhoeal visit.

Surveillance for adverse events was done for 14 days after each dose in the treatment centres. A serious adverse event was defined according to Khan and colleagues.\textsuperscript{18} The causal relationship of adverse events to vaccination was judged by the study physicians.

Specimens were tested for \textit{Vibrio cholerae} including O1 and O139 serogroups and Inaba and Ogawa serotypes. Biotype was ascertained for all O1 isolates, and the genetically encoded biotype of the cholera toxin was identified as previously described.\textsuperscript{13} Specimens were also tested for enterotoxigenic \textit{Escherichia coli} by multiplex PCR and further confirmed by immunodiagnostic methods as previously described.\textsuperscript{14–16}

A cholera episode was defined as a diarrhoeal episode with no passage of bloody stools, if a faecal specimen yielded \textit{V. cholerae} O1 or O139 and a follow-up check at home, done within 7 days of discharge, confirmed that the participant had visited the treatment centre for diarrhoea on the recorded date of presentation. An enterotoxigenic \textit{E. coli} diarrhoeal episode was defined as a diarrhoeal episode in which no component visit yielded \textit{V. cholerae} O1 or O139 and a faecal specimen yielded enterotoxigenic \textit{E. coli}.

Outcomes
The primary outcome was overall protective effectiveness of the vaccine assessed by the risk of severely dehydrating cholera during 2 years of follow-up, defined by the presence of at least two of the following signs and symptoms: sunken eyes, dry tongue, thirst, irritable condition, less active than usual, skin-pinch goes back slowly, low volume of radial pulse along with inability to drink, or uncountable or absence of radial pulse. Enterotoxigenic \textit{E. coli} diarrhoea was a secondary outcome, studied to assess whether the analysis of vaccine protection against cholera was biased by the absence of allocation masking and whether the behaviour change intervention conferred protection against enterotoxigenic \textit{E. coli}. For the main analysis, we included all residents present at the time of the second dose, irrespective of their vaccination status or eligibility for vaccination. In another secondary analysis, we assessed the total effectiveness of the vaccine for all two-dose recipients from the vaccine only group and vaccine plus behaviour change group and all participants aged 1 year or older in the non-intervention group.

Statistical analysis
We calculated sample size by methods described elsewhere.\textsuperscript{8} We calculated the intra-cluster correlation for cholera hospital admissions for 2008, and 2009. We assumed 65\% efficacy and 65\% coverage, yielding 42\% overall protective efficacy, with a one-sided test (\(\alpha=0.05\)), 80\% power, incidence of 1-6 cases per 1000 people per year, 25\% yearly attrition, and 2 years of post-vaccination surveillance. On the basis of these assumptions, we calculated that we would need 236,340 participants (78780 in each group).

To test for bias,\textsuperscript{9} we assessed the protection conferred by the vaccine against enterotoxigenic \textit{E. coli} diarrhoea, which should not be protected by the vaccine but is clinically similar and transmitted in a similar fashion to cholera. An absence of protection against enterotoxigenic \textit{E. coli} diarrhoea would suggest that bias is an unlikely explanation for apparent vaccine protective effectiveness.

The follow-up start date in the vaccinated clusters was 14 days after the second dose for two-dose recipients, and 14 days after the median date of the second dose in the cluster for others. The follow-up start date for members of the non-vaccinated clusters was 14 days after the median date of the second dose in the vaccination cycle for the
nearest vaccinated cluster. Deferring the follow-up start date until 14 days after the second dose was based on what is presumed to be the optimum time needed for development of a good immune response to vaccination, an assumption made in assessing protective efficacy of this and other oral cholera vaccines. We considered cholera episodes up to 716 days after the start of follow-up.

