Vaccine 37 (2019) 3668–3676

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Effectiveness of oral cholera vaccine in preventing cholera among fishermen in Lake Chilwa, Malawi: A case-control study

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ARTICLE INFO

Article history:
Received 24 October 2018
Received in revised form 4 May 2019
Accepted 13 May 2019
Available online 24 May 2019

Keywords:
Cholera
Vaccine
Thermostability
Effectiveness
Hard-to-reach populations
Malawi

ABSTRACT

Background: In response to a cholera outbreak among mobile, difficult-to-reach fishermen on Lake Chilwa, Malawi in 2016, a novel vaccine distribution strategy exploited the proven vaccine thermostability. Fishermen, while taking the first vaccine dose under supervision, received the second dose in a sealed bag, and were told to drink it two weeks later. This study assessed short-term vaccine protection of this strategy.

Methods: Patients with diarrhoea admitted to health facilities around lake were interviewed and a stool sample collected for PCR testing. Vaccine effectiveness was assessed in a case-control test-negative design by comparing cases (PCR-positive for *V. cholerae* O1) and controls (patients with diarrhoea but PCR-negative) and with the screening method that compared the proportions of vaccinated among cholera cases versus the general fishermen population.

Results: Of 145 study participants, 120 were fishermen living on the lake. Vaccine effectiveness at three-months was 90.0% [95% CI: 38.8; 98.4] among fishermen and 83.3% [95% CI: 20.8; 96.5] among all participants in the case-control test-negative design, and 97.5% [95% CI: 90.9; 99.3] with the screening method.

Conclusion: This strategy was effective in providing short-term protection in fishermen against cholera. Further research is needed to determine the adding value of the second dose and to identify the optimal vaccination strategies for different contexts.

1. Introduction

Supplies of safe and effective oral cholera vaccines (OCV) are becoming increasingly available for both preventive and reactive immunization campaigns, including through a global OCV stockpile [1,2]. The World Health Organization (WHO) recommends the use of the killed whole-cell OCV in humanitarian emergencies, endemic countries, and in response to outbreaks [3]. These recommendations are supported by a growing body of evidence that showed high protection (56% efficacy and 83% effectiveness in the first year after vaccine administration) that can last up to five years [4,5].

However, cholera vaccination campaigns can be logistically challenging, as they typically require two vaccine distributions two weeks apart. Although most of the vaccine recipients seroconvert after the first dose, the second dose is expected to act as booster and extend the duration of vaccine protection, especially in the under-five population [4,6]. Furthermore, the manufacturers recommend storing OCV in standard cold-chain conditions (2–8 °C) [7], despite studies showing that OCV remains equally immunogenic after exposure to elevated temperatures for long time. A study conducted in Bangladesh in 1994 found that the whole-cell component of the vaccine was stable and immunogenic after six months at 42 °C [8]. A more recent study, also from Bangladesh, measured vaccine stability and immunogenicity after 14 days'

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https://doi.org/10.1016/j.vaccine.2019.05.044
0264-410X/

exposure to 42 °C and showed similar results [9]. With the support of these evidences and the stability data provided by the manufacturer, WHO in February 2018 (after the work presented in this paper was carried out) pre-qualified the use of Shanchol™ (Shantha, Sanofi Group) in controlled temperature chain, with a maximum of 40 °C for up to 14 days [10].

Previous OCV mass campaigns had already taken advantage of the vaccine high thermostability to reduce costs and logistical complexity. For example, large campaigns were conducted in Guinea, Juba and Lusaka using a flexible cold-chain strategy [11–13]. Vaccines vials were kept under cold-chain conditions while in central storage, and then transferred into cold boxes without passive cold chain (i.e., icepacks) on the day of delivery. The Vaccine Vial Monitor (VVM) were checked before vaccine administration, and very few vaccine doses had to be discarded due to invalid indicators [11–13].

The high thermostability of OCV opens the door to other new strategies that further reduce the logistic complexity and costs of vaccine delivery without jeopardizing its effectiveness. One such novel strategy was used in response to a cholera outbreak that started in December 2015 in and around Lake Chilwa (Fig. 1) [14], a known hotspot for cholera on the border between Malawi and Mozambique [15]. The outbreak hit predominantly fishermen who settle in unsanitary floating huts (traditionally called '*zimboweras*'), many of them a journey of three hours or more by canoe to the shore—making it difficult for them to seek health care, or to return to a vaccination point for a second dose.

The vaccination campaign, which took place in February and March 2016 (Fig. 1), employed a strategy that eliminated the second visit. Fishermen received the second vaccine dose in a sealable bag while taking the first one under supervision, and were told to drink it two weeks later. The second dose therefore remained out of cold chain for two weeks. The oral cholera vaccine Shanchol™ (Shantha Biotechnics, Hyderabad, India), lot numbers SCN 014A15, SCN 015A15 and SCN 016A15, was used.

Assessment of this campaign found that it was successful in reaching a two-dose vaccine coverage of 78.8% [14]. Nevertheless, this novel vaccine distribution strategy needed to be assessed on whether it affected the effect in protecting the population. The study described here assessed the vaccine effectiveness among the population living in Lake Chilwa, with a special focus on the fishermen settled in the *zimboweras*.

