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

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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.**

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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] 30–40% patients with cholera have severe dehydration, which can be fatal if not promptly treated with intravenous fluids.[2] The financial cost of cholera to patients in Bangladesh can be very high.[3]

In 2001, WHO prequalified the licensed killed oral cholera vaccine Dukoral (Valneva; Stockholm, Sweden) for purchase by UN organisations.[4] 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.[4] 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 trial[5] 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.[6] 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.[7]

Cholera is endemic in Bangladesh, and the entire population is at risk. Outbreaks of cholera in Dhaka, Bangladesh spike in spring and autumn,[8] with additional outbreaks during floods.[2] 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.[1] 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.

See Online for appendix

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.

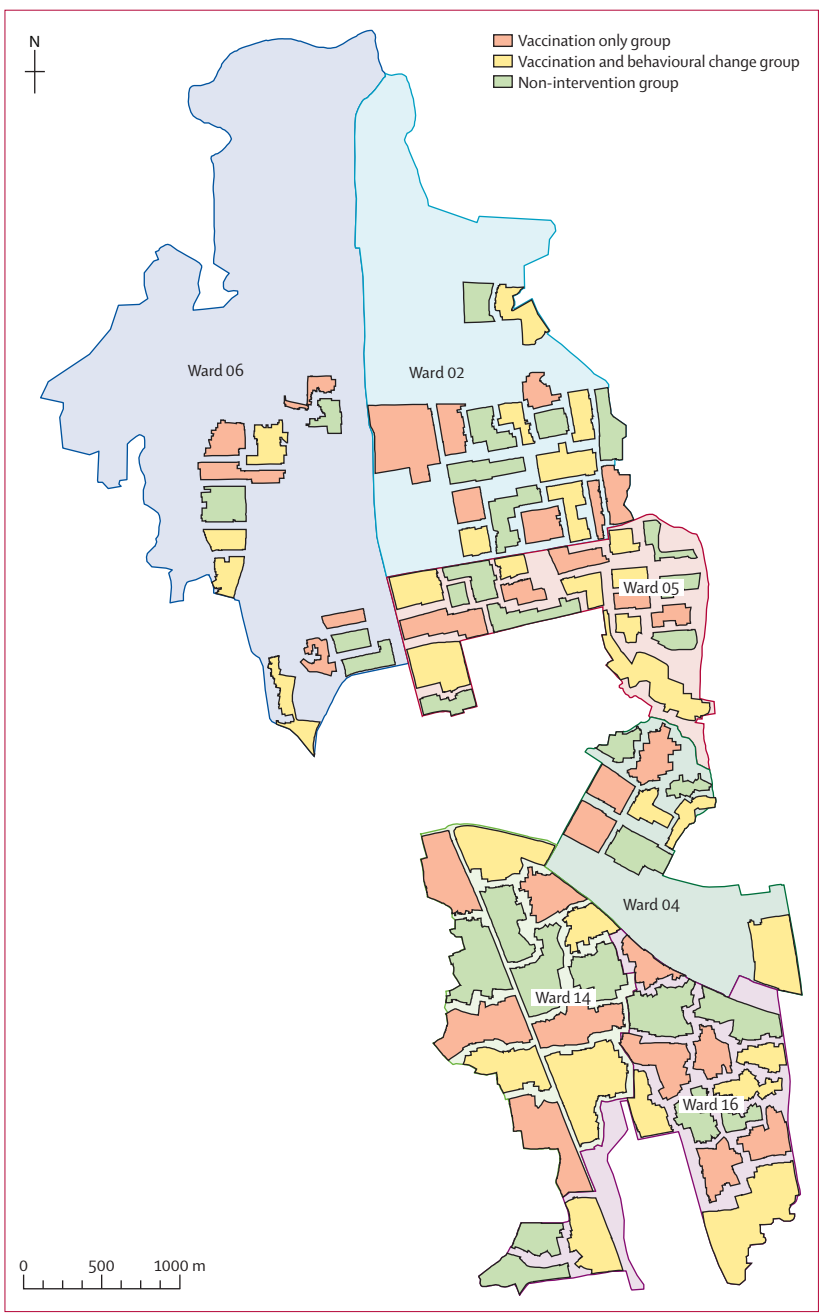

Figure 1: Study site showing the 90 clusters allocated to the three groups of the trial

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

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.