We did survival analyses censoring individuals who died or migrated out before the end of follow-up. We assessed time-to-event by Kaplan-Meier analysis and then fitted unadjusted and adjusted Cox proportional hazards models after testing for multicollinearity and heterogeneity of vaccine protection in these subgroups by analysing two-way interaction terms between the assigned group and subgroup variables in the models. Our protocol specified the use of one-tailed p values and CIs, because we had no reason to suspect that vaccinated clusters would have a higher risk of cholera than unvaccinated clusters. To enhance the interpretability of our primary analyses for readers who prefer two-tailed tests, we provide both one-tailed and two-tailed p values and CIs for the primary analyses. The threshold of significance for individual estimates of protective effectiveness was p less than 0.05 (one-tailed) with corresponding one-sided 95% CI; and that for assessing heterogeneity of protective effectiveness between subgroups was p less than 0.05 (two-tailed) with corresponding two-tailed 95% CIs. We did the statistical analyses with SAS (version 9.3).

Role of the funding source

The study was registered at ClinicalTrials.gov number, NCT01339845.

Vaccination only group

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Non-intervention</th>
<th>Overall effectiveness (crude estimate)</th>
<th>Overall effectiveness (adjusted estimate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants (n)</td>
<td>Cholera episodes (n)</td>
<td>Person-days of follow-up</td>
<td>Incidence (cases per 100 000 person-days; 95% CI)</td>
</tr>
<tr>
<td>All individuals</td>
<td>94675</td>
<td>65</td>
<td>41 809 947</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-4.9</td>
<td>9440</td>
<td>8</td>
<td>39 980 993</td>
</tr>
<tr>
<td>5-14.9</td>
<td>19 393</td>
<td>9</td>
<td>9 011 812</td>
</tr>
<tr>
<td>≥15</td>
<td>65 842</td>
<td>51</td>
<td>28 800 042</td>
</tr>
<tr>
<td>Year of follow-up</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>94675</td>
<td>41</td>
<td>25 176 059</td>
</tr>
<tr>
<td>Second</td>
<td>53 170</td>
<td>24</td>
<td>16 633 888</td>
</tr>
</tbody>
</table>

(Table 2 continues on next page)
### Table 2: Occurrence of cholera with severe dehydration and cumulative overall protection by the killed oral cholera vaccine during 2 years of follow-up

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Year of follow-up</th>
<th>Intervention</th>
<th>Non-intervention</th>
<th>Overall effectiveness (crude estimate)</th>
<th>Overall effectiveness (adjusted estimate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Participants (n)</td>
<td>Cholera episodes (n)</td>
<td>Person-days of follow-up</td>
<td>Incidence (cases per 100 000 person-days; 95% CI)</td>
</tr>
<tr>
<td>1·0–4·9</td>
<td>First</td>
<td>92 539</td>
<td>55</td>
<td>40 553 587</td>
<td>0·1356 (0·1041 to 0·1766)</td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>53 501</td>
<td>21</td>
<td>15 893 093</td>
<td>0·1231 (0·0826 to 0·1827)</td>
</tr>
</tbody>
</table>

PE=protective effectiveness. *For the overall interaction between the assigned group and subgroup variables in the model. †Adjusted for closer distance from the household to the nearest icddr,b hospital, age at zero time (years), individuals having reported diarrhoea within 6 months at the time of household registration, individuals living in a household having one room only, percentage of individuals in the cluster living in their own house, and percentage of individuals in the cluster having a flushing toilet. ‡‡‡Adjusted for closer distance from the household to the nearest icddr,b hospital, individuals having reported diarrhoea within 6 months at the time of household registration, individuals living in a household having one room only, percentage of individuals in the cluster living in their own house, and percentage of individuals in the cluster with a flushing toilet, and individuals living in a household using safe water source. §§Adjusted for closer distance from the household to the nearest icddr,b hospital, individuals having reported diarrhoea within 6 months at the time of household registration, individuals living in a household having one room only, percentage of individuals in the cluster living in their own house, and percentage of individuals in the cluster living in their own house. ¶¶Adjusted for closer distance from the household to the nearest icddr,b hospital, individuals having reported diarrhoea within 6 months at the time of household registration, individuals living in a household having one room only, percentage of individuals in the cluster living in their own house, and percentage of individuals in the cluster sharing water source with others, and individuals living in a household using safe water source. ††Adjusted for closer distance from the household to the nearest icddr,b hospital, individuals having reported diarrhoea within 6 months at the time of household registration, individuals living in a household having one room only, percentage of individuals in the cluster living in their own house, and individuals living in a household using safe water source. ## Adjusted for closer distance from the household to the nearest icddr,b hospital, individuals having reported diarrhoea within 6 months at the time of household registration, individuals living in a household knowing about cholera vaccine, individuals living in a household having one room only, percentage of individuals in the cluster living in their own house, and percentage of individuals in the cluster living in their own house, and individuals living in a household using safe water source. ¶¶¶Adjusted for closer distance from the household to the nearest icddr,b hospital, individuals having reported diarrhoea within 6 months at the time of household registration, individuals living in a household knowing about cholera vaccine, individuals living in a household having one room only, percentage of individuals in the cluster living in their own house, and individuals living in a household using safe water source.}