2. Methods

2.1. Study setting and design

The fisher community on Lake Chilwa comprises approximately 6000 individuals, mainly young men.

The OCV campaign was described elsewhere [14]. Briefly, for the population living in *zimboweras*, the vaccine was distributed in nineteen communal shelters (larger and slightly better equipped *zimboweras*). After having taken the first vaccine dose in the distribution point, fishermen took the second one by themselves 2 weeks later after having kept it with them or in their *zimbowera* in a sealable bag. The mean temperature inside a *zimbowera* during the two weeks between the first and second dose was 27.1 °C (75th percentile 29.3 °C, maximum temperature 33.7 °C). The OCV campaign in the wider region also incorporated a standard two-dose vaccine delivery strategy using fixed distribution sites for the population in villages within two kilometres from the lake shore, and a second-dose self-administration under a simplified cold-chain for the population living on six lake islands.

Vaccine effectiveness was evaluated using a case-control test negative design as the main study design, and a screening method [16]. Both methods use the same definitions for cholera cases and exposure to the vaccine (see definitions below).

We used the test-negative as the main study design as this approach has been suggested as a valid method to estimated vaccine effectiveness of OCV [17] and other vaccines [18] and it is consider more robust than the screening method to obtain unbiased estimates of the vaccine protection [19,20]. The test-negative design compares the odds of vaccination between patients with diarrhoea who tested positive for cholera (cases) and patients with diarrhoea who tested negative for cholera (controls), as described below [17,21]. Patients were recruited from eight health facilities located on the Lake Chilwa shore (Fig. 2) that were primarily used by the *zimbowera* fishermen. The study started on 7 March 2016, soon after the end of the vaccination campaign and ended on 11

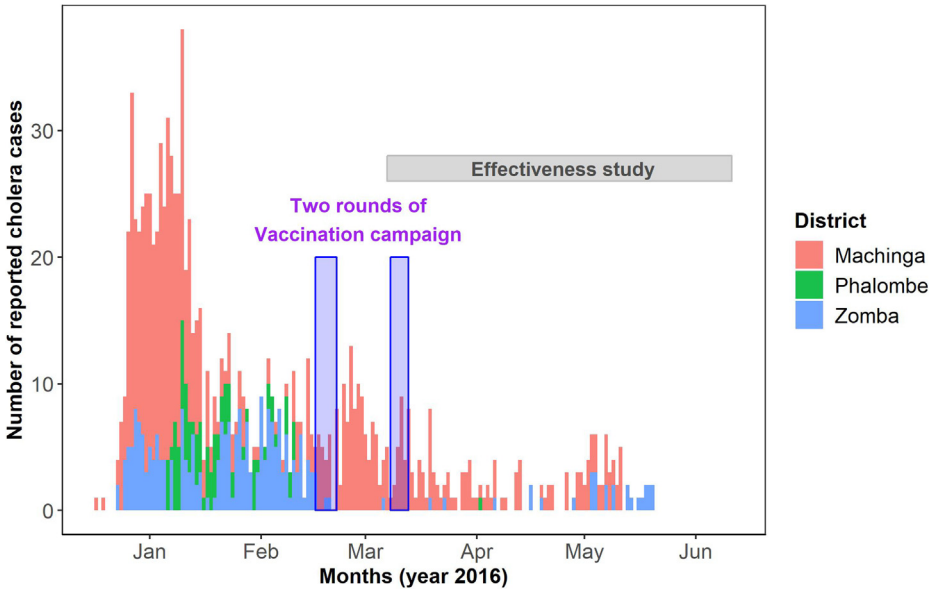

Fig. 1. Epidemic curve of reported cholera cases and timeline of the two vaccination campaign rounds (blue shadows) and of the effectiveness study (grey shadow). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