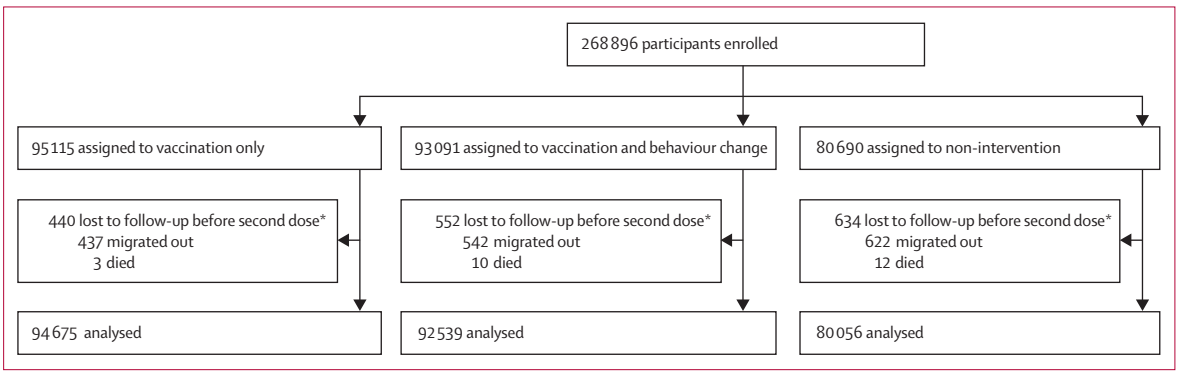

Figure 2: **Trial profile**
*Median date of second dose for recipients of one dose or no doses.

	Vaccination only group (n=94 675)	Vaccination and behavioural change group (n=92 539)	Non-intervention group (n=80 056)
Mean age (SD; years)	23·9 (15·8)	23·9 (15·7)	24·1 (16·0)
Male participants	45 677 (48·2%)	45 164 (48·8%)	39 264 (49·0%)
Diarrhoea within previous 6 months	12 657 (13·4%)	12 143 (13·1%)	11 189 (14·0%)
Diarrhoea within previous 48 h	1177 (1·2%)	1175 (1·3%)	1003 (1·3%)
Mean time living in the area (SD; months)	67·9 (116·9)	63·8 (110·5)	74·1 (121·7)
Lived in study area for less than 1 year	43 174 (45·6%)	42 104 (45·5%)	32 424 (40·5%)
Live in own house	19 892 (21·0%)	18 945 (20·5%)	20 075 (25·1%)
Households using safe water source (household tap)	4493 (4·7%)	5083 (5·5%)	4228 (5·3%)
Live in a household with a specific place for waste disposal	76 146 (80·4%)	77 634 (83·9%)	61 943 (77·4%)
Live in a household with a flushing toilet	65 499 (69·2%)	74 260 (80·2%)	62 149 (77·6%)
Live in a household with a concrete roof	83 263 (87·9%)	78 239 (84·5%)	67 532 (84·4%)
Live in a household with only one room	78 173 (82·6%)	74 522 (80·5%)	64 679 (80·8%)
Sharing kitchen with other households	82 207 (86·8%)	83 486 (90·2%)	66 536 (83·1%)
Live in a household sharing water source with others	61 378 (64·8%)	65 514 (70·8%)	48 563 (60·7%)
Live in a household using treated water (boiled, filtered, or chemical treatment)	48 512 (51·2%)	53 525 (57·8%)	42 276 (52·8%)
Live in a household that knows about cholera vaccine	5944 (6·3%)	7895 (8·5%)	6432 (8·0%)
Live in household close (less than the median distance) to the nearest icddr,b hospital	44 246 (46·7%)	45 708 (49·4%)	39 377 (49·2%)
Mean number of individuals per household (SD)	4·7 (2·0)	4·7 (1·9)	4·8 (1·9)
Median distance to the nearest icddr,b hospital (IQR)	1792 (1121–2266)	1792 (1307–2306)	1802 (994–2414)
Mean percentage of children younger than age 5 years in the cluster (SD)	10·0% (1·1)	10·0% (0·9)	10·1% (1·0)
Mean percentage of male participants in the cluster (SD)	48·3% (1·3)	48·8% (1·1)	49·0% (0·9)
Mean percentage of individuals using safe water source in the cluster (SD)	4·7% (4·4)	5·5% (4·7)	5·3% (3·6)
Mean percentage of individuals living in their own house in the cluster (SD)	21·0% (17·8)	20·4% (18·0)	25·0% (24·7)
Mean percentage of individuals using specific place for waste disposal in the cluster (SD)	80·4% (25·6)	83·9% (20·9)	77·4% (24·2)
Mean percentage of individuals using flushing toilet in the cluster (SD)	69·2% (28·2)	80·2% (15·3)	77·6% (21·5)

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.[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.[10] The causal relationship of adverse events to vaccination was judged by the study physicians.

