Effectiveness of One Dose of Oral Cholera Vaccine in Response to an Outbreak: A Case-Cohort Study

Effectiveness of one dose of oral cholera vaccine in response to an outbreak: a case-cohort study

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Summary

Background **Oral cholera vaccines represent a new effective tool to fight cholera and are licensed as two-dose regimens with 2–4 weeks between doses. Evidence from previous studies suggests that a single dose of oral cholera vaccine might provide substantial direct protection against cholera. During a cholera outbreak in May, 2015, in Juba, South Sudan, the Ministry of Health, Médecins Sans Frontières, and partners engaged in the first field deployment of a single dose of oral cholera vaccine to enhance the outbreak response. We did a vaccine effectiveness study in conjunction with this large public health intervention.**

Methods **We did a case-cohort study, combining information on the vaccination status and disease outcomes from a random cohort recruited from throughout the city of Juba with that from all the cases detected. Eligible cases were those aged 1 year or older on the first day of the vaccination campaign who sought care for diarrhoea at all three cholera treatment centres and seven rehydration posts throughout Juba. Confirmed cases were suspected cases who tested positive to PCR for *Vibrio cholerae* O1. We estimated the short-term protection (direct and indirect) conferred by one dose of cholera vaccine (Shanchol, Shantha Biotechnics, Hyderabad, India).**

Findings **Between Aug 9, 2015, and Sept 29, 2015, we enrolled 87 individuals with suspected cholera, and an 898-person cohort from throughout Juba. Of the 87 individuals with suspected cholera, 34 were classified as cholera positive, 52 as cholera negative, and one had indeterminate results. Of the 858 cohort members who completed a follow-up visit, none developed clinical cholera during follow-up. The unadjusted single-dose vaccine effectiveness was 80·2% (95% CI 61·5–100·0) and after adjusting for potential confounders was 87·3% (70·2–100·0).**

Interpretation **One dose of Shanchol was effective in preventing medically attended cholera in this study. These results support the use of a single-dose strategy in outbreaks in similar epidemiological settings.**

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Introduction

Oral cholera vaccines are a feasible and effective tool for cholera outbreak response.[1,2] Currently, there are three WHO-prequalified oral cholera vaccines; one primarily for travellers (Dukoral, Janssen, Beerse, Belgium), and two that are better adapted for delivery through mass campaigns in outbreaks, Shanchol (Shantha Biotechnics, Hyderabad, India, prequalified in November, 2012) and Euvichol (EuBiologics, Seoul, South Korea, prequalified in December, 2015). Two doses of any of these killed whole-cell vaccines provide high levels of direct protection probably lasting at least 5 years and some herd protection.[2–6]

In 2015, fewer than 4 million doses of oral cholera vaccine were produced, with most purchased by the global oral cholera vaccine stockpile, managed by the International Coordinating Group, comprised of Médecins Sans Frontières, UNICEF, the International Federation of the Red Cross and Red Crescent Societies, and WHO.[7] Although production will probably have increased in 2016, global availability will continue to be dwarfed by the more than 2 billion people at risk of cholera.[8] Delivery of two oral cholera vaccine doses separated by at least 2 weeks presents logistical challenges for achieving adequate coverage in areas at most risk where populations are highly mobile and the epidemic focus might rapidly shift.

Epidemiological evidence, although minimal, suggests that one oral cholera vaccine dose might provide moderate protection from cholera.[2,4,9,10] Immunogenicity studies also suggest that a single dose of oral cholera vaccine elicits a similar (vibriocidal) antibody response to two doses provided 2–4 weeks apart.[11,12] Modelling results suggest that when vaccine supply is limited, vaccinating twice the number of people with a single dose will often save more lives than providing the full two-dose regimen to a smaller population during an outbreak.[13] The global shortage of oral cholera vaccine, which will probably persist for years, coupled with the challenges of delivering two doses in some settings, might make a single dose, if effective, an attractive regimen.