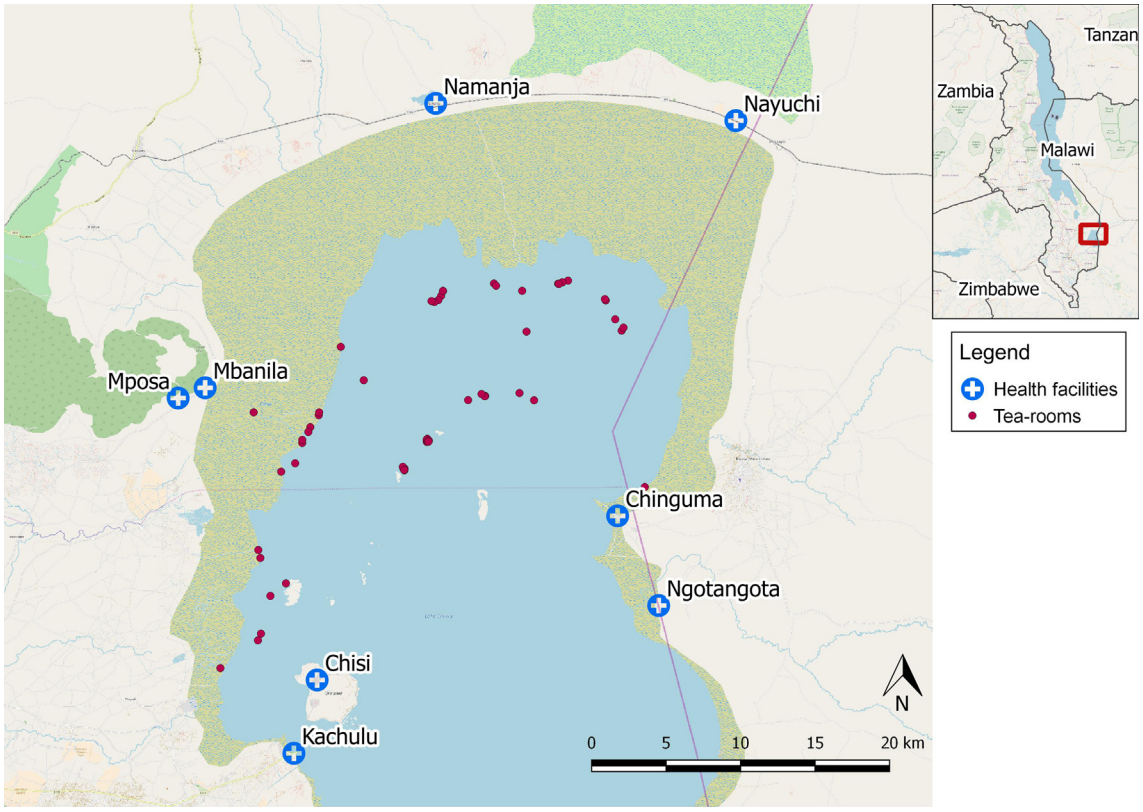

Fig. 2. Location of health facilities participating in the study and of *zimbowera* tea-rooms.

June 2016 after which no rapid diagnostic test (RDT) positive cholera patients were detected for the three consecutive weeks [22].

The screening method is based on two measures: (i) the proportion of cholera cases vaccinated; and (ii) the proportion of vaccinated individuals in the general population. This second estimate was obtained from a vaccination coverage survey carried out between 21 March and 6 April 2016 [14].

The primary analysis for both methods included only fishermen living the *zimboweras*. The secondary analyses for both methods included estimates of vaccine effectiveness among the whole population eligible for vaccination during the campaign, regardless of where they resided.

2.2. Definitions of case, control, and exposure

A suspected cholera case was defined as any person who presented to one of the eight health facilities with diarrhoea (three or more loose or watery stools in 24 h). A confirmed case was a suspected cholera case with a positive PCR result for *V. cholerae* O1. A control was a suspected cholera case with a negative PCR result for *V. cholerae* O1.

A "one-dose vaccinated" individual was a person who reported having taken only one OCV dose during the vaccination campaign; a "two-dose vaccinated" individual was a person who reported having taken two OCV doses with a two-week interval. People who took two doses and reported having vomited one were classified as having taken one dose. For the primary analysis, *zimbowera* fishermen were further categorised as those who took the vaccine in a tea-room (corresponding to the "self-administered second dose out of cold chain") and those who took the vaccine in the shore or in an island.

People for whom it was not possible to clearly assess their case status and/or exposure to vaccine were excluded from the analyses. Reasons for this ambiguity were: (i) the PCR result was missing; (ii) information on vaccination status was missing; (iii) having spit or vomited the only dose of vaccine taken; (iv) having spit or vomited both vaccine doses, if two doses were taken; (v) having taken the only dose less than 7 days before being admitted to the health facility.

2.3. Interview procedures

Study participants were interviewed at the health facility by a health surveillance assistant (HAS) who received a two-day training on the study procedures. The following information was collected on a standardised questionnaire: sex, age, profession, place of residence in the past week, socio-economic data, history of diarrhoeal episodes and contact with cholera suspects, type of water and food consumed, use of soap, toilets and latrine, and specific information about the cholera vaccine intake (number of doses and location where the patient received the first and second doses). Those who reported being vaccinated were asked to show their vaccination cards. Information was collected when the patient felt sufficiently well to answer to the questions.

The HSA activity was supervised by two nurses who visited regularly the health facilities during the study period. The health facilities reporting most of the cases were visited either every day or every second day.

2.4. Laboratory procedures

Stool specimen was collected in a clean bucket. Two drops of the specimen were then placed on a Whatman 903 protein saver filter paper and left to dry, and four drops of stool were placed in alkaline peptone water (APW) for enrichment. After four hours of incubation at room temperature following the manufacturer

instructions [23], two drops of the enrichment broth were placed on the filter paper for case-control classification.