Specimens were tested for *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.[13] Specimens were also tested for enterotoxigenic *Escherichia coli* by multiplex PCR and further confirmed by immunodiagnostic methods as previously described.[14–16]

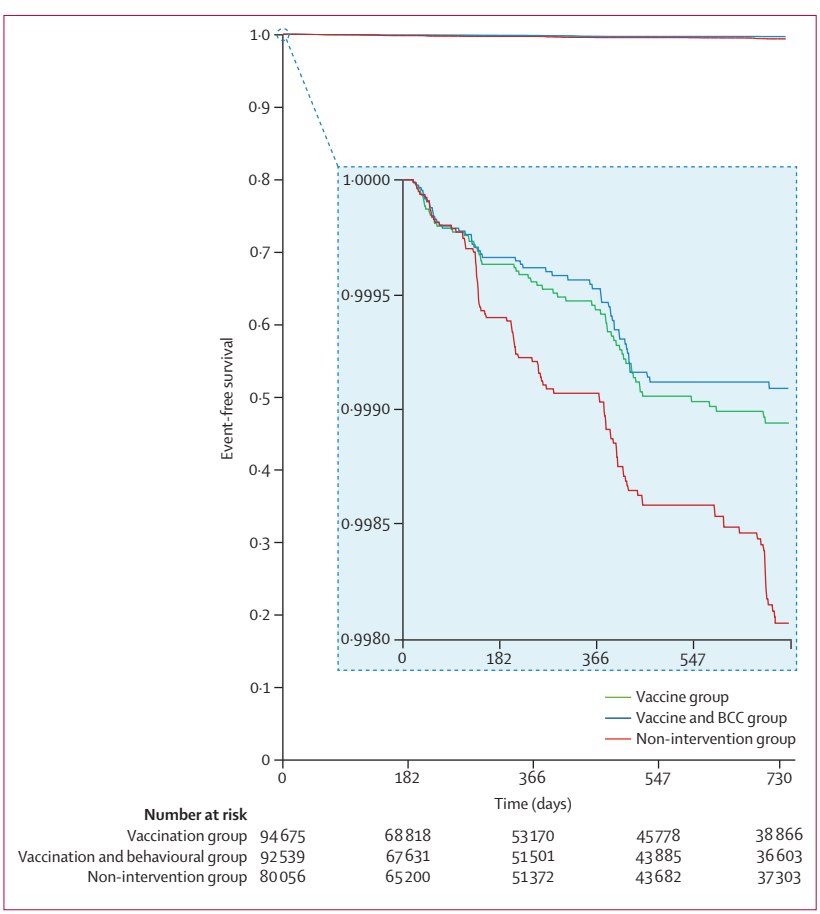

Figure 3: **Kaplan-Meier assessment of overall effectiveness**

A cholera episode was defined as a diarrhoeal episode with no passage of bloody stools, if a faecal specimen yielded *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 *E coli* diarrhoeal episode was defined as a diarrhoeal episode in which no component visit yielded *V cholerae* O1 or O139 and a faecal specimen yielded enterotoxigenic *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 *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 *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.[17] 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 (α=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 (78 780 in each group).

To test for bias,[18] we assessed the protection conferred by the vaccine against enterotoxigenic *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 *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,[6,19,20] 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 (2 years after receipt of the second dose).

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 regression models after testing for multicolinearity and verifying that the proportionality assumptions were fulfilled for all independent variables.[21–23] We estimated the hazard ratios (HRs) by exponentiation of the coefficient for the group variable in these models. We calculated vaccine protective effectiveness as: (1−HR)×100. We used robust sandwich variance estimates to account for the design effect of cluster randomisation, allowing inferences for vaccine effectiveness at the individual level.[24] To calculate protective effectiveness adjusting for covariates, we included the stratification variable (distance to the hospitals) in the model irrespective of its statistical significance. Additionally, baseline variables that were associated with time to event at p less than 0·10 in bivariate analyses were candidates as covariates in the model. To help avoid over-fitting the models, we used a backward elimination algorithm to select covariates associated with time to event at p less than 0·10.

