Universidad de Valladolid

FACULTAD DE MEDICINA

ANATOMÍA PATOLÓGICA, MICROBIOLOGÍA, MEDICINA PREVENTIVA Y SALUD PÚBLICA, MEDICINA LEGAL Y FORENSE

TESIS DOCTORAL:

Feasibility, acceptability and effectiveness of killed whole cell *V cholerae* vaccine as part of the response to epidemics

Presentada por FRANCISCO JAVIER LUQUERO ALCALDE
para optar al grado de
doctor por la Universidad de Valladolid

Dirigida por los doctores:
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A mi padre y a mi madre

Universidad de Valladolid

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(Art. 2.1. c de la Normativa para la presentación y defensa de la Tesis Doctoral en la UVa)

D. **José María Eiros Bouza**, profesor del departamento de Anatomía Patológica, Microbiología, Medicina Preventiva y Salud Pública, Medicina Legal de la Facultad de Medicina de la Universidad de Valladolid.

CERTIFICA:

Que ha dirigido el trabajo titulado **"Feasibility, acceptability and effectiveness of killed whole cell *V cholerae* vaccine as part of the response to epidemics,** realizado por el Licenciado en Medicina y Cirugía Francisco Javier Luquero Alcalde y que reúne todos los requisitos científicos y formales para ser presentado y defendido ante el tribunal correspondiente.

Y para que así conste a todos los efectos, firmo en Valladolid a 19 de Marzo de 2014.

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List of Abbreviations

AEFI: Adverse Events Following Immunization

BivWC: bivalent inactivated killed whole cells *V cholerae* O1 and *V cholerae* O139 vaccine

CI: confidence interval

CFR: Case Fatality Ratio

CRF: Case Report Form

cAMP: cyclic adenosine monophosphate

CT: Cholera Toxin

DNA: Deoxyribonucleic acid

DEFF: Design Effect

GAVI: Global Alliance for Vaccines and Immunization

Guinea: Republic of Guinea

HIV: Human Immunodeficiency Virus

IQR: inter-quartile range

IV: intravenous fluid

LPS: Lipopolysaccharide

MoH: Ministry of Health

MSF: Médecins sans Frontières

OCV: Oral cholera vaccines

OD: Optical Density

OMS: Organización Mundial de la Salud

ORS: oral rehydration solution

PCR: Polymerase Chain Reaction

RDT: Rapid Diagnostic Tests

SD: Standard Deviation

TCP: Toxin-Coregulated Pilus

V cholerae: *Vibrio cholerae*

VIP: vibrio Pathogenicity Island

VVM: Vaccine Vial Monitor

WC: whole cells

WC-rBS: monovalent inactivated killed whole cells *V cholerae* O1 vaccine plus recombinant cholera toxin B subunit

WHO: World Health Organization

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SUMMARY

1. Background and rationale

Cholera is an acute intestinal diarrhoeal disease with profuse watery diarrhoea, vomiting, rapid dehydration and high lethality in absence of adequate treatment. Current cholera outbreaks are caused by *Vibrio cholerae* 01 El Tor.

Provision of safe water and proper sanitation are without doubt the long-term and only solution for cholera control. However, controlling cholera globally is far from being achieved; the disease burden is increasing with large-scale outbreaks reported in the past several years, such as those in Haiti and Zimbabwe. Current outbreak response interventions focus on case management and access to health care, as well as the immediate provision of safe water and hygiene promotion. However, current outbreak control activities have proven insufficient to avoid massive numbers of cases and deaths in recent large-scale outbreaks. The adequate treatment of cases for example, although crucial to decrease mortality, has a limited impact in controlling disease spread. Oral cholera vaccines (OCV), which have the potential to reduce the number of cases and minimize the spread of disease, could be an important addition to the cholera response arsenal.

The World Health Organization (WHO) prequalifies the OCV Dukoral (SBL Vaccine/Crucell, Sweden) and Shanchol (ShantaBiotechnics, Hyderabad, India). Both are killed whole cell *V cholerae* O1 vaccines; Shanchol also contains *V cholerae* O139 and Dukoral the recombinant cholera toxin B subunit. The two vaccines share a good safety and efficacy profile with an estimated protection of 60–85% for 2–3 years. Although, recommended by WHO (including in response to outbreaks since 2010), their use as public health tools has been limited. Specifically, questions about the acceptability, feasibility, effectiveness, cost and potential diversion of resources have discouraged the use of OCV for outbreak control.