Enriched and non-enriched dried filter papers were sent for PCR testing using a standard courier service at the Laboratory of Enteric Diseases for the Center for Immunization Research at Johns Hopkins University (Baltimore, USA). DNA was extracted using chelex-100 (Bio-Rad) as described previously [24]. PCR amplification was then performed to detect the outer membrane protein of *V. cholerae (ompW)*, for species confirmation; the cholera toxin A gene (*ctxA)*, to assess the toxigenic potential of the strain; and the *rfb* gene, to identify the O1 and O139 serogroups [25,26]. All negative samples were tested for the presence of 16S ribosomal DNA, as a control to assess the quality of sample preservation and absence of PCR inhibitors.

The enrichment broth sample was also tested using the cholera rapid diagnostic test (RDT) Crystal VC (Span Diagnostics, Surat, India) following the manufacturer's instructions. The RDT result was used to monitor the epidemic and to decide when to stop recruiting patients for the study, but not for case-control classification.

2.5. Case-control test-negative design

With the assumption of 70% of the target population been fully vaccinated and a conservative protective effectiveness of 65% (based on current evidence [22,27–29]), 60 confirmed cholera cases and 60 confirmed non-cholera controls were required for the study to have 80% power to detect a significant protective effect, with a type I error of 0.05.

We compared the odds of vaccination between cases and controls using bivariate and multivariate logistic regression models, with case-control status as the dependent variable and vaccination status as the main independent variable. We calculated the vaccine effectiveness as (1-odds ratio) × 100. The independent variable took into consideration both the vaccination status and the location where the individual received the vaccine and was classified as "not vaccinated", "vaccinated on the shore/island", and "vaccinated in a tea-room." Variables with a p-value < 0.2 in the bivariate analysis were progressively added in the multivariate analysis. Statistical models were compared using the likelihood ratio test for goodness of fit and the Akaike information criterion for parsimony. Robust standard errors and 95% confidence intervals (95% CI) were calculated using bootstrap with 5000 replicates. A statistically significant association was defined as one with a p-value below 0.05.

A secondary analysis was carried out by including all patients in the study (i.e. including patients from the shore or islands' villages). For this analysis, origin of the patients (*zimbowera* or shore/island) was taken into account by including a stratification variable using the svy command in Stata/SE, version 13 (StataCorp, College Station TX).

2.6. Screening method

Vaccine effectiveness with the screening method were estimated using the following formula:

$$VE = \frac{\mathrm{PPV} - \mathrm{PCV}}{\mathrm{PPV} * (1 - \mathrm{PCV})}$$

where VE is the vaccine effectiveness; PPV is the proportion of the population vaccinated (i.e., vaccine coverage) and PCV is the proportion of confirmed cholera cases vaccinated.

To compare the proportion of vaccinated people among cases versus the general population using a homogenous population, this analysis was restricted to the *zimboweras* fishermen cases who reported being present during the vaccination campaign.

Confidence intervals were estimated by logistic regression as proposed by Farrington [30]. We took into account the cluster design of the coverage survey by using the *svy* command in Stata/SE.

2.7. Ethics considerations

The study protocol was approved by the Malawi National Health Sciences Research Committee (approval number NHSRC # 16/2/1545) and by the French Committee Comité de Protection des Personnes Ile de France XI in Saint-Germain-en-Laye (Reference number 16021).

The process of obtaining written informed consent started with an information note read in the local language (Chichewa). For each patient responding to the suspected case definition, after emergency care was provided, the HSA in charge of the study described the study and asked the patient for consent to participate. All patients were recruited after a written consent was obtained. All data were entered and analysed anonymously.

3. Results

3.1. Study description and baseline characteristics of participants

Overall, 236 patients responding to the suspected cholera case definition were admitted to the eight health facilities; of these, 85 patients were not included in the study, the majority (45 patients) because a stool sample was not collected or because the trained staff to collect the stool sample and interview the patients (30 patients) was absent. Of the 151 included patients, 116 patients had a PCR-positive result for *V. cholerae* O1 and were classified as cases for the vaccine effectiveness analysis, while 35 had a PCR-negative result and were classified as controls. No patients died in health facilities. Furthermore, six patients were excluded from the analysis because they either reported having vomited the only vaccine dose taken (2 patients) or took the vaccine less than seven days before admission to the health facility (4 patients) (Fig. 3). The weekly distribution of patients admitted according to their study classification is shown in Fig. 4.

A higher proportion of confirmed cholera cases were male (98.2% versus 81.8% among control, $p < 0.001$) and lived in a *zimbowera* (94.6% versus 42.4% among controls, $p < 0.001$). The median age among cases and controls was 25 and 29 years, respectively. Cases had more frequently watery diarrhoea ($p < 0.001$) and severe dehydration ($p < 0.001$) than controls. Cases and controls also showed significant differences in the type of water source and the type of toilet used in the week before becoming ill (Table 1). No significant differences were identified with respect to the time from onset of symptoms to consultation, the use of antibiotics, or exposure to a person suffering from diarrhoea (Table 1).