Research in context

Evidence before this study

We did a PubMed search between Jan 1, 1980, and Jan 1, 2016, for studies published in English using the search terms "('cholera vaccine' OR 'cholerae vaccine') AND (efficacy OR effectiveness) AND oral", with 19 of the 213 manuscripts having primary assessments of killed whole cell oral cholera vaccine, including studies from Haiti, Bangladesh, India, Guinea, Mozambique, Peru, Vietnam, and Tanzania (excluding human challenge studies and those of travellers). A phase 3 clinical trial in Kolkata, India, a cholera-endemic setting, estimated the 5-year cumulative efficacy of Shanchol, the vaccine used in the global cholera stockpile and the main focus of this study, to be 65%. Post-licensure effectiveness studies of Shanchol conducted in outbreak settings estimated the (direct) effectiveness of the full two-dose regimen at 87% after 6 months (in Guinea) and 63% after 24 months. No published studies in our search had a primary endpoint of efficacy or effectiveness after one dose of oral cholera vaccine, although four field studies included estimates for the effectiveness of a single dose, ranging from 33–67%, but none of these were statistically significant.

Added value of this study

This study provides evidence of the effectiveness of a single dose of killed whole cell oral cholera vaccine (Shanchol) against symptomatic confirmed cholera in an outbreak.

Implications of all the available evidence

These results, combined with evidence from previous two-dose studies, suggest that a single dose oral cholera vaccine regimen might provide a practical and cost-effective approach for rapidly protecting populations during an outbreak. More research is needed to understand how protection might vary across settings with different levels of cholera endemicity, the duration of protection from a single-dose, and the added value of booster doses provided months to years after a primary dose.

Cholera outbreaks are reported every 1–5 years in South Sudan. In 2014, an outbreak of *Vibrio cholerae* O1 Inaba struck the country, causing 6269 suspected cases and 156 deaths.[14] Within this outbreak, oral cholera vaccine campaigns were implemented throughout the country and primarily focused on displaced person camps, including two on the outskirts of Juba where roughly 20 000 individuals were vaccinated with a two-dose regimen.[14]

In May, 2015, less than a year from the end of this outbreak, a cholera case was detected in a camp of internally displaced people in the capital, Juba. A *V cholerae* O1 Inaba outbreak was officially declared in June and Médecins Sans Frontières along with the South Sudan Ministry of Health and the National Cholera Taskforce made the decision to integrate oral cholera vaccine into the cholera response, complementing water, sanitation, and hygiene; case management; and surveillance activities. Only being able to secure around 250 000 doses of Shanchol from the oral cholera vaccine stockpile for the 500 000–1 million-person city of Juba, the Ministry of Health and partners agreed to offer a single dose of oral cholera vaccine to high-risk areas of the city to rapidly immunise as many as possible with an aim of maximising the public health benefit.

We did a vaccine effectiveness study in conjunction with this large public health intervention. We present the first vaccine effectiveness estimates for a single dose of oral cholera vaccine provided in response to an outbreak.

Methods

Study design and participants

Decisions about where to target the limited available vaccine within the city were made through a consultative process based on (1) evidence of continuous transmission from the most recent situation reports; (2) cumulative attack rates at the time of decision making; (3) general living conditions including water and sanitation access; and (4) the estimated population size in each neighbourhood. The mass vaccination campaign lasted from July 31, 2015, to Aug 5, 2015. Additional groups were targeted from Aug 13, 2015, to Sept 1, 2015, including neighbours of cholera cases, a military camp, prisoners, and health-care workers. Ultimately, 165 000 people were vaccinated with a single dose of oral cholera vaccine in this campaign, which targeted a different population from those previously conducted.[14]

Enhanced surveillance for cholera began at the start of the outbreak and consisted of standardised line listing, including demographic and clinical data, of each patient seeking care for suspected cholera at any health facility within the city and daily reporting to the National Cholera Taskforce. Line lists were intended to help track the epidemic progression and to guide cholera control and case-management interventions. The surveillance network for the study included all three cholera treatment centres providing inpatient care and the seven oral rehydration posts, providing care for patients with mild suspected cholera throughout the city during the study period. At each site, the line lists were maintained by clinic staff and study staff were either posted full-time or made daily visits to each. When there were few cases at the end of the study, staff made regular phone calls to the sites for updates, and visited when any new suspected cases arrived.