Dukoral showed 84% short-term protection (six months) under field conditions, and has been successfully used both in Asia and Africa. Conversely, the effectiveness of Shanchol

under field conditions needs to be determined as the efficacy of the vaccine has been only measured under experimental conditions in Kolkata, India. Furthermore, as the trial was not designed to evaluate the short-term, but rather long-term protection (at two, three and five years), the protection offered by Shanchol within the first months after vaccination remains unknown. Shanchol has important difference compared with Dukoral; its price is considerably lower (1.85 vs. 5.25 US$ per dose), it does not require buffer and occupies lower storage volume, which reduces the logistic burden to implement mass vaccination campaigns. Evidence about the protection conferred by Shanchol in the first months after administration under field conditions is essential when considering its use for outbreak response. This is especially true at a time when WHO and its partners are in the process of creating a cholera vaccine stockpile for emergency use.

In 2012, the Ministry of Health (MoH) of the Republic of Guinea, with the support of Médecins Sans Frontières (MSF), organized the first mass vaccination campaign using a two-dose oral cholera vaccine (Shanchol) as an additional control measure to respond to an on-going nationwide epidemic. This was also the first time that Shanchol was used in a mass vaccination campaign on the African continent. This project proposal aimed to gain evidence on the use of OCV to diminish cholera consequences in epidemic situations, trying also to identify critical elements for scaling up its use. Furthermore, it intended to enable the assessment of whether a reactive cholera vaccine intervention in selected, high-risk areas is a feasible, acceptable and effective strategy to reduce morbidity and mortality during future cholera outbreaks.

2. Objectives

2.1. Overall Objective

To evaluate the feasibility, acceptability and effectiveness of a mass vaccination campaign using the oral cholera vaccine Shanchol in response to an outbreak in order to identify critical elements for scaling up its use in real life situations.

This overall objective was addressed through the following specific objectives:

2.2 Specific Objectives

- Feasibility assessment of the mass vaccination campaign
    - To describe the mass vaccination campaign procedures
    - To monitor number of doses administered, the time of administration, the vaccine wastage and the costs.

- Assessment of the acceptability of the mass vaccination campaign by the population
    - To estimate the percentage of people vaccinated in the first and the second round of the mass vaccination campaign, by age group (1-4 years, 5-14 years and over 15 years old)
    - To estimate the percentage of people who received two doses of vaccine, by age group (1-4 years, 5-14 years and over 15 years old)
    - To estimate the dropout rate between the two rounds
    - To describe the reasons for not being vaccinated during the different vaccination opportunities
    - To describe the acceptability of the oral cholera vaccine used during the mass vaccination campaign.

- Surveillance of adverse events following immunization
    - To describe the adverse effects following immunization

- Evaluation of the performance of the cholera rapid diagnostic test Crystal in vaccinated individuals
    - To estimate the proportion of positive results of a cholera rapid diagnostic tests in recipients of the cholera vaccine at different time points after vaccination
    - To estimate the mean time to become negative (in those with an initial positive test after vaccination

- Estimate of the vaccine effectiveness
    - To estimate vaccine effectiveness of two complete doses of the oral bivalent cholera vaccine Shachol
    - To determine the presence or absence of bias related with the health seeking behavior that can affect the vaccine effectiveness estimates

3. Material and Methods

3.1 Feasibility of the mass vaccination campaign

A descriptive analysis of mass vaccination procedures was conducted through direct observation and on-site recording of the following information in specific registers:

- Composition and organization of vaccination teams and other participating personnel
- Number of doses administered
- Vaccine wastage

- Average time of administration and time spent in vaccination sessions
- Logistical problems faced, including transportation and storage of vaccines and water
- Availability of safe water
- Waste managment
- Overall direct costs incurred

3.2. Acceptability of the mass vaccination campaign by the population

We performed a cross-sectional cluster survey and implemented adverse event surveillance. The study population included individuals older than 12 months, eligible for vaccination, and residing in the areas targeted for vaccination (Forécariah and Boffa, Guinea). Data sources were household interviews with verification by vaccination card.

3.3. Adverse events following immunization

Surveillance of adverse events following immunization was implemented in the sites where the mass vaccination campaigns were carried out as well as in the health centers and health posts of the areas targeted by the mass vaccination campaigns for 14 days following each vaccination round. The following data were collected using a standardized form age, sex, pregnancy, history of allergies, vaccination date, consultation date, date of onset of the symptoms, type of symptoms, and clinical outcome (recovery, transfer or death).

3.4. Performance of the cholera rapid diagnostic test crystal in vaccinated individuals

A total of 108 vaccinated individuals, selected systematically among all persons older than one year, were included at vaccination sites and 106 were included in the analysis. Stools samples of this cohort of vaccinated participants were collected and tested with the rapid diagnostic test every day until the test was negative for two consecutive visits or for a maximum of 7 days.

3.5 Vaccine effectiveness

We conducted a matched case-control study between May 20 and October 19, 2012. Suspected cholera cases were confirmed by rapid test, control subjects were selected among neighbors of the same age and sex as the case-patients. The odds of vaccination were compared between case-patients and control-subjects in bivariate and adjusted conditional logistic regression models. Vaccine effectiveness was calculated as 1-odds ratio per 100.