3.2. Vaccine effectiveness: case-control test negative design

Among the 145 study participants included in the analysis, 120 reported being fishermen and staying in the *zimboweras* when they became ill. Among these 120 fishermen 106 were cases and 14 were controls, and 11 (9.2%) reported having taken at least one dose of vaccine (8 among cases and 3 among controls), including 8 (6.7%) who took two doses. Six fishermen—4 among cases and 2 among controls—took the first dose in a tea-room and self-administered the second dose. Using the best model adjusting for potential confounders, the vaccine effectiveness was 90.0% [95% CI: 38.8; 98.4] for the vaccine taken in a tea-room and 91.3% [95% CI: 63.7; 97.9] for the vaccine taken on the shore or island (Table 2).

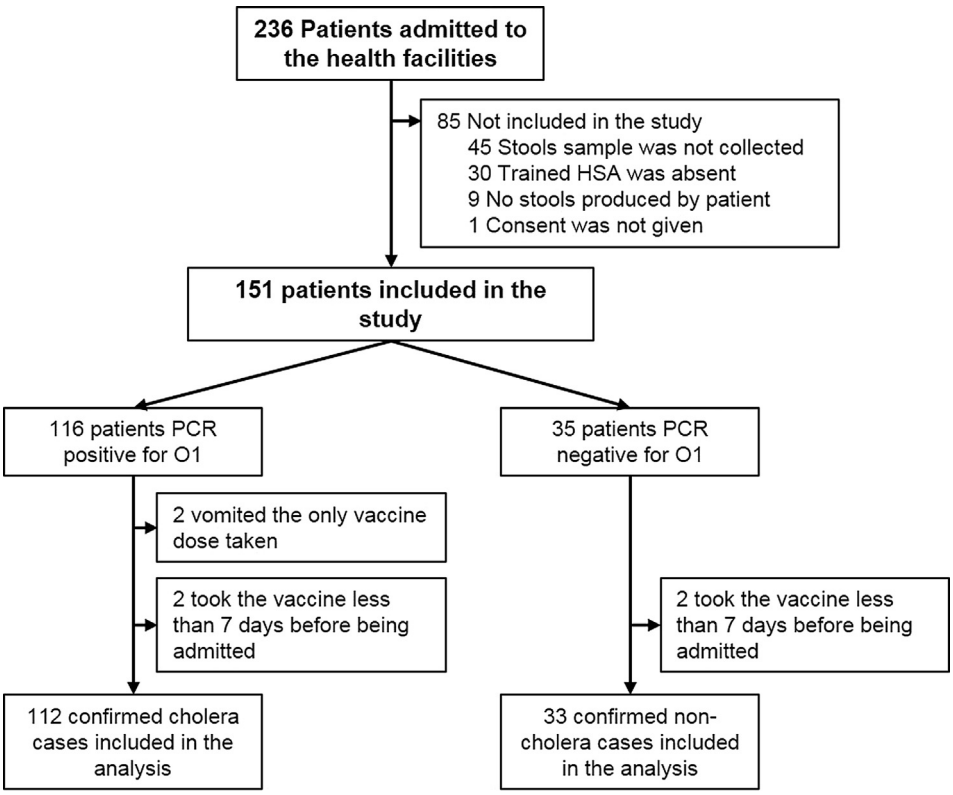

Fig. 3. Study participants flowchart.

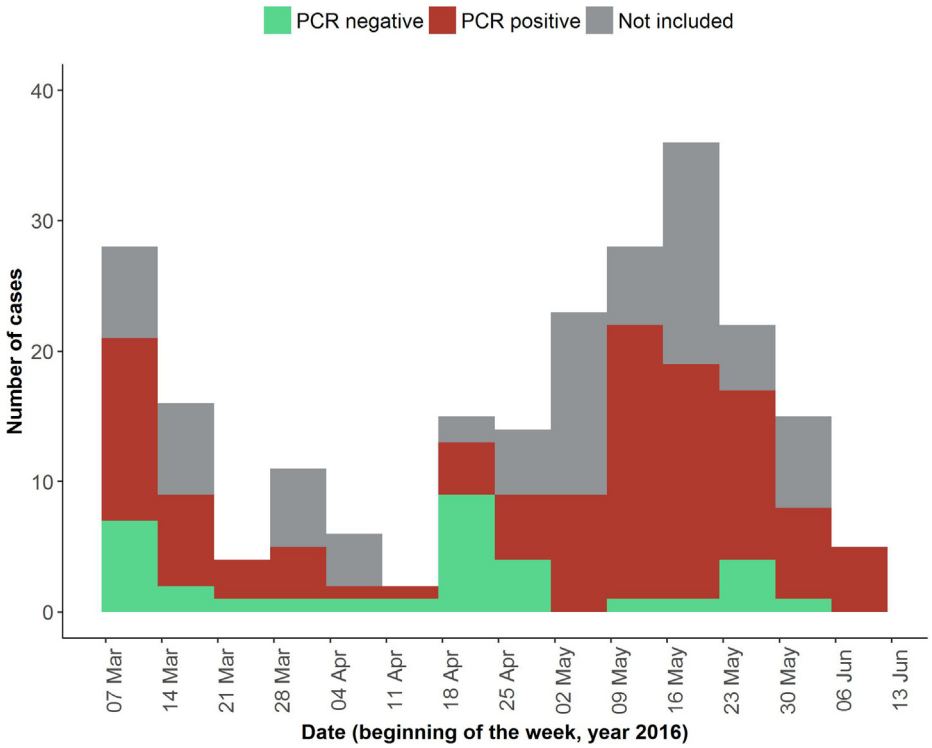

Fig. 4. Distribution of suspected cases of cholera over the study period, Lake Chilwa, Malawi, March-June 2016.