**Results**

There were 267 270 participants in the 90 clusters who were present at the time of the second dose (figures 1, 2), and vaccine coverage (recipients of two doses) was 65% (61 970/95 115) in the vaccination only group and 66% (61 689/93 091) in the vaccination and behavioural change group. Table 1 shows baseline characteristics. 154 498 (58%) of 267 270 participants migrated out or died before completing 2 years of follow-up: 55 809 (35%) of 94 675 in the vaccination only group, 55 936 (60%) of 92 539 in the vaccination and behavioural change group, and 42 753 (53%) of 80 056 in the non-intervention group. We recorded no serious adverse events during 14 days after vaccination; however, we recorded 95 adverse events during follow-up: 44 in the vaccination group and 51 in the non-intervention group. We recorded no serious adverse events during 14 days after vaccination; however, we recorded 95 adverse events during follow-up: 44 in the vaccination group and 51 in the non-intervention group.
We detected 528 cholera episodes. All cases were *V cholerae* O1 El Tor biotype; only six isolates were Inaba serotype. 226 (43%) of 528 patients were severely dehydrated (only one isolate from these patients was Inaba serotype); 65 in the vaccination only group, 55 in the vaccination and behavioural change group, and 106 in the non-intervention group, for the analysis of overall protection. Significant overall vaccine protection against severely dehydration cholera was evident in the vaccination only group and 45% (95% CI lower bound 18%, p=0·0024) in the vaccination and behavioural change groups (figure 3). Adjusted cumulative 2-year overall protection of the vaccine was not significantly different between the vaccination only group and the vaccination and behavioural change group (p=0·50). Although the point estimates of overall protection differed by age group (table 2), they were not significantly different in either the vaccination only group (p\textsubscript{interaction}=0·39) or the vaccination and behavioural change group (p\textsubscript{interaction}=0·25). Similarly, although the point estimate for overall protection was higher in the second year than in the first year of follow-up (table 2), the difference was not significant in either the vaccination only group (p\textsubscript{interaction}=0·67) or the vaccination and behavioural change group (p\textsubscript{interaction}=0·85).

The appendix shows details of the study population for analysis of total effectiveness. We recorded 34 episodes of severely dehydration cholera in the vaccination only group, 30 in the vaccination and behavioural change group (table 2). The occurrence of cholera was not significantly different between the vaccination only group and the vaccination and behavioural change group (p=0·50). Although the point estimates of overall protection differed by age group (table 2), they were not significantly different in either the vaccination only group (p\textsubscript{interaction}=0·39) or the vaccination and behavioural change group (p\textsubscript{interaction}=0·25). Similarly, although the point estimate for overall protection was higher in the second year than in the first year of follow-up (table 2), the difference was not significant in either the vaccination only group (p\textsubscript{interaction}=0·67) or the vaccination and behavioural change group (p\textsubscript{interaction}=0·85).
### Table 3: Occurrence of cholera with severe dehydration and cumulative total protection by the killed oral cholera vaccine during 2 years of follow-up