We also analysed protective effectiveness by age group at zero time and by year of follow-up. We assessed 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).

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

Role of the funding source

The funder helped to design and plan the study. They had no role in data collection, data analysis, data interpretation, or writing of this report. The corresponding author had full

	Intervention				Non-intervention				Overall effectiveness (crude estimate)			Overall effectiveness (adjusted estimate)		
	Participants (n)	Cholera episodes (n)	Person-days of follow-up	Incidence (cases per 100 000 person-days; 95% CI)	Participants (n)	Cholera episodes (n)	Person-days of follow-up	Incidence (cases per 100 000 person-days; 95% CI)	PE (%)	95% CI (one-sided; two-sided)	p value (one-sided; two-sided)	PE (%)	95% CI (one-sided; two-sided)	p value (one-sided; two-sided)
Vaccination only group														
All individuals	94 675	65	41 809 947	0·1555 (0·1219 to 0·1982)	80 056	106	39 327 744	0·2695 (0·2228 to 0·3260)	42%	22; 17 to 60	0·0014; 0·0029	37%†	18; 13 to 55	0·0024; 0·0048
Age (years)											..			0·39*
1·0–4·9	9440	8	3 998 093	0·2001 (0·1001 to 0·4001)	8081	18	3 852 132	0·4673 (0·2944 to 0·7417)	57%	16; 4 to 81		52%‡	8; −4 to 78	
5·0–14·9	19 393	6	9 011 812	0·0666 (0·0299 to 0·1482)	16 688	12	8 564 265	0·1401 (0·0796 to 0·2467)	52%	−24; −49 to 85		33%§	−59; −88 to 76	
≥15·0	65 842	51	28 800 042	0·1771 (0·1346 to 0·2330)	55 287	76	26 911 347	0·2824 (0·2255 to 0·3536)	37%	16; 11 to 56		34%¶	11; 6 to 53	
Year of follow-up											..			0·67*
First	94 675	41	25 176 059	0·1629 (0·1199 to 0·2212)	80 056	62	23 468 300	0·2642 (0·2060 to 0·3389)	39%	5; −4 to 64		31%‖	−6; −15 to 58	
Second	53 170	24	16 633 888	0·1443 (0·0967 to 0·2153)	51 372	44	15 859 444	0·2774 (0·2065 to 0·3728)	48%	16; 9 to 70		45%**	16; 9 to 67	

(Table 2 continues on next page)

	Intervention				Non-intervention				Overall effectiveness (crude estimate)			Overall effectiveness (adjusted estimate)		
	Participants (n)	Cholera episodes (n)	Person-days of follow-up	Incidence (cases per 100 000 person-days; 95% CI)	Participants (n)	Cholera episodes (n)	Person-days of follow-up	Incidence (cases per 100 000 person-days; 95% CI)	PE (%)	95% CI (one-sided; two-sided)	p value (one-sided; two-sided)	PE (%)	95% CI (one-sided; two-sided)	p value (one-sided; two-sided)
(Continued from previous page)														
Vaccination and behavioural change group														
All individuals	92 539	55	40 553 587	0·1356 (0·1041 to 0·1766)	80 056	106	39 327 744	0·2695 (0·2228 to 0·3260)	50%	29; 24 to 67	0·0006; 0·0012	45%††[7]	24; 19 to 63	0·0011; 0·0022
Age (years)											..			0·25*
1·0–4·9	9253	13	3 881 974	0·3349 (0·1945 to 0·5767)	8081	18	3 852 132	0·4673 (0·2944 to 0·7417)	28%	−37; −55 to 67		23%‡‡	−46; −65 to 64	
5·0–14·9	18 733	5	8 601 834	0·0581 (0·0242 to 0·1397)	16 688	12	8 564 265	0·1401 (0·0796 to 0·2467)	58%	−5; −25 to 86		41%§§	−43; −70 to 80	
≥15·0	64 553	37	28 069 779	0·1318 (0·0955 to 0·1819)	55 287	76	26 911 347	0·2824 (0·2255 to 0·3536)	53%	33; 28 to 70		49%¶¶	28; 23 to 67	
Year of follow-up											..			0·85*
First	92 539	34	24 660 494	0·1379 (0·0985 to 0·1930)	80 056	62	23 468 300	0·2642 (0·2060 to 0·3389)	48%	18; 10 to 70		44%‖‖	15; 7 to 66	
Second	53 501	21	15 893 093	0·1321 (0·0862 to 0·2027)	51 372	44	15 859 444	0·2774 (0·2065 to 0·3728)	52%	12; 2 to 77		45%***	2; −10 to 73	