We used a case-cohort approach to estimate vaccine effectiveness, combining information on vaccination status and disease outcomes from a random cohort recruited from throughout the city of Juba with that from all cases of cholera detected, irrespective of cohort membership, following established methods.[15–17] This design allowed us to take into account the variable person-time at risk in vaccinated and unvaccinated states

and provide a vaccine effectiveness estimate incorporating some degree of indirect protection due to the inclusion of unvaccinated individuals in both vaccine-targeted and non-targeted areas.

Suspected cases were all individuals aged 1 year and older on the first day of the vaccination campaign who sought care for diarrhoea at participating health structures; who had three or more loose stools in the preceding 24 h; who had lived in the same residence during the preceding 2 weeks (within a 2 h drive of central Juba); and who had not visited a health facility for diarrhoea before Aug 1, 2015, but reported that they would do so for severe diarrhoea.

Cohort participants were eligible for inclusion in the trial if they were aged 1 year or older on the first day of the vaccination campaign; resided in the same household in Juba for at least the preceding 2 weeks; and had not visited a health facility for diarrhoea before Aug 1, 2015 (start of vaccination campaign), but reported that they would do so for severe diarrhoea.

Procedures

On admission, study staff collected a stool sample using a clean, unused container. Once the patient was in a stable condition, they were approached by a study team member and asked for written consent to participate. If no consent was provided, the stool sample was tested locally and results were used for routine surveillance. The protocol for this study was approved by the South Sudan ethical review board and the Johns Hopkins Bloomberg School of Public Health institutional review board.

Cohort participants were selected through a multistage random spatial sampling process. We divided the city into 350 m × 350 m grid cells and selected grid cells (45 in vaccinated and 45 in unvaccinated areas) randomly with weights proportional to the number of buildings within each (using recent digitised aerial imagery). For each chosen cell, we randomly (uniform distribution) selected ten points to visit. Study staff visited the closest residential door to each point. At each selected household (people sleeping under the same roof and sharing meals every day for at least the previous 2 weeks), all household members, not just those present, were enumerated, and one person was randomly chosen and asked for written consent to participate in the study.

Study staff attempted to follow-up each cohort member at the end of the epidemic to ask about episodes of diarrhoea, both medically attended and non-medically attended. At the end of the study, we cross-checked (using name, sex, and age) the study participants with the master national line list of suspected cholera cases.

All cohort members and cases were asked a series of questions from standardised pre-piloted questionnaires to capture data on their personal and household demographics, their date of arrival in Juba if not from there, recent health status, history of cholera vaccination (over the past 2 years), and data for other potential confounders including access to water, sanitation, and health care. Study staff were trained to ascertain vaccination status by first describing the vaccination campaign and showing a photo of someone taking an oral cholera vaccine. They then asked the person if they were vaccinated along with details of when and where. Vaccination cards were requested from all individuals reporting to have been vaccinated. Since individuals with suspected cholera were unlikely to have vaccination cards in their possession at the health facilities when recruited, study staff visited households to ascertain vaccination status.

Individuals were considered vaccinated 10 days after reporting to have fully ingested one dose of oral cholera vaccine based on published immunological scientific literature suggesting that the peak vibriocidal antibody response after infection or vaccination occurs 9–11 days after exposure.[18–21]

Samples were tested in-country and at two international laboratories with culture-dependent and culture-independent methods (appendix). First, stool was tested on-site after a 4–6 h-enrichment in alkaline peptone water with a rapid dipstick test (Crystal VC, Arkray Healthcare Pvt, Surat, India). Both direct and enriched specimens were placed on filter paper (Whatman 903 211 Protein Saver Card, GE Healthcare, Cardiff, UK) for PCR analyses at Johns Hopkins University.[22] In parallel, stool was placed onto wet filter paper disks for culture at the South Sudan National Public Health Laboratory and both culture and PCR at Institut Pasteur, with standard methods.[23,24] To maximise sensitivity while maintaining high specificity, confirmed cases were defined as suspected cases who were positive by at least one PCR test. To minimise the risk of misclassification, cholera-negative cases were those testing negative by all tests including PCR, culture, and enriched rapid test.