In the secondary analysis that included all 145 patients admitted to the study, the adjusted vaccine effectiveness was 88.9% among those who took at least one dose and 83.3% among those who took both doses (Table 2).

3.3. Vaccine effectiveness: screening method

The estimated vaccine effectiveness using the screening method was 97.5% (Table 3) when comparing the proportion of vaccinated

Table 1
Characteristics of PCR-confirmed cholera and non-cholera patients included in the OCV effectiveness study.

	Non-cholera controls N = 33		Cholera cases N = 112		
	n	(%)	n	(%)	*p*
Clinical characteristics					
Type of diarrhoea					
Loose	12	(36.4)	4	(3.6)	*<0.001*
Watery	21	(63.6)	108	(96.4)	
Dehydration at admission					
No dehydration	8	(24.2)	4	(3.6)	*<0.001*
Moderate	21	(63.6)	33	(29.5)	
Severe	4	(12.1)	75	(67.0)	
Antibiotic use <2 days prior to admission					
No	22	(66.7)	81	(72.3)	*0.521*
Yes	11	(33.3)	31	(27.7)	
Antibiotic at health facility					
No	33	(1 0 0)	104	(92.9)	*0.199*
Yes	0	(0)	8	(7.1)	
Delay from onset of symptoms to consultation at health facility					
Same day	20	(60.6)	71	(63.4)	*0.931*
1-2 days	12	(36.4)	37	(33.0)	
3 + days	1	(3.0)	4	(3.6)	
Socio-demographic characteristics					
Sex					
Male	27	(81.8)	110	(98.2)	*<0.001*
Female	6	(18.2)	2	(1.8)	
Median age (IQR)	29	(25–37)	25	(20–35)	*0.080**
Current job					
Fishermen	12	(36.4)	94	(83.9)	*<0.001*
Fish businessman	3	(9.1)	4	(3.6)	
Farmer	14	(42.4)	8	(7.1)	
Vendor	0	(0.0)	2	(1.8)	
Pupil/student	2	(6.6)	3	(2.7)	
Too young for school	1	(3.0)	0	(0.0)	
Missing	1	(3.0)	1	(0.9)	
Highest education level					
Cannot read/write	5	(15.2)	21	(18.8)	*0.837*
None but can read/write	1	(3.0)	2	(1.8)	
Primary school	25	(75.8)	77	(68.8)	
Secondary school	2	(6.1)	11	(9.8)	
Unknown	0	(0.0)	1	(0.9)	
Owning (economic indicators)					
Radio	22	(66.7)	69	(61.6)	*0.684*
Motorbike	0	(0.0)	3	(2.7)	*0.329*
Mobile phone	14	(42.4)	48	(42.9)	*1.000*
Generator	1	(3.0)	7	(6.3)	*0.218*
Canoe	5	(15.2)	44	(39.3)	*0.010*
Boat	5	(15.2)	25	(22.3)	*0.349*
Behaviour characteristics					
Place of residence in the 7 days before being ill					
Zimbowera	14	(42.4)	106	(94.6)	*<0.001*
Island	5	(15.2)	1	(0.9)	
Shore	14	(42.4)	5	(4.5)	
Arrival in the place where the person fell ill					
Before or during vaccination campaign	19	(57.6)	29	(25.9)	*0.004*
After vaccination campaign	10	(30.3)	51	(45.5)	
Unclear/Unknown	4	(12.1)	32	(28.6)	
Contact with someone suffering from diarrhoea					
Staying with someone who currently has diarrhoea	1	(3.0)	10	(8.9)	*0.581*
Staying with someone who had diarrhoea within the previous week	2	(6.1)	8	(7.1)	*1.000*
Met a friend or neighbour who was suffering from diarrhoea	3	(9.1)	17	(15.2)	*0.399*
Source of water in the previous week					
Lake/river/pond	12	(36.4)	83	(74.1)	*<0.001*
Filtered lake water	4	(12.1)	15	(13.4)	*1.000*
Chlorinated lake water	6	[18.2)	20	(17.9)	*1.000*
Borehole	15	(45.5)	14	[12.5)	*<0.001*
Shallow well	4	(12.1)	1	(0.9)	*0.010*
Piped water	2	(6.1)	0	(0.0)	*0.051*
Consumption of food from market/street vendor or tea-room					
Never	15	(45.5)	31	(27.7)	*0.212*
At least once in past week	14	(42.4)	67	(59.8)	
Every day in past week	4	(12.1)	12	10.7)	
Do not know/remember	0	(0.0)	2	(1.8)	

(*continued on next page*)

Table 1 (*continued*)

	Non-cholera controls N = 33		Cholera cases N = 112		
	n	(%)	n	(%)	*p*
Type of toilet mostly used in past week					
Latrine	17	(51.5)	6	(5.4)	*<0.001*
Lake	14	(42.4)	105	(93.8)	
Bush	2	(6.1)	1	(0.9)	
Use of soap after toileting and before eating					
Never	14	(42.4)	52	(46.4)	*0.367*
Rarely	17	(51.5)	42	(37.5)	
Frequently	1	(3.0)	5	(4.5)	
Always	1	(3.0)	13	(11.6)	

Fisher's exact test, unless otherwise stated

Table 2
Estimated OCV effectiveness by vaccination site and patients' place of residence at onset of illness (unadjusted, adjusted and multilevel adjusted analysis).