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 individuals living in a household using safe water source. ‡Adjusted for closer distance from the household to the nearest icddr,b hospital, individuals living in a household knowing about cholera vaccine, individuals living in their own house, and individuals living in a household having specific place for waste disposal. §Adjusted for closer distance from the household to the nearest icddr,b hospital, individuals 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 living in their own house in the cluster, individuals living in a household having concrete roof, individuals living in a household 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 knowing about cholera vaccine, individuals living in a household having one room only, percentage of individuals in the cluster living in their own house, 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, age at zero time (years), 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 their own house, individuals living in a household sharing water source with others, individuals living in a household using treated water, and individuals living in a household using safe water source. ††Adjusted for closer distance from the household to the nearest icddr,b hospital, age at zero time (years), 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 living in study area for less than 1 year, and individuals living in a household with a specific place for waste disposal. §§Adjusted for closer distance from the household to the nearest icddr,b hospital, individuals living in a household knowing about cholera vaccine, individuals living in a household with only one room, percentage of individuals in the cluster living in their own house, and percentage of individuals in the cluster with a flushing toilet. ¶¶Adjusted for closer distance from the household to the nearest icddr,b hospital, individuals living in a household with only one room, percentage of individuals in the cluster living in their own house, individuals living in a household 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, 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, individuals having reported diarrhoea within 48 h at the time of household registration, individuals living in study area for less than 1 year, individuals living in a household sharing water source with others, individuals living in a household using treated water, and individuals living in a household using safe water source.

Table 2: **Occurrence of cholera with severe dehydration and cumulative overall protection by the killed oral cholera vaccine during 2 years of follow-up**

access to all the data in the study and had final responsibility for the decision to submit the manuscript for publication. Shantha's role was limited to supplying the vaccine.

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 (59%) 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 vaccination and behavioural change group. The most common were acute watery diarrhoea, vomiting, abdominal pain, and fever.

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 dehydrating cholera was evident in the vaccination only group and the vaccination and behavioural change groups (figure 3). Adjusted cumulative 2-year overall protection of the vaccine was 37% (95% CI lower bound 18%, p=0·0024) in the vaccination only group and 45% (95% CI lower bound 24%, p=0·0011) 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 ($p_{interaction}$=0·39) or the vaccination and behavioural change group ($p_{interaction}$=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 ($p_{interaction}$=0·67) or the vaccination and behavioural change group ($p_{interaction}$=0·85).

The appendix shows details of the study population for analysis of total effectiveness. We recorded 34 episodes of severely dehydrating cholera in the vaccination only group, 30 in the vaccination and behavioural change