See Online for appendix

Statistical analysis

In sample size calculations (PASS, version 14) assuming a vaccine effectiveness of 50%, 80% vaccine coverage in the targeted areas, and 15% loss to follow-up, we estimated that we needed to enrol 900 cohort members (450 each from vaccine-targeted and non-vaccine-targeted areas) and have 155 cases of cholera for 80% power to detect a significant vaccine effectiveness at an α level of 0·05. In addition to the case-cohort design, we also attempted to estimate vaccine effectiveness with a "test-negative" design and the direct vaccine effectiveness with a matched case-control study, which were selected a priori as secondary analyses (appendix). We estimated unadjusted and adjusted hazard ratios (HR) of medically attended cholera, comparing those who received the vaccine to those who did not, and calculated the vaccine effectiveness with the association: vaccine effectiveness = 1 − HR. We used proportional hazards models with vaccination as an independent variable and

For more on **digitised aerial imagery** see http://wiki.openstreetmap.org/wiki/WikiProject_South_Sudan

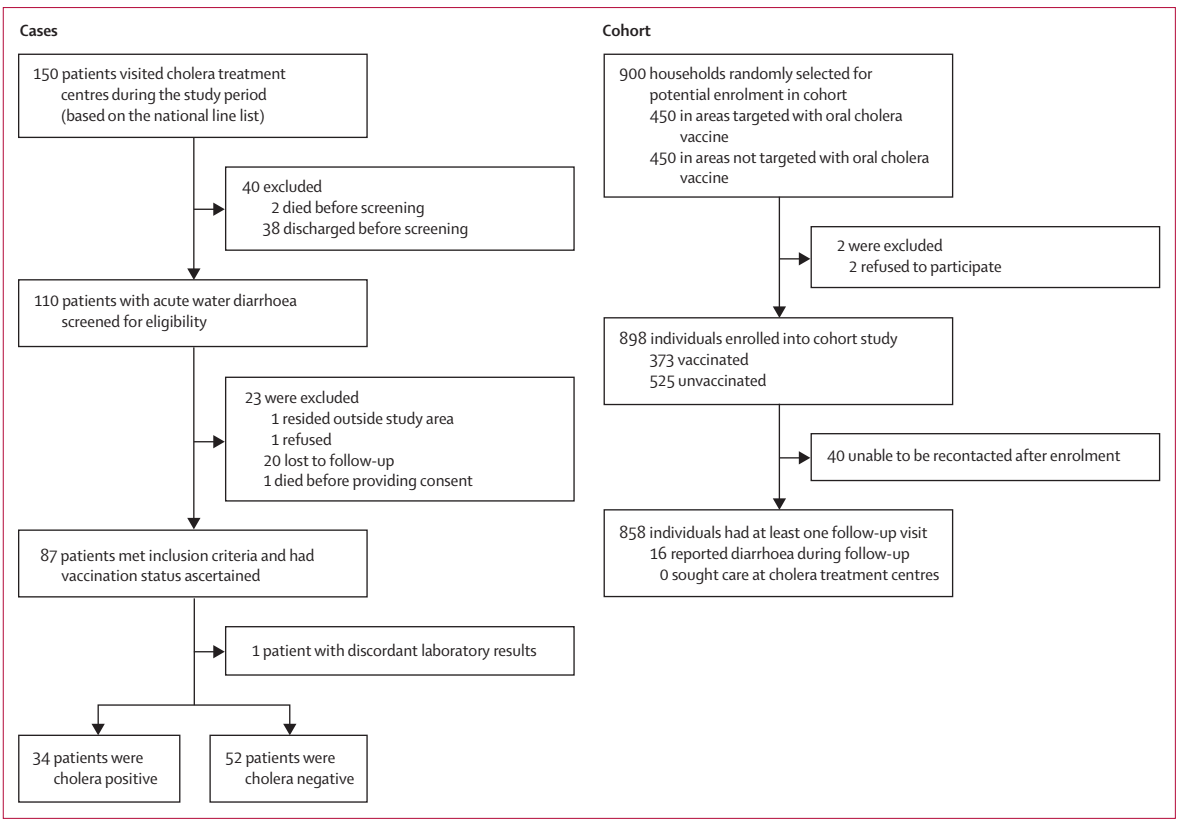

Figure 1: **Study enrolment overview**
Enrolment of cases outside the cohort (left) and follow-up within the cohort (right) are shown.

a time origin of Aug 5, 2015 (the end of the mass campaign), following established case-cohort methods.[25] We assumed that individuals in the cohort reporting diarrhoea during follow-up, who did not have a positive cholera test result recorded at a cholera treatment centre, remained at risk of cholera after the episode(s).