<table>
<thead>
<tr>
<th>Year of follow-up</th>
<th>Interventions</th>
<th>Non-interventions</th>
<th>Total effectiveness (crude estimate)</th>
<th>Total effectiveness (adjusted estimate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Participants</td>
<td>Cholera cases</td>
<td>Person-days of follow-up</td>
<td>Incidence cases per 100 000 person-days; 95% CI</td>
</tr>
<tr>
<td></td>
<td>(n)</td>
<td>episodes (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>61689</td>
<td>23</td>
<td>16 909 884</td>
<td>0.1260 (0.0904 to 0.2047)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second</td>
<td>36 202</td>
<td>7</td>
<td>11 346 698</td>
<td>0.0657 (0.0294 to 0.1294)</td>
</tr>
</tbody>
</table>

PE-protective effectiveness. *For the overall interaction between the assigned group and subgroup variables in the model. †Adjusted for closer distance from the household to the nearest icddr,b hospital, at zero time (years), individuals having reported diarrhoea within 6 months at the time of household registration, individuals living in a household with only one room, and percentage of individuals in the cluster living in their own house. ‡‡Adjusted for closer distance from the household to the nearest icddr,b hospital, individuals living in study area for less than 1 year, individuals living in their own house, individuals living in a household with only one room, and percentage of individuals in the cluster living in their own house. §§Adjusted for closer distance from the household to the nearest icddr,b hospital, individuals having reported diarrhoea within 6 months at the time of household registration, individuals living in a household with only one room, and percentage of individuals in the cluster living in their own house. ¶¶Adjusted for closer distance from the household to the nearest icddr,b hospital, individuals having reported diarrhoea within 6 months at the time of household registration, individuals living in a household with only one room, and percentage of individuals in the cluster living in their own house. **Adjusted for closer distance from the household to the nearest icddr,b hospital, age at zero time (years), individuals having reported diarrhoea within 6 months at the time of household registration, individuals living in a household with only one room, and percentage of individuals in the cluster living in their own house. ***Adjusted for closer distance from the household to the nearest icddr,b hospital, age at zero time (years), individuals having reported diarrhoea within 6 months at the time of household registration, individuals living in a household with only one room, and percentage of individuals in the cluster living in their own house.

### Discussion

Our results show that even with moderate coverage, the incidence of severely dehydrating cholera was reduced by oral cholera vaccination in the study population, irrespective of vaccination status, when vaccine was administered via routine government services in a densely populated urban setting. Furthermore, patient presentations with life-threatening cholera were reduced. Cholera with severe dehydration imposes a major burden on the population under study, and is also associated with considerable financial losses at the household level. Management of such patients involves aggressive fluid replacement, and almost all cholera deaths occur among these cases. Young children are especially vulnerable to severe cholera in endemic settings.\(^2\) Addition of the behavioural change intervention to vaccination seemed to add little to protection against severely dehydrating cholera. The difference between protection in the vaccination only and the vaccination and behavioural
change clusters cannot be interpreted as the protection of the water and hygiene interventions without vaccination, because this difference was conditional on receipt of cholera vaccine by participants in both types of clusters. Nevertheless, the effect of clean water, sanitation, and hygiene on the incidence of cholera is an important topic for future study.

The point estimate for total protective effectiveness in this study was slightly lower than that in the Kolkata trial of the same vaccine, although the 95% CIs of the two estimates overlap, so chance variation cannot be ruled out as an explanation for the difference. However, only 9% of participants migrated in the Kolkata study during the 2 years of follow-up compared with 58% in the present study. Comparison of pre-migration rates of cholera among those who migrated out versus rates among those who did not give no indication that outmigration directly affected protection. However, a high rate of migration could have two effects that could have reduced vaccine protection: firstly, influx of non-vaccinees into vaccinated clusters could have diluted vaccine coverage; and second, migration of vaccinees into non-intervention clusters could have contaminated the control group. Therefore, we believe that our estimates of vaccine protection are conservative compared with a mass vaccination programme in a large geographic population, within which most migrations would occur.