	Intervention				Non-intervention				Total effectiveness (crude estimate)			Total effectiveness (adjusted estimate)		
	Participants (n)	Cholera episodes (n)	Person-days of follow-up	Incidence (cases per 100 000 person-days; 95% CI)	Participants (n)	Cholera episodes (n)	Person-days of follow-up	Incidence (cases per 100 000 person-days; 95% CI)	PE (%)	95% CI (one-sided; two-sided)	p value (one-sided; two-sided)	PE (%)	95% CI (one-sided; two-sided)	p value (one-sided; two-sided)
Vaccination only group														
All individuals	61 970	34	28 852 319	0·1178 (0·0842 to 0·1649)	78 518	105	38 580 815	0·2722 (0·2248 to 0·3295)	57%	37; 32 to 72	0·0001; 0·0003	53%†	34; 29 to 68	0·0001; 0·0003
Age (years)											..			0·72*
1·0–4·9	6271	7	2 782 390	0·2516 (0·1199 to 0·5277)	6543	17	3 105 203	0·5475 (0·3403 to 0·8807)	54%	1; –14 to 82		44%‡	–17; –35 to 77	
5·0–14·9	15 335	5	7 478 641	0·0669 (0·0278 to 0·1606)	16 688	12	8 564 265	0·1401 (0·0796 to 0·2467)	52%	–33; –61 to 86		33%§	–64; –94 to 77	
≥15·0	40 364	22	18 591 288	0·1183 (0·0779 to 0·1797)	55 287	76	26 911 347	0·2824 (0·2255 to 0·3536)	58%	38; 33 to 73		56%¶	36; 31 to 72	
Year of follow-up											..			0·71*
First	61 970	21	17 051 313	0·1232 (0·0803 to 0·1889)	78 518	61	23 015 633	0·2650 (0·2062 to 0·3406)	53%	23; 15 to 75		49%‖	17; 10 to 71	
Second	37 083	13	11 801 006	0·1102 (0·0640 to 0·1897)	50 398	44	15 565 182	0·2827 (0·2104 to 0·3799)	61%	30; 21 to 80		60%**	31; 23 to 79	
Vaccination and behavioural change group														
All individuals	61 689	30	28 256 582	0·1062 (0·0742 to 0·1518)	78 518	105	38 580 815	0·2722 (0·2248 to 0·3295)	61%	44; 40 to 75	<0·0001; <0·0001	58%††	41; 37 to 72	<0·0001; <0·0001
Age (years)											..			0·33*
1·0–4·9	6100	8	2 642 836	0·3027 (0·1514 to 0·6053)	6543	17	3 105 203	0·5475 (0·3403 to 0·8807)	45%	–11; –27 to 76		43%‡‡	–10; –25 to 74	
5·0–14·9	14 973	3	7 164 869	0·0419 (0·0135 to 0·1298)	16 688	12	8 564 265	0·1401 (0·0796 to 0·2467)	70%	10; –11 to 92		57%§§	–26; –54 to 88	
≥15·0	40 616	19	18 448 877	0·1030 (0·0657 to 0·1615)	55 287	76	26 911 347	0·2824 (0·2255 to 0·3536)	64%	43; 37 to 79		62%¶¶	40; 34 to 77	

(Table 3 continues on next page)

	Intervention				Non-intervention				Total effectiveness (crude estimate)			Total effectiveness (adjusted estimate)		
	Participants (n)	Cholera episodes (n)	Person-days of follow-up	Incidence (cases per 100 000 person-days; 95% CI)	Participants (n)	Cholera episodes (n)	Person-days of follow-up	Incidence (cases per 100 000 person-days; 95% CI)	PE (%)	95% CI (one-sided; two-sided)	p value (one-sided; two-sided)	PE (%)	95% CI (one-sided; two-sided)	p value (one-sided; two-sided)
(Continued from previous page)														
Year of follow-up											..			0·07*
First	61 689	23	16 909 884	0·1360 (0·0904 to 0·2047)	78 518	61	23 015 633	0·2650 (0·2062 to 0·3406)	49%	21; 15 to 69		44%‖‖	17; 10 to 65	
Second	36 202	7	11 346 698	0·0617 (0·0294 to 0·1294)	50 398	44	15 565 182	0·2827 (0·2104 to 0·3799)	78%	52; 45 to 91		76%***	48; 40 to 90	

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 with only one room, individuals living in their own house, percentage of individuals in the cluster living in their own house, individuals living in a household using safe water source. ‡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, percentage of individuals in the cluster using a flushing toilet, and individuals living in a household having specific place for waste disposal. §Adjusted for closer distance from the household to the nearest icddr,b hospital, individuals 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 with only one room, percentage of individuals in the cluster living in their own house, individuals living in a household 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, 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, individuals having reported diarrhoea within 48 h at the time of household registration, individuals living in study area for less than 1 year, individuals living in their own house, individuals living in a household using safe water source, individuals living in a household sharing water source with others, and individuals living in a household using treated water. ††Adjusted for closer distance from the household to the nearest icddr,b hospital, age at zero time (years), individuals living in a household with only one room, individuals living in their own house, individuals living in a household using treated water, and individuals living in a household using safe water source. ‡‡Adjusted for closer distance from the household to the nearest icddr,b hospital, individuals living in study area for less than 1 year, and individuals living in their own house. §§Adjusted for closer distance from the household to the nearest icddr,b hospital, individuals living in a household with only one room, percentage of individuals in the cluster living in their own house, and percentage of individuals in the cluster using a flushing toilet. ¶¶Adjusted for closer distance from the household to the nearest icddr,b hospital, individuals living in a household with only one room, individuals living in their own house, individuals living in a household using treated water, and individuals living in a household using safe water source. ‖‖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, individuals living in study area for less than 1 year, individuals living in their own house, individuals living in a household using treated water, and individuals living in a household using safe water source.