Weights were used in all analyses to account for sampling design with relative population estimates derived from digitised satellite imagery layers. CIs were estimated with 5000 bootstrap replicates. We explored violations of non-proportionality of hazards visually and using Schoenfeld residuals, with no violations detected.[26] Several adjusted candidate models, consistent with previous scientific literature and expert opinion, were proposed by co-investigators of the study. These models and variants of models selected by stepwise selection were compared by Akaike Information Criteria (appendix).[27] To understand the robustness of our estimates, we estimated the vaccine effectiveness using models that accounted for potential differences in the baseline hazard of cholera infection in different areas of the city. We also estimated vaccine effectiveness with alternative definitions of vaccination (including only those with a vaccination card), confirmed cases, timing of vaccine protection (2–14 days after ingestion), and alternative methods for accounting for diarrhoea cases within the cohort (appendix).

With use of the cholera-negative suspected cases, we also did a bias indicator study to help ascertain whether receipt of vaccine was associated with a change in risk of medically attended non-cholera diarrhoea. To control for potential biases in health-seeking behaviour, we restricted this analysis to those seeking care before Sept 1, 2015 (when all but one cholera treatment centre closed and the health-care-seeking patterns changed throughout the city). With the use of the same methods as the primary analysis, we assessed the effectiveness of a single dose of oral cholera vaccine against non-cholera diarrhoea. Analyses were done with R software, version 3.2.3, and the survival package.

Role of the funding source

The funder of this study had staff (co-authors of this manuscript) who had a role in study design, study execution, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

Between Aug 9, 2015, and Sept 29, 2015, 110 individuals with suspected cholera visited health facilities for