	Controls		Cases		Vaccine effectiveness					
					Unadjusted			Adjusted[a]		
	n	(%)	n	(%)	%	[95% CI]	p	%	[95% CI]	p
Fishermen in *zimboweras*	**14**		**106**							
Not vaccinated	11	(78.6)	98	(92.4)	Ref.	–		Ref.	–	
Vaccinated with at least one dose:										
- on the shore/island	1	(7.1)	4	(3.8)	55.1	[−101.6; 90.0]	0.296	91.3	[63.7; 97.9]	0.001
- in a tea-room	2	(14.3)	4	(3.8)	77.6	[−16.1; 95.7]	0.075	90.0	[38.8; 98.4]	0.013
Vaccinated with:										
- one dose	1	(7.1)	2	(1.9)	77.6	[−5.0; 95.2]	0.058	94.5	[78.7; 98.6]	<0.001
- two doses	2	(14.3)	6	(5.7)	66.3	[−60.0; 92.9]	0.171	88.4	[33.2; 98.0]	0.016
All patients admitted to the study[b]	**33**		**112**							
Not vaccinated	23	(69.7)	104	(92.9)	Ref.	–		Ref.	–	
Vaccinated with at least one dose	10	(30.3)	8	(7.1)	82.3	[44.8; 94.3]	0.003	85.1	[42.3; 96.2]	0.006
Vaccinated with:										
- one dose	4	(12.1)	2	(1.8)	88.9	[46.6; 97.7]	0.006	88.9	[35.8; 98.1]	0.014
- two doses	6	(18.2)	6	(5.4)	77.9	[11.2; 94.5]	0.033	83.3	[20.8; 96.5]	0.024

[a] Adjusted for owning a radio and for the type of toilet used.
[b] Stratified analysis by patient's place of residence in the week when he/she fell ill as stratum.

Table 3
Vaccine effectiveness among confirmed cholera cases living in *zimboweras* using the screening method.

	Proportion of vaccinated participants				Vaccine effectiveness	
	Among Cases		Among the General population[a]			
	n/N	(%)	%	[95% CI]	%	[95% CI]
Present during the vaccination campaign						
- At least one dose	3/25	(12.0)	84.7	[78.0; 89.7]	97.5	[90.9; 99.3]
- One dose	1/25	(4.0)	5.9	[2.6; 12.9]	88.3	[−7.3; 98.7]
- Two doses	2/25	(8.0)	78.8	[69.8; 85.7]	98.2	[91.7; 99.6]
All						
- At least one dose	8/106	(7.6)	72.5	[63.9; 79.8]	96.9	[92.9; 98.6]
- One dose	2/106	(1.9)	5.1	[2.3; 11.0]	89.0	[42.9; 97.9]
- Two doses	6/106	(5.7)	67.5	[58.9; 76.0]	97.5	[93.7 ; 99.0]

[a] Data from the vaccine coverage survey [14].

individuals among cases who were present in the lake during the vaccination campaign (25 patients) and the proportion of vaccinated individuals among the *zimbowera* fishermen present during the vaccination campaign [14]. A similar estimate (96.9%) was obtained when effectiveness was calculated with all confirmed cases coming from the lake (regardless of reported date of arrival) and the vaccine coverage calculated two weeks after the end of the vaccination campaign (at the time of the coverage survey).

4. Discussion

WHO and others have repeatedly stressed the need to simplify strategies for implementing mass OCV campaigns [3]. Here we demonstrate that a novel strategy incorporating two simplifications—self-administration of the second vaccine dose and removal of this dose from cold chain two weeks prior to self-administration—achieved comparable high level of short-term effectiveness as did other recent two-dose campaigns conducted elsewhere without either of these simplifications [21,29]. Both design methods used in this study, the test-negative case-control design and screening method, showed similar vaccine effectiveness estimates.

Another simplified strategy used in recent emergency responses to cholera outbreaks is one-dose OCV campaigns, although understanding of the relative effectiveness of one versus two doses in different contexts is still limited. Our study was ultimately underpowered to assess whether addition of the out-of-cold-chain second dose led to better protection against cholera than the first

dose alone, since the final sample size and number of individuals vaccinated were too small. Nevertheless, previous studies showed that a single dose of vaccine is sufficient to provide high short-term protection [22,31,32], but as yet there is limited data on its longer-term protection. A study conducted recently in Bangladesh showed that a single-dose of OCV offers similar levels of protection than the two-dose schedule up to two years in an adult population frequently exposed to cholera, but protection was not demonstrated among the children under-five years of age [33].