4. Results

We administered 312,650 doses of vaccine during two vaccination rounds in two coastal districts in Guinea. The feasibility, timeliness of implementation, and delivery cost were similar to those of other mass vaccination campaigns.

In total 5,248 people were included in the household-based surveys, 3,993 in Boffa and 1,255 in Forécariah. Overall, 89.4% [95%CI:86.4–91.8%] and 87.7% [95%CI:84.2–90.6%] were vaccinated during the first round and 79.8% [95%CI:75.6–83.4%] and 82.9% [95%CI:76.6–87.7%] during the second round in Boffa and Forécariah respectively. The two dose vaccine coverage (including card and oral reporting) was 75.8% [95%CI: 71.2–75.9%] in Boffa and 75.9% [95%CI: 69.8–80.9%] in Forécariah respectively. Vaccination coverage was higher in children. The main reason for non-vaccination was absence. No severe adverse events were notified.

A total of 94.3% of cholera vaccine recipients had a positive test after vaccination; all except one of these positive results were reactive only with the O139 antigen. The mean time to become negative in those with an initial positive result after vaccination was 3.8 days.

Overall, 40 case-patients and 160 control-subjects were included in the vaccine effectiveness study for the primary analysis between June 8 and October 19, 2012. Vaccination with two complete doses was associated with significant protection against cholera, in the crude analysis and after adjustment for potentially confounders (86.6%; 95% confidence interval:

56.7 to 95.8%; P value=0.001). In the sub-analysis including only cases that were culture and/or PCR confirmed, vaccination with two complete doses was also associated with significant protection against cholera (91.6%; 95% confidence interval: 58.6 to 98.3%; P value=0.002).

5. Conclusions

- The implementation of the first vaccination campaigns with a prequalified oral cholera vaccine in response to epidemics is a feasible strategy.

- The vaccination campaign was well accepted by the population, and high vaccination coverage was achieved despite the short time available for preparation, the two-dose schedule, the remote rural setting, and the highly mobile population.

- The oral cholera vaccine Shanchol is safe when administered in mass vaccination campaigns.

- The rapid test Crystal can be used normally as soon as 24 hours after vaccination in a context of O1 epidemics, which represent the vast majority of cases, and after a period of five days in areas where *V cholerae* O139 is present.

- The effectiveness of Shanchol when used in response to outbreaks is high, which supports the addition of vaccination as an outbreak response tool.

This evidence should serve to strongly recommend the addition of OCV among the tools to be used in response to epidemics, alongside efforts to improve provision of safe water and sanitation and access to cholera treatment. In addition it served to support the creation of an oral cholera vaccine stockpile for emergency use by the World Health Organization (WHO) and partners. This work has been also considered by the Global Alliance for Vaccines and

Immunization (GAVI) in order to include the oral cholera vaccine among the vaccine supported for introduction in the next coming years.

RESUMEN EN ESPAÑOL

1. Introducción y justificación

El cólera es una enfermedad intestinal aguda que se presenta con diarrea acuosa profusa, vómitos, y deshidratación rápida, acompañado de una alta letalidad en ausencia de un tratamiento adecuado. Actualmente los brotes de cólera están causados principalmente por la cepa *Vibrio cholerae* 01 El Tor.

El suministro de agua potable y un saneamiento adecuado son sin duda la única solución a largo plazo para el control del cólera. Sin embargo, el control del cólera a nivel mundial está lejos de ser una realidad, la carga de la enfermedad está aumentando con brotes de gran amplitud declarados en los últimos años, como los de Haití en 2010 y Zimbabue en 2009. Las intervenciones usuales de respuesta frente a los brotes epidémicos de cólera se centran en el manejo clínico de los pacientes y la mejora del acceso a la atención médica, así como en el suministro de agua potable y la promoción de medidas de higiene. Sin embargo, estas actividades han demostrado no ser suficientes para evitar un elevado número de casos y muertes en los últimos brotes epidémicos. El tratamiento adecuado de los casos por ejemplo, aunque crucial para reducir la mortalidad, tiene un impacto limitado en el control de la propagación de las epidemias. Las vacunas contra el cólera, que tienen el potencial de reducir el número de casos y reducir al mismo tiempo la propagación de la enfermedad, podrían ser una herramienta adicional al arsenal de respuestas a los brotes de cólera.