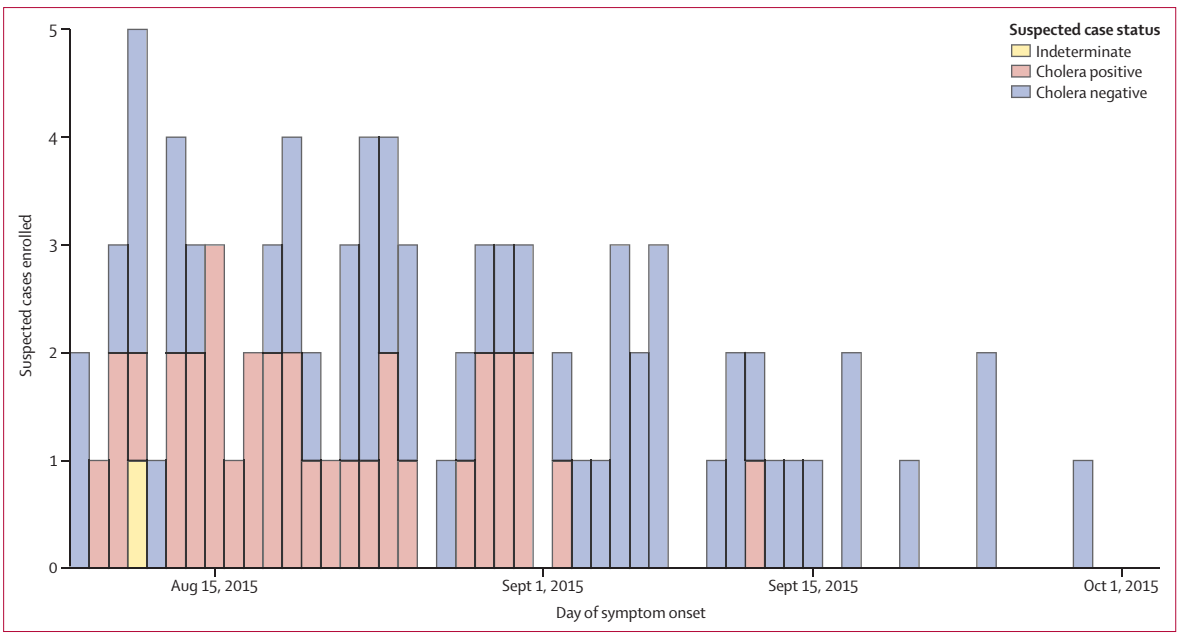

Figure 2: **Suspected cholera cases enrolled by date of symptom onset and cholera confirmation status**

treatment in Juba (figure 1, 2). Of these, 87 were enrolled in the study. We also enrolled 898 cohort members from throughout the city, including 450 from vaccine-targeted areas and 448 from non-targeted areas starting on Aug 23, 2015 (figure 1).

Among the 87 enrolled participants with suspected cholera, 34 participants were classified as cholera positive (confirmed), 52 were classified as cholera negative (negative to all tests), and one remained unclassified because of discordant laboratory results. No cholera cases were detected within the cohort through self-report or matching with the national cholera line list, although 16 (of the 858 successfully contacted for follow-up) reported having diarrhoea during the follow-up period.

Individuals with confirmed cholera were aged between 2 and 48 years old with a mean age of 23·1 years (SD 11·96) and a median age of 25 years (IQR 17–29). Individuals with cholera came from both vaccine-targeted (n=20) and non-targeted (n=14) areas throughout the city, although most of those from vaccinated areas lived close to the border between vaccine-targeted and unvaccinated areas. Among the confirmed cases, only two (6%) individuals were vaccinated at the time of symptom onset.

Within the cohort, 290 (64%) of 450 individuals living in vaccine-targeted areas and 83 (19%) of 448 living in non-targeted areas were vaccinated (through targeted vaccination after the mass campaign). Although vaccinated and unvaccinated participants of the case-cohort study (including cases) generally appeared similar at baseline, some differences were apparent (table 1). Vaccinated individuals tended to be younger, more often female than male, live in larger households, more likely to treat their drinking water, more likely to share a latrine with someone who had severe diarrhoea in the week before the interview, and less likely to have improved sanitation than unvaccinated individuals.[28]

In both unadjusted and adjusted analyses, vaccination with one-dose of oral cholera vaccine was associated with significant protection from medically attended cholera (table 2). We estimated the unadjusted single-dose vaccine effectiveness to be 80·2% (95% CI 61·5–100·0), and after adjusting for potential confounders, 87·3% (70·2–100·0). In a bias indicator analysis, we found that reported ingestion of one dose of oral cholera vaccine was not significantly associated with medically attended non-cholera diarrhoea risk (vaccine effectiveness 17·6% [95% CI –48·7 to 67·1]).