Both the case-control design and the screening method have limitations to consider in interpreting these results. The screening method relied on a comparison between two metrics that were assessed at different times. The vaccine coverage survey showed that coverage among the *zimbowera* community dropped in the weeks after the vaccination campaign ended, due to the arrival of unvaccinated fishermen from the inland [14]. For this reason the screening method analysis was restricted to the population that reported being on the lake during the vaccination campaign, but had the drawback of decreasing its sample size.

On the other hand, the case-control test-negative design makes it possible to control for differences in health-seeking behaviour and other risk factors for cholera. This analysis was, however, hindered by the small number of cholera PCR-negative patients, so we were not able to reach the required sample size for controls. This was most likely due to preferential admission of patients with severe diarrhoea, despite repeated reinforcement to the health care personnel that all patients who reported acute diarrhoea, regardless of the severity, should be included in the study. Another possibility is a lower frequency of health-seeking behaviour for non-severe diarrhoea patients among the *zimbowera* population. Fishermen must make a long journey to the shore before reaching the nearest health facility, and they likely do so only when they feel severely ill. Conversely, the study may have missed some severe cholera cases, as suggested by the fact that two fishermen were brought dead from the lake to the health facility. More profoundly than as a study limitation, these events highlight once more the importance of preventive measures for this population who have very limited access to health care.

A further limitation was that the study did not include all eligible patients, due to the absence in some staff shifts of a person that was trained to collect the stool samples and to interview the patients. This limitation affected the precision of the estimates, although the a-posteriori power remain over 80% considering the higher than assumed estimate of the vaccine effectiveness as well as the higher than planned number of positive cases included in the study. In relation to possible systematic bias linked with the low number of controls included in the study, it is important to notice that these patients were not included considering their characteristics, rather as a result of qualified staff to collect the samples or to interview the patients; therefore we expect that the sample was representative of all the patients attending the health facilities and should not have biased the vaccine effectiveness estimate.

An additional limitation for both methods was the short length of the study that ended after three months when no more cholera cases were detected. This limitation made this study unable to determine the effective contribution of the second vaccine dose on the long-term protection. To clarify this question, in addition to a longer follow up, it will be important to better monitor the intake of the second dose as well as the viability of the vaccine vial monitor, to ensure that the vaccines are still potent at the time of the vaccine intake; although vaccinations conducted among difficult to reach populations, like the one described here, can make complex answering this question.

Although the vaccination campaign provided many fishermen with protection against cholera, it is important to keep in mind that protection at both the individual and population levels can only be achieved with high levels of vaccine coverage, which can vary based on the accessibility and other factors related to the setting or the age and gender groups. Our study shows that most of the confirmed cholera cases were among fishermen who arrived at Lake Chilwa after the vaccination campaign had ended. This highlights the challenge of maintaining high vaccination coverage in a highly mobile population and should be considered in the planning of future campaigns. An additional rationale for maintaining high vaccine coverage in the Lake Chilwa setting is its particular ecosystem as a closed body of water, which could act as a reservoir for *V. cholerae* [34] and a major source of infection through consumption of contaminated water. If so, the herd protection offered by the vaccine might be lower than in settings where human-to-human transmission plays a more prominent role [35], thus increasing the need for individual protection. From these perspectives, and based on the reassuring acceptability and effectiveness shown by the strategy used in Lake Chilwa, the self-administration of the second OCV dose should be considered in other settings as a potential way to improve full vaccination coverage, to reduce the vaccine implementation cost and to ensure that the individual level of vaccine acquired protection is maximized.

In conclusion, despite several challenges in evaluating vaccine effectiveness in this particular context, this study shows that vaccination incorporating two key simplifications was effective in providing short term protection in fishermen against cholera. This study was too limited in time to determine the effective contribution of the second vaccine dose. We hope that futures long-term effectiveness studies will help to elucidate whether a self-administered second dose contributes to longer-lived cholera vaccine protection.

Declaration of Competing Interest

All authors declare no conflict of interests.

Acknowledgments

We would like to express out deep gratitude to all patients who accepted to participate to the study.

Many thanks to all the HSA at the health facilities: Felix Dausi, Duncan Limbe, James Chikapa, Eric Nkandawire, Francis Sandalamu, Mac Maida, Fabiano Selevasio, James Kaziphombe, James Chikaoonda, Peter Mkwate, Jimmy Nyalapa, Magret Matewere, Emily German, Austin John, Khama Daudi. Many thanks to Patrick Kumwenda and Gravel Maulidi, nurse supervisors of the field activity, for their exceptional work. Many thanks to Patricia Kahn for the wise suggestions in editing this manuscript.

Funding

This work was funded by Médecins Sans Frontières (MSF), Operational Centre Paris. MSF national and international personnel collaborated and supported the implementation of the study.

Previous presentations

Results of this work were presented internally in MSF meetings, but not in a public conference.

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