Similar to the Kolkata trial, we reported higher vaccine protection against cholera in the second year than that in the first year, although the difference was not significant. The reason for this higher protective effectiveness in the second year, if real, remains unclear, although it could be related to post-vaccination boosting by natural cholera infections in the field site. Future modelling studies might help clarify this possibility. We did not detect significant differences in vaccine protection by age, similar to the Kolkata trial, although in both studies point estimates for protection were lower in children younger than 5 years.

Data related to acceptability, cost, and feasibility of Shanchol in Bangladesh are encouraging. A limitation of our trial was that we did not evaluate the effect of the behavioural change intervention per se on cholera. We did not include such an assessment because it would have required a much larger, four-arm factorial design, in which one group would receive only the behavioural change intervention, and because the primary purpose of this trial was to assess routine public health implementation of the vaccine. Furthermore the behavioural change intervention is not a routine intervention used in public health programmes in Bangladesh.

We did not use a placebo for the control group, so our study could not be masked. However, our analyses of protection against enterotoxigenic E coli diarrhoea suggest that bias was not the explanation for our findings of protection against cholera in the vaccination only group. Evidence of protection against enterotoxigenic E coli diarrhoea in the vaccination and behavioural change group could mean that the slightly increased protection against cholera in this group was caused by bias, but could also have resulted from protection against enterotoxigenic E coli by the behavioural change intervention.

Vaccine coverage did not differ from earlier trials of oral cholera vaccines. Few patients refused vaccination. The less than ideal coverage among the targeted population was probably because the study design prevented us from using mass media to inform participants. Previous analyses suggest that factors contributing to people not participating in the study include age (18–29 years), sex (male), and being employed (in garment and other industries).

Cholera is now endemic in more than 50 countries and causes substantial mortality and economic costs in some
of the world’s poorest nations. Our findings support earlier studies showing that killed oral cholera vaccine is effective for both children and adults against cholera of life-threatening severity even in a highly mobile urban population (panel). To obtain the full combined benefit of direct and herd protection by this vaccine in such a population, large geographic populations will have to be targeted so that most migrations occur within the targeted area. Alternatively, although possibly more difficult, vaccination could continuously target immigrants. These findings should assist policy makers in formulating rational vaccination programmes for cholera in highly mobile, high-risk urban populations.

Contributors
FQ, JDC, SPL, AC, and MAI contributed to the study design and managed and supervised the project. SPL, LU, FB, and SKB were responsible for the behavioural change component of the study. FQ, FC, AIK, AS, and IAK contributed equally to the implementation and supervision of the study. AR and SAS contributed to the delivery of the study in the field. YAY, NCS, AUS, AK, JDC, and MAI analysed the data. YAB and TRB participated in the microbiological part of the study. All others (MIC, MAs, AA, AK, BKR, MJU, JAMK, AIC) supported the feasibility study in the different components. All authors participated in the writing of the manuscript and had full access to the data in the study. All authors saw and approved the final version of the manuscript.

Declaration of interests
We declare no competing interests.

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References
Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Appendix 1 Field site selection, GIS and Census

Field site selection: We conducted the study in urban slums in Mirpur, within the Dhaka metropolitan area, which has an estimated population of over 3 million people. The highest hospitalization rates of patients with cholera seeking care at the icddr,b diarrhoeal hospitals are from this area. Six wards of Mirpur (2, 4, 5, 6, 14 and 16) were selected for this study based on data of high cholera incidence rates in the last few years preceding the study (Appendix 4). The study targeted 268,896 residents classified as 'high risk' residents in these wards. The high risk residents were persons who met one or more of the five criteria for selection of the participants for the study. These included, living in overcrowded conditions, unsafe water use, poor sanitation, unhealthy and unhygienic living conditions, sharing of toilet and kitchen. Overcrowding was defined as three or more adults living in one room. Unsafe water use was defined as the lack of clean water for drinking and for washing utensils, poor sanitation was defined as the lack of a sanitary latrine such as a pour flush latrine, water sealed latrine, or improved pit latrine as well as with direct connection to a sewer line or septic tank. Unhealthy and unhygienic living conditions included water sources, kitchens, or toilets which were shared. The overall condition of each dwelling was assessed by field interviewers trained in assessing these five components for selection of participants.