Sensitivity analyses with alternative assumptions and models yielded similar results, and we had too few cases of cholera to reliably estimate vaccine effectiveness from the secondary study designs (appendix). With use of the test-negative design, we estimated the unadjusted vaccine effectiveness to be 60·0% (95% CI –123·0 to 94·8) and adjusted vaccine effectiveness to be 75·9% (–89·2 to 97·7), which were not statistically different from our primary estimates. In the matched case-control design, aimed at measuring the direct vaccine effectiveness, we had only seven matched case-control sets contributing to our unadjusted estimate of 33·6% (–318·5 to 89·5) and adjusted estimate of 36·5% (–401·7 to 89·4).

Discussion

In this first field use of a single dose of oral cholera vaccine, we found the regimen to be effective in preventing medically attended cholera disease during an

	Unvaccinated (n=557)	Vaccinated (n=375)	p value
Age (years)	23·8 (15·33)	19·3 (14·77)	<0·0001
Sex			
Male	306 (55%)	153 (41%)	..
Female	251 (45%)	222 (59%)	<0·0001
Mean number of people in the household	5·2 (2·77)	6·22 (3·83)	<0·0001
Had mobile telephone in household	454 (82%)	314 (84%)	0·38
Number of households where all school-aged children were in school*	230/287 (80%)	199/261 (76%)	0·27
Time to nearest clinic (min)	25·9 (16·8)	25·25 (16·74)	0·56
Cases of cholera in household in the past week	14 (3%)	11 (3%)	0·70
Main drinking water source in the past week considered an "improved water source"†	94 (17%)	49 (13%)	0·11
Water treatment (sometimes or always)	390 (70%)	311 (83%)	<0·0001
Ate street food in the past week	170 (31%)	127 (34%)	0·27
Had soap available (self-report)	486 (87%)	321 (86%)	0·47
Had improved sanitation (self-report)†	345 (62%)	186 (50%)	0·0002
Shared latrine with someone with severe diarrhoea in the past week	16 (3%)	25 (7%)	0·01
Shared water with someone with cholera case in the past week	17 (3%)	7 (2%)	0·25

Data are mean (SD), n (%), or n/N (%). p values refer to the Wald test. Anyone who was vaccinated for at least 1 day of the study period was classified as vaccinated for this table and everyone else as unvaccinated; this included both cohort members and cases from outside the cohort. All variables were based on self-reports. *384 households had no individuals aged 5–15 years reported. †Defined by WHO/UNICEF Joint Monitoring Program.

Table 1: **Baseline characteristics**

	Unvaccinated group (30 001 person-days)	Vaccinated group (13 591 person-days)	Unadjusted vaccine effectiveness (95% CI)	Adjusted vaccine effectiveness (95% CI)*
Number of cholera cases	32	2	80·2% (61·5–100·0)	87·3% (70·2–100·0)

*Adjusted for age, sex, household size, number of cholera cases in the household in the past week, improved drinking water source, drinking water treatment (sometimes or always) in the past week, whether street food was eaten in the last week, and whether soap was available.

Table 2: **Overview of vaccine effectiveness results**

For more on the **WHO/UNICEF Joint Monitoring Program** see http://www.wssinfo.org/definitions-methods/watsan-categories/

outbreak. These findings suggest that one dose of oral cholera vaccine, half the present regimen, might be an effective, practical tool in outbreaks where a rapid reduction in short-term cholera risk is needed.

Although this study represents the first field use and effectiveness study of a single-dose regimen, it also adds to the evidence provided by a randomised trial and secondary analyses from previous two-dose effectiveness studies.[2,9,10,29] The individually randomised trial, done in Bangladesh, estimated the 6-month direct efficacy of this regimen to be 40% against all types of medically attended cholera and 63% against severely dehydrating cholera.[10] The two-dose effectiveness studies, not powered to estimate the single dose effect, estimated the direct single-dose effectiveness to be 33–67% with CIs that included zero.[2,4,9,29] Secondary analyses of the direct effectiveness from our study (appendix) support the notion that the true direct vaccine effectiveness might fall within this range.