La Organización Mundial de la Salud (OMS) precalifica actualmente dos vacunas contra el cólera: Dukoral (SBL Vacuna / Crucell, Suecia) y Shanchol (ShantaBiotechnics, Hyderabad, India). Ambas vacunas están compuestas por células enteras inactivadas de *V cholerae* O1; Shanchol también contiene *V cholerae* O139 y Dukoral una subunidad B recombinante de la toxina del cólera. Las dos vacunas comparten un buen perfil de seguridad y eficacia con una protección estimada de 60-85% durante 2-3 años. Aunque, recomendadas por la OMS, su uso como herramientas de salud pública ha sido muy limitado; dudas sobre la

aceptabilidad, el potencial desvío de recursos, el coste y la factibilidad de la implementación de campañas de vacunación en masa han desalentado su uso.

Dukoral ha mostrado una protección a corto plazo (seis meses) del 84% utilizada en condiciones de campo tanto en Asia como en África. Por el contrario, la eficacia de Shanchol en condiciones de campo se desconoce aún ya que la eficacia de la vacuna se ha medido sólo en condiciones experimentales en Calcuta, India. Además, el ensayo clínico de Calcuta no fue diseñado para evaluar la protección a corto plazo, sino la protección a largo plazo (a los dos, tres y cinco años), por lo que la protección ofrecida por Shanchol en los primeros meses después de la vacunación sigue siendo desconocida.

Shanchol tiene importantes diferencias en comparación con Dukoral, su precio es considerablemente más bajo (1,85 vs. 5,25 dólares EE.UU. por dosis), no requiere búfer y ocupa un volumen de almacenamiento menor, lo que reduce la carga logística para llevar a cabo campañas de vacunación en masa. Es por esto que la evidencia sobre la protección que confiere Shanchol en los primeros meses después de su administración en condiciones de campo es esencial para considerar su uso en la respuesta a brotes epidémicos.

Este estudio pretende evaluar diferentes aspectos de la la primera utilización en respuesta a una epidemia de una vacuna oral contra el cólera precalificada por la OMS. Esta fue también la primera vez que Shanchol ha sido utilizada en el continente africano, donde se registra cada año el mayor número de muertes ligadas al cólera. En 2012, el Ministerio de Salud de la República de Guinea, con el apoyo de Médicos Sin Fronteras, organizó una campaña de vacunación en masa con vacuna oral contra el cólera (Shanchol) como una medida de control adicional en la respuesta ante una epidemia de esta enfermedad. Con los diferentes estudios incluidos en este manuscrito buscamos obtener evidencia sobre el uso de vacunas orales para disminuir las consecuencias del cólera en situaciones epidémicas, tratando también de identificar los elementos críticos para extender su uso a otros contextos. Asimismo, buscamos evaluar si la vacunación contra el cólera en respuesta a brotes

epidémicos es una estrategia viable, aceptable y eficaz para reducir la morbilidad y la mortalidad durante futuras epidemias de cólera.

2. Objetivos

2.1. Objetivo general

Evaluar la factibilidad, aceptabilidad y eficacia de una campaña de vacunación en masa con la vacuna oral contra el cólera Shanchol en respuesta a un brote epidémico con el fin último de identificar los elementos críticos para ampliar su uso en situaciones epidémicas reales.

Este objetivo general fue abordado a través de los siguientes objetivos específicos:

2.2 Objetivos Específicos

- Evaluación de la factibilidad de la campaña de vacunación en masa
    - Describir los procedimientos para implementar la campaña de vacunación en masa
    - Estimar el número de dosis administradas, el tiempo de administración, la pérdida de vacunas y los costes asociados.
- Evaluación de la aceptabilidad de la campaña de vacunación en masa por la población
    - Estimar el porcentaje de personas vacunadas en las dos rondas de vacunación, por grupo de edad (1-4 años, 5-14 años y mayores de 15 años)
    - Estimar el porcentaje de personas que recibieron dos dosis de la vacuna, por grupo de edad (1-4 años, 5-14 años y mayores de 15 años)
    - Estimar la tasa de abandono entre las dos rondas de vacunación
    - Describir las razones para no vacunarse durante las diferentes oportunidades de vacunación

- Vigilancia de eventos adversos tras la inmunización
    - Describir los efectos adversos tras la vacunación
- Evaluación del test rápido Crystal para el diagnóstico de cólera en individuos vacunados
    - Estimar la proporción de resultados positivos en las personas vacunadas contra el cólera a diferentes intervalos de tiempo después de la vacunación
    - Estimar el tiempo medio para la obtención de un resultado negativo (en aquellos con una prueba positiva inicial) después de la vacunación
- Estimación de la efectividad de la vacuna
    - Estimar la eficacia de la vacuna oral bivalente contra el cólera (Shanchol) tras la administración de dos dosis completas
    - Determinar la presencia o ausencia de sesgo sobre las estimaciones de la eficacia de la vacuna relacionado con la búsqueda de tratamiento en caso de diarrea

3. Material y Métodos

3.1 Factibilidad de la campaña de vacunación en masa

Se llevó a cabo un análisis descriptivo de los procedimientos de vacunación en masa mediante a partir de la siguiente información recogida en los puntos de vacunación mediante el uso de registros destinados a tal efecto:

- Composición y organización de los equipos de vacunación y demás personal que participó en la campaña de vacunación
- El número de dosis administradas
- Proporción de vacunas perdidas
- El tiempo medio de administración y el tiempo utilizado para completar las sesiones de vacunación

- Problemas logísticos que se encontraron, incluyendo el transporte, el almacenamiento de vacunas y la distribución de agua
- Disponibilidad de agua potable
- Gestión de residuos
- Los costes totales directos

3.2 Aceptabilidad de la campaña de vacunación en masa por la población

Se realizó una encuesta transversal con muestreo en conglomerados. La población de estudio incluyó a individuos mayores de 12 meses, elegibles para la vacunación, y que residían en las zonas seleccionadas para la vacunación (Forécariah y Boffa, Guinea). Las fuentes de datos fueron las entrevistas realizadas en los hogares, con verificación de los carnets de vacunación.

3.3 Eventos adversos tras la inmunización

La vigilancia de eventos adversos tras la vacunación se llevó a cabo en los puntos de vacunación donde se implementaron las campañas de vacunación en masa, así como en los centros de salud y puestos de salud de las zonas objeto de las campañas de vacunación durante los 14 días sucesivos a cada ronda de vacunación. Los datos a continuación se recogieron a través de registros estandarizados: edad, sexo, embarazo, antecedentes de alergias, fecha de vacunación, fecha de consulta, la fecha de inicio de los síntomas, tipo de síntomas y severidad, y resultado clínico (curación, referencia a un hospital o muerte).

3.4 Rendimiento del test de diagnóstico rápido de cristal de cólera (Crystal) en los individuos vacunados

Un total de 108 personas vacunadas se incluyeron en dos puntos de vacunación. Los sujetos fueron sistemáticamente seleccionados entre todas las personas mayores de un año presentes en los puntos de vacunación. De los 108 individuos, 106 fueron incluidos en el análisis. Se recogieron muestras diarias de heces y se testaron con el test de diagnóstico

rápido para el cólera hasta que la prueba fue negativa durante dos visitas consecutivas o durante un máximo de 7 días.

3.5 Efectividad de la vacuna

Se realizó un estudio de casos y controles. Los casos sospechosos de cólera fueron confirmados por pruebas de diagnóstico rápido, se seleccionó una muestra de sujetos control entre los vecinos de la misma edad y sexo que los casos. Las odds de vacunación se compararon entre los caos y los controles con modelos de regresión logística condicional bivariados y ajustados por posibles factores de confusión. La efectividad de la vacuna se calculó como 1-odds ratio por 100.

4. Resultados

Durante las campañas de vacunación en masa en dos distritos litorales de la República de Guinea se administraron 312.650 dosis de la vacuna contra el cólera en dos rondas de vacunación. La factibilidad, el tiempo de implementación y el coste de la campaña son similares a los de otras campañas de vacunación en masa implementadas en respuesta a epidemias (como el sarampión y la meningitis).

En total 5.248 personas fueron incluidas en las encuestas poblacionales: 3.993 en Boffa y 1.255 en Forécariah. En general, el 89,4% [IC 95%:86.4-91 0,8%] y 87,7% [IC 95% :84.2-90 0,6%] fueron vacunados en la primera vuelta y el 79,8% [IC 95% :75.6-83 0,4%] y 82,9% [95% CI :76.6-87 0,7%] en la segunda ronda en Boffa y Forécariah respectivamente. La cobertura de dos dosis de la vacuna (incluyendo la información verificada en carnet de vacunación y verbalmente) fue 75,8% [IC 95%: 71,2-75,9%] en Boffa y 75,9% [IC 95%: 69,8-80,9%] en Forécariah respectivamente. La cobertura de vacunación fue mayor en los niños. La razón principal para no vacunarse fue la ausencia durante la campaña de vacunación. No se notificó ningún evento adverso grave.

Entre las personas que recibieron la vacuna del cólera, un 94.3% tuvo un resultado positivo en el test de diagnóstico rápido para el cólera; todos estos resultados positivos excepto uno fueron exclusivamente positivos al antígeno O139. El tiempo medio para obtener un resultado negativo en los pacientes con un resultado positivo después de la vacunación inicial fue de 3,8 días.

En total, 40 casos y 160 de controles fueron incluidos en el análisis principal de efectividad de la vacuna. La vacunación con dos dosis completas se asoció con una protección significativa contra el cólera en el análisis crudo y tras ajustar por potenciales factores de confusión (efectividad: 86,6%, IC 95%: 56,7-95,8%, valor p = 0,001). En el sub-análisis incluyendo sólo los casos que tuvieron un resultado positivo en el cultivo y / o en la PCR, la vacunación con dos dosis completas también se asoció con una protección significativa contra el cólera (efectividad: 91,6%, IC 95%: 58,6 a 98,3%, valor p = 0,002).