A subsequent validation assessment (n=600; 100 dwellings from each ward) demonstrated that 95% of identified dwelling units were accurately classified.

GIS and Census: Prior to vaccination, a de jure census survey was conducted from April to September 2010 to enumerate population from the high risk communities according to their regular residence, to map the households living in the area, to assign unique study identification numbers, and to assess socioeconomic status, water use, sanitation, and hygienic characteristics. About 64% of the dwelling units were being classified as high risk population and the households living in these units were enumerated. Verbal informed consent was obtained prior to the interviews. The biannual census survey was conducted using a paperless system; the data were entered directly into personal digital assistants (PDAs) during interviews. The geographic locations of the households as well as other geographic features of interest including health clinics were also collected during the census survey, and created a geographic information system (GIS) database with those geographic features. Subsequently, door-to-door routine visits biannually were made to collect vital demographic events to update the population data. The
demographic events included birth, migration-in (the origin from outside the study area), migration-out (the destination to outside the study area), death, and internal movements (origin and destination within the study area). Just before vaccination the targeted high risk individuals within the clusters were given unique identification numbers and cards. Vaccination status and doses were recorded on the cards as well as in the vaccination record forms. After the biannual census updates on PDAs, and if during the process, cards were found to have been lost, these were replaced in the next census update.

Appendix 2

**Vaccine and Behaviour Change Communication Interventions**

**Vaccine Delivery**

Groups of clusters were phased for enrollment into the trial over five cycles (in Appendix 3). Vaccination was targeted in sixty intervention clusters (30 VAC and 30 VBCC clusters) through fixed outreach sites in the community, within each cluster. There were provisions for additional sites, mobile and house to house mop ups to provide enough opportunities for the participants to get two doses of vaccine. The mop-up campaign was conducted around the end of the second round of vaccination, and some people received a single dose during the campaign. These individuals were not given the second dose and were not included in the analysis. The schedule of dosing was designed to ensure that at least 14 days transpired between dose one and two for any vaccine. “Zero time” was defined as the date of dose 1 for vaccine recipients, and at the median date of dose 1 of the cycle of vaccination in the clusters for non-vaccinees. The information on vaccine coverage, was obtained by the comparison of the list of eligible participants in each arm with the data of those who actually got the vaccine. These data were available from the computerized vaccination database.

**Behaviour Change Communication Intervention**

Handwashing intervention- Participants in the VBCC were trained on how to make soapy water by the Community Health Workers (CHW) and were encouraged to either make soapy water or purchase bar soap for regular hand washing. Hand washing after defecation, after cleansing a child's anus and during food preparation were especially emphasized. The CHWs visited each compound at least three times during each of the first two months to troubleshoot any difficulties with the hand washing station and to encourage adoption of the hand-washing habit among household members. Hand-washing promotion began two months after vaccination and activities
to encourage target practices among household members included demonstration of hardware use and maintenance, hand washing methods and suggested key times, using health and non health messages. Special activities were conducted with the mothers of children less than five years old to encourage young children about handwashing with soap. Older children (6-13 years) participated in preparing soapy water and home water treatment with chlorine. CHWs conducted activities directed at children approximately every two months that included games, songs, picture coloring debating quizzes on flip chart contents and annual participation in Global Hand Washing Day and World Water Day.