Our vaccine effectiveness estimates are higher than previous estimates for several potential reasons. First, in addition to the direct effects of the vaccine, our estimates include indirect effects, which might be substantial even with low vaccine coverage.[5,6] Second, these estimates represent the effectiveness over a much shorter period of time (up to 2 months) than other studies. Finally, although we enhanced cholera surveillance, it probably remained less sensitive than surveillance in other countries, such as Bangladesh and India, where vaccine effectiveness in other studies was assessed. Therefore, our estimates might reflect the effectiveness of oral cholera vaccine to prevent severe medically attended disease as opposed to all medically attended cholera disease.

The effectiveness of one dose of oral cholera vaccine is likely to depend on historical population-level exposure to cholera, with immunologically primed populations likely to benefit more than those who have never had cholera before. A trial[10] of a single-dose regimen found that young children were less protected than adults, which might be attributable to historical exposure. This study was done during an outbreak only 1 year after a previous large outbreak, suggesting that for some, one dose might have simply acted as a booster after natural exposure. More work is needed to understand how a single dose can protect populations not previously exposed to cholera, including young children, through both direct and indirect effects.

The observational nature of this study and the challenging field conditions present several limitations for interpreting our results. Numerous quality control procedures were in place to ensure that interviewers correctly ascertained the participants' vaccination status and vaccination date, although nearly 50% of participants did not have their vaccination cards. The verbal reports of vaccination used in the main analysis could have been affected by vaccination coverage and cholera incidence surrounding participants. However, in sensitivity analyses excluding those without a vaccination card, we find our main vaccine effectiveness estimates to be similar to those reported here (appendix). Although our cohort was meant to be representative of the underlying population at risk of seeking care for suspected cholera, it is possible that differences in health-care-seeking behaviour across the city could have affected our estimates. Given that our estimates with the test-negative design, which controls for some biases related to care-seeking behaviour, were similar to, although lower than, our main estimates, this effect was unlikely to be large.

Vaccinated and unvaccinated individuals in this study had baseline differences that we attempted to control for with regression models. However, these models come with many simplifying assumptions. Data for covariates were collected through self-report and could have been

affected by social desirability and other biases. Our estimates could have residual confounding by measured and unmeasured covariates after adjustment; however, the stability of our effectiveness estimates across multiple adjusted models (appendix) provides some reassurance.

On the basis of previous cholera field-effectiveness studies, we anticipated having only a few true cases enrolled in the study, with only a few of these being from vaccinated areas where a comparison of cholera in vaccinated and unvaccinated people could be made. We used a case-cohort design, which can be more efficient and allowed us to make use of cases from both vaccine-targeted and non-targeted areas in estimating vaccine effectiveness. We enrolled 34 confirmed cases, which was far less than our target number of cases, and did not allow us to make stratified estimates by age. Although these results are promising and qualitatively consistent with other published estimates, more evidence from field studies is needed to confirm the generalisability of these results. Several questions remain, including how long protection from a single-dose lasts beyond the 2-month timeframe of this study and how the effectiveness of a single dose varies between people and settings with different historical exposure to cholera.

We found a single dose of oral cholera vaccine to be effective in preventing cholera during an epidemic in Juba, South Sudan. Although these analyses come with several shortcomings, sensitivity and bias-indicator analyses point towards our results being robust. These results support the use of a single-dose regimen in response to outbreaks in populations who are at high risk of cholera, where the priority is to rapidly provide protection to populations at risk, particularly when vaccine supply is limited.

Contributors

ASA and FJL contributed to the study design and managed and supervised the study. LAP, FT, FG, LO, SC, IC, AEL, ASA, and FJL contributed to the implementation and supervision of the study. ASA, FG, JL, and FJL analysed the data. LLD, BKB, M-LQ, A-LP, LO, AKD, DAS, and M-LQ participated in the microbiological aspects of the study. All other authors had a role in facilitating the study and providing ad-hoc technical support. All authors participated in the writing of the manuscript, had full access to the data in the study, and saw and approved the final version of the manuscript.

Declaration of interests

We declare no competing interests.

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