5. Conclusiones

- La primera utilización de una vacuna oral contra el cólera precualificada por la OMS en respuesta a una epidemia fue una estrategia viable.

- La campaña de vacunacion fue bien aceptada por la población, y la elevada cobertura vacunal se logró a pesar del poco tiempo disponible para la preparación, la pauta de dos dosis, un entorno rural de difícil acceso y una población con gran movilidad.

- La vacuna contra el cólera oral Shanchol es segura cuando se administra en las campañas de vacunación en masa.

- El test diagnóstico rápido Crystal se puede utilizar normalmente tras la vacunación en un contexto de epidemias de *V cholerae* O1, que representan la gran mayoría de

los casos, y después de un período de cinco días en las zonas donde *V cholerae* O139 está presente.

- La eficacia de Shanchol cuando se utiliza en respuesta a los brotes es alta, lo que apoya la inclusión de la vacunación como una herramienta de respuesta situaciones epidémicas.

La evidencia presentada en este trabajo sirvió para recomendar la incorporación de vacunas orales contra el cólera entre las herramientas de respuesta a las epidemias de cólera. Así mismo, sirvió a la creación por parte de la OMS y sus colaboradores de un stock de vacunas contra el cólera para uso en situaciones de emergencia sanitaria. Este trabajo ha sido considerado por la Global Alliance for Vaccines and Immunisation (GAVI) para incluir las vacunas contra el cólera entre las vacunas cuya introducción será apoyada en los próximos años.

1. INTRODUCTION

1.1 History of cholera and global disease burden

Cholera is an infectious disease that produces acute profuse watery diarrhea, vomiting, rapid dehydration and high mortality in absence of adequate treatment. Cholera is caused by the gram-negative bacteria *Vibrio cholerae* and it is one of the oldest diseases affecting humans [1–4]. The earliest reports of dehydrating diarrhea were recorded in Sanskrit in the 5th century BC. Hippocrates also reported the symptoms of cholera in several documents. The disease has existed in the Indian subcontinent for centuries. The first contemporary reporting of epidemic cholera was from Garcia del Huerto, a Portuguese physician working in India. During the epidemic that affected London between 1849 and 1854, John Snow proposed that cholera was a communicable disease and that stool contained infectious material. He suggested that this infectious material could contaminate drinking water supplies, resulting in transmission of cholera. Filippo Pacini, working independently in Italy in 1854, was the first to observ comma-shaped forms under a microscope in cholera stools. In 1884, Robert Koch first isolated *V cholerae* in pure culture in Egypt and India.

Six pandemics have been registered between 1817 and 1923. All of them started in the Ganges delta and were caused by *V cholerae* O1, Classical biotype. The ongoing 7th pandemic is caused by *V cholerae* O1, El Tor biotype, which was first reported in Indonesia in 1961, reached the Indian subcontinent in 1966 and then spread to the Middle East. It reached Africa in 1970 and extended rapidly throughout the continent, creating new endemic zones that had not been affected by cholera for over a century. It took another 20 years for the 7th pandemic to reach the Americas: the first cases were reported in Peru in 1991 and within one year the disease spread throughout Latin America. A new strain appeared in 1992: *V cholerae* O139 (Bengal). This new strain has in principle the potential to emerge as the 8th pandemic and to replace *V cholerae* O1 El Tor.

Although cholera has disappeared from the diseases affecting the high income countries, controlling cholera globally is far from being achieved and it remains one of the main causes of morbidity and mortality in the poorest areas of the world [5,6]. Moreover, the disease burden is increasing with large-scale outbreaks reported in the past several years, such as those in Haiti and Zimbabwe [7].. Every year an estimated 3 million cases of cholera and about 100,000 deaths occur worldwide [7]. The reported cholera case fatality rates remain high in many countries particurally among vulnerable groups and high-risk areas [8]. The burden of the 7th pandemic has shifted from South and Central America to the African continent. Currently African countries account for the highest proportion of cholera cases and deaths reported worldwide. However, it is likely that a high underreporting of cases occurs in Southern Asian countries

1.2 Causal agent

The *Vibrio cholera*, part of the family *Vibrionaceae*, is a Gram-negative, non-spore forming, comma-shaped bacterium, 1.4—2.6μm long. The *V cholerae* is capable of having both, a respiratory and a fermentative metabolism. The bacterium is oxidase-positive, reduces nitrate and is motile through a single, sheathed, polar flagellum [1,2,9]. The *V cholerae* has two circular chromosomes, with 4 million base pairs of DNA sequence and 3,885 predicted genes. Chromosome 1 has ~3 million base pairs and chromosome 2 has ~1 million base pairs [10]. The first chromosome contains the crucial genes for toxicity, regulation of toxicity and relevant cellular functions. The *V cholerae* contains a genomic island of pathogenicity called vibrio pathogenicity island (VPI) and is lysogenized with phage DNA that contains the genome of the cholera toxin (CT), which makes the bacterium pathogenic [10].