Table 3: **Occurrence of cholera with severe dehydration and cumulative total protection by the killed oral cholera vaccine during 2 years of follow-up**

group, and 105 in the non-intervention group, for analysis of total effectiveness. The appendix shows event-free survival curves. The adjusted cumulative 2-year total protection was 53% (95% CI lower bound 34%; p=0·0001) in the vaccination only group and 58% (95% CI lower bound 41%; p<0·0001) in the vaccination and behavioural change group (table 3). We detected no difference in the occurrence of cholera between these two groups (p=0·67). The point estimates of cumulative 2-year total protection differed by age group, but the differences were not significant for either the vaccination only group or the vaccination and behavioural change group (table 3). Likewise, total protection by year of follow-up was not significantly different for the vaccination only group or the vaccination and behavioural change group (table 3).

Assessment of the vaccine against enterotoxigenic *E coli* diarrhoea, showed no overall vaccine protection in the vaccination only group (protective effectiveness 1%, 95% CI lower bound –23%; p=0·46) or in the vaccination and behavioural change group (protective effectiveness 14%, 95% CI lower bound –10%; p=0·15). Total vaccine protection against enterotoxigenic *E coli* diarrhoea was not significant in the vaccination only group (protective effectiveness 5%, 95% CI lower bound –21%; p=0·36). However, we did record significant protection in the vaccination and behavioural change group (protective effectiveness 30%, 95% CI lower bound 7%; p=0·02).

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.[25] However, only 9% of participants migrated in the Kolkata study during the 2 years of follow-up[5] 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 gave no indication that outmigration directly affected protection. However, a high rate of migration could have two effects that could have reduced vaccine protection: first, 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.[5] 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.[5]

Data related to acceptability, cost, and feasibility of Shanchol in Bangladesh are encouraging.[3,10,26] 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[10] 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

***Panel:* Research in context**

Systematic review

In 2011, a Cochrane review[27] assessed data from seven large efficacy trials with five variations of a killed whole-cell oral cholera vaccine. Based on this review, the overall vaccine efficacy of oral cholera vaccine in the first year was 52% and in the second year was 62%. No reviews of effect of provision of safe water and encouragement of handwashing on cholera incidence have been published. We searched PubMed and WHO for reports about cholera and oral cholera vaccines from 1985 onwards, with the terms "oral cholera vaccine", "Dukoral", "Shanchol", "whole cell inactivated cholera vaccine", "cholera vaccine efficacy", "cholera vaccine effectiveness", and "safe water and hand washing interventions". We identified assessments of killed whole-cell oral cholera vaccines in Bangladesh, India, Guinea, Mozambique, Peru, Vietnam, and Zanzibar. A phase 3 assessment of the efficacy of pre-emptive administration of the WHO-prequalified, killed oral cholera vaccine Shanchol in urban Kolkata, India, where cholera is endemic, showed that the vaccine was safe and conferred 65% protection during 5 years of follow-up.[6] A post-licensure effectiveness assessment of Shanchol was done in Guinea, where the vaccine was administered reactively during a cholera epidemic.[28] Protective efficacy was 87% during 6 months of follow-up. Neither of these studies assessed the effectiveness of this vaccine against endemic cholera when given pre-emptively through routine government health services. No studies have assessed the marginal additional protection of including an intervention to improve water quality and personal hygiene.

Interpretation

At 2 years of follow-up, we noted 37% overall protection against severely dehydrating cholera in the vaccination clusters, and 45% overall protection in vaccination and behavioural change clusters, irrespective of vaccination status within the clusters. For participants who received two doses of the vaccine, total effectiveness was 53% in the vaccination only group and 58% in the vaccination and behavioural change group. Our study extends earlier findings by assessing population-level, overall protection of the Shanchol vaccine against severely dehydrating cholera, including participants and non-participants and given in a realistic programme of pre-emptive vaccination through routine government services. The findings suggest that the behavioural interventions to improve water quality and personal hygiene afforded little additional protection against cholera. These findings support earlier studies that showed that the vaccine is effective for both children and adults against cholera of life-threatening severity even in a highly mobile urban population setting. They also provide guidance to policy makers in south Asia about whether to implement Shanchol in poor urban areas where cholera is endemic.

of the world's poorest nations.[2] Our findings support earlier studies[5,6] 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 MAl 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 MAl 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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