**Liquid chlorine-based household drinking water treatment** - Four months following vaccination, the CHWs returned to each compound to promote the use of a liquid chlorine-based household drinking water treatment method. Each drinking water station included a chlorine dispenser. Since the dose of chlorine in the dispenser and the volume of the reservoir was preset, a standard dose of sodium hypochlorite, high enough to treat contaminated drinking water but low enough not to cause excessive smell or taste, was delivered. About 3 ml of chlorine was added per 5 liter of drinking water. Community residents were encouraged to drink treated water. Messages emphasized health benefits, avoidance of missed days at work due to illness, nurturing the healthy development of children, and the importance of personal cleanliness. The CHWs visited each compound at least three times during each of the first two months after placement of the chlorine dispenser to troubleshoot any difficulties with the chlorine dispenser and to encourage regular consumption of treated water. Within 4 weeks of completion of vaccination, BCC hardware was implemented. After full implementation of interventions the CHWs visited households twice per month to observe hand washing and home chlorination practice of the participants and reinforced messages to encourage practices. They also refilled chlorine dispensers with sodium hypochlorite every one or two months depending on consumption.

**Appendix 3: Cycles of vaccination**

The vaccination was conducted in cycles in each round of vaccination. There were five cycles as shown below:

| Cycle | Vaccine clusters | Vaccine plus BCC clusters | Non-intervention clusters
<table>
<thead>
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<td>50, 51, 56, 58, 62, 63</td>
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</tbody>
</table>

*The cycle for the non-intervention clusters were determined based on the nearest vaccine/vaccine plus BCC cluster.

Appendix 4 The study area in Mirpur, Dhaka City, Bangladesh. The ward numbers are given inside the ward boundary.
Appendix 5: CONSORT for assembling the population for evaluating total effectiveness

268,896 individuals at zero time

95,115 individuals assigned to vaccine arm
- 23,703 individuals did not receive vaccine
  - 1598 under 1 year old
  - 593 consent not given
  - 788 pregnant
  - 120 acute illnesses
  - 20604 absent

93,091 individuals assigned to vaccine plus BCC arm
- 22,658 individuals did not receive vaccine
  - 1565 under 1 year old
  - 607 consent not given
  - 832 pregnant
  - 159 acute illnesses
  - 19495 absent

80,690 individuals assigned to non-intervention arm
- 1546 under 1 year old
- 71,412 individuals received one dose
- 70,433 individuals received one dose
- 61,970 individuals received two doses
- 61,689 individuals received two doses
- 79,144 individuals at study begin date

71,412 individuals received one dose
- 227 individuals were lost before date of dose 2*
  - 225 migrated out
  - 2 died
- 9,221 individuals did not receive vaccine
  - 5 under 1 year old
  - 593 consent not given
  - 155 pregnant
  - 72 acute illnesses
  - 8,392 absent
  - 4 incomplete doses

70,433 individuals received one dose
- 626 individuals were lost before date of dose 2*
  - 614 migrated out
  - 12 died
- 8,517 individuals did not receive vaccine
  - 4 under 1 year old
  - 499 consent not given
  - 130 pregnant
  - 91 acute illnesses
  - 7,789 absent
  - 4 incomplete doses

79,144 individuals at study begin date
- 221 individuals were lost before date of dose 2*
  - 221 migrated out
  - 0 died
- 23,703 individuals did not receive vaccine
  - 607 under 1 year old
  - 832 consent not given
  - 788 pregnant
  - 120 acute illnesses
  - 19495 absent

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78,518 individuals analyzed
* The date of dose 2 for the two-dose recipients, or the median date of dose 2 of the cycle of vaccination for no- or one-dose recipients.
† assessed by the vaccinators during the time of vaccination
Appendix 6: Kaplan-Meier estimates of the cumulative risk of not having severe dehydrated cholera among target population (total effectiveness analysis)

<table>
<thead>
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<th>Days of follow-up</th>
<th>Vaccine arm</th>
<th>Vaccine plus BCC arm</th>
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<td>78,518</td>
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<td>35</td>
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