The optimal growing conditions include salty water, alkaline media and warm temperatures. Although *V cholerae* can as well survive at low temperatures and it is able to

grow in water of low salinity if it is warm and rich in organic nutrients. The bacteria does not survive to acid media and boiling. The optimal conditions for growing are found in estuarine environments, where zooplankton and shellfish are abundant [11,12] *V cholerae* enters in a viable but non-culturable form in water [3,13,14]

The serological classification of *V cholerae* is based in differences in the sugar composition of the heat-stable surface O-antigen (lipopolysaccharide). Currently, more than 200 different O serogroups have been described. Thus far, strains belonging to O group 1 (O1) are responsible for all the cholera pandemics. Strains that do not agglutinate with the O1-antiserum are called non-O1; they can cause only sporadic infections and do not have the potential to cause epidemics.

The strains of the serovar O1 consist of two biotypes, classical and El Tor. Antigenic factors allow further differentiating into serotypes. Both El Tor and Classic biotypes are divided into 3 serotypes: Ogawa (A and B antigens), Inaba (A and C antigens) and Hikojima (A, B and C antigens). The three serotypes can co-exist during an epidemic because of the flexibility of the bacteria to mutate between serotypes. Only recently, cholera infections in India and Bangladesh which subsequently were also reported in several parts of the Asia were caused by a novel non-O1 strain, the O139 Bengal [15,16]. The strain O139 Bengal closely resembles biotype El Tor of the serovar O1 and it most likely derived from *V cholerae* O1 El Tor by lateral transfer of a genomic island substituting the O139 for the O1 antigen [17–19].

Although early isolates of *V cholerae* O1 were susceptible to most antibiotics, *V cholerae* O139 and recent isolates of *V cholerae* O1 El Tor have acquired antibiotic resistance. Resistance to co-trimoxazole and streptomycin, which is mediated by the acquisition of an SXT element, has been described in most of the isolates in the last decade [20]. In the last year additional resistance has been described against tetracycline, erythromycin, ciprofloxacin, chloramphenicol, nalidixic acid or imipenem [21–25].

1.3 Pathogenesis

The clinical characteristics of the disease caused by *V cholerae* O1 and O139 strains are equivalent. Both serogroups produce the clinical signs and symptoms by producing an enterotoxin that promotes the secretion of fluid and electrolytes into the lumen of the small intestine [9].

To reach the small intestine, the *V cholerae* needs to be ingested in enough quantity. Most of the bacteria are killed by the gastric acid, but some of them arrive at the small intestine, where they colonize the endothelium cells. Therefore the use of antacids and histamine receptor blockers increases the risk of cholera infection and predisposes patients to more severe disease as a result of reduced gastric acidity. The same applies to patients with chronic gastritis secondary to *Helicobacter pylori* infection or those with gastrostomy. Retinol deficiency has been also associated with a higher risk of severe diseases [26,27]. As well, individuals with O blood group are prone to more severe diseases [28–31]. It has been suggested that virulence of *V cholerae* can be modulated by enteropathogenic bacteria and parasites [32,33].

The main virulence factors of *V cholerae* are the CT, a pilus that is required for colonization (toxin-coregulated pilus - TCP) and a membrane complex that regulates the production of TCP (ToxR). The genes of the CT are encoded within the genome of a bacteriophage (CTXφ) [34]. The classical and El Tor strains have different version of the CTXφ. The surface receptor of the CTXφ is the TCP. The genome of the TCP is encoded in a vibrio pathogenicity island (VPI) [35,36].

The enterotoxin is a protein molecule composed of 5 B subunits and 2 A subunits [37]. The B subunits are responsible for binding to a ganglioside (monosialosyl ganglioside, GM1)

receptor located on the surface of the cells that line the intestinal mucosa. The activation of the A1 subunit by adenylate cyclase is responsible for the net increase in cyclic adenosine monophosphate (cAMP). cAMP blocks the absorption of sodium and chloride by the microvilli and promotes the secretion of chloride and water [38–40]. The result is an isotonic watery diarrhea. Unless the lost fluid and electrolytes are replaced adequately and rapidly, the infected person may develop shock from intense dehydration and acidosis from loss of bicarbonate [9].

1.4 Epidemiology

Cholera is a disease of poverty and closely linked to poor sanitation and a lack of clean drinking water. Humans are the main natural host for *V cholerae* and constitute the main cholera reservoir. Nonetheless, *V cholerae* is naturally present in the environment and the existence of environmental reservoirs in association with copepods and other zooplankton has been documented in salty water and estuaries. The disease is transmitted by fecal-oral route.

Cholera spreads both as an endemic disease and in epidemics. Endemic cholera has been defined by the World Health Organization (WHO) as the occurrence of fecal culture-confirmed cholera diarrhea in a population in at least three of the past five years [7]. Cholera occurs endemically in south and south-east Asia and in Africa, where it may also cause major outbreaks. Young children living in endemic areas are the most frequently affected by the disease, but any age group may suffer cholera infection, especially during epidemics [41].

John Snow, one of the founders of modern epidemiology, showed the importance of descriptive epidemiology in cholera epidemics, emphasizing the importance of the

consideration of space, to target prevention and control activities [42]. Today, resources and tools for mapping are available; however, the description of place in cholera epidemics remains poor and examples of studies using spatial technologies in the medical literature are limited [43–51]. Some recent examples in cholera outbreaks where the spatial component was analyzed have shown the existence of "hotspots" where some sub-populations are exposed at higher risk of infection [52–55]. In addition, it has been suggested that these hotspots can contribute in a higher extent to the progression of the epidemics [56]. The identification of high risk areas is crucial to properly target prevention and control strategies (see Annex 1: related publications 1 and 2).

The work conducted by John Snow in London in the 19th century also established contaminated water as the main route of cholera transmission [40] . Nonetheless, since then John Snow himself and others have suggested that other routes of transmission are also important. Studies have shown the importance of the foodborne transmission in cholera outbreaks [57,58] and the preventive effect of using acidifiers in food [59]. Other means of transmission, such as certain burial practices, are also possible [60], and secondary cases at household level can be involved thereby maintaining transmission(see Annex 1: related publication 3) [61–64].

The possibility of direct person-to-person transmission through poor hygiene, patients' fluids or infected clothes has been considered less important due to the high infectious dose (>105 vibrios) needed to produce infection under laboratory conditions [65,66]. The infectious dose of *V cholerae* required to cause clinical disease varies by the mode of administration. Recent studies have suggested that *V cholerae* from human stool is hyperinfectious compared with laboratory-grown bacteria (700-fold increase), which means a lower infectious dose and therefore a higher probability of human-to-human transmission [67–69]. The incubation period varies from 12 hours to 5 days [17].

1.5 Diagnosis

Cholera diagnosis relies on the microbiological identification of the pathogen by stool culture, which remains the gold standard to confirm the diagnosis. However, this procedure requires laboratory infrastructure, adequate transport procedures and trained staff [70]. As rapid diagnostic tests (RDT) require less time, a minimum laboratory infrastructure and basic technical skills, they are used to confirm cholera outbreaks in places where high laboratory standards are difficult to obtain [71].

In 2003, the Institut Pasteur developed a cholera RDT based on the qualitative detection of the lipopolysaccharide (LPS) antigen of both, Vibrio cholerae O1 and O139 serogroups, from stool specimens. This test uses one-step, vertical-flow immunochromatography principle and monoclonal antibodies against the core and O-specific polysaccharides of each serogroup for capture and detection of antigens [72,73]. The O1 specific antigenic determinant is common to Ogawa and Inaba serotypes [73,74] and the one for O139 is common to both O139 capsular polysaccharide and LPS. This cross-reactivity between O139 LPS and capsular polysaccharide explains that antibodies react with both encapsulated and non-encapsulated *V cholerae* O139 strains [75]. The RDT is produced by Span Diagnostics (Surat, India) under the trade name Crystal VC [70,76]. Several evaluations have shown good sensitivity, ranging from 92% to 100% [72,77,78]. In contrast, the specificity was lower and most evaluations in field conditions have shown specificities from 71% to 77% when compared with culture as the gold standard [76–79]. Nevertheless, the use of culture as gold standard may underestimate specificity, and re-analysis of the data using statistical methods for evaluation with an imperfect gold standard showed that the specificity could be around 85% [80]. After these evaluations, the manufacturer SPAN changed the test presentation (order of the lines and addition of a dilution buffer), but the test in this new version has not been formally evaluated. This test is widely used for epidemiological purposes during outbreaks. However, given that the RDT Crystal VC detects the LPS antigens of *V cholerae* O1 and O139 in feces, which are also contained in the oral vaccine Shanchol, the stools of

vaccinated individuals could potentially become positive by the rapid test due to the vaccine only, in the absence of viable bacteria.

1.6 Clinical features

The typical presentation of cholera is a sudden onset of profuse painless watery stools, sometimes rice-water like, often accompanied by vomiting [81]. There is no fever. Dehydration appears within 12 to 24 hours. Cholera can cause as high as 20 to 50% mortality if case management is not adequate. Conversely, mortality is low (<2%) if well treated [9].

In moderate forms of the disease there are frequent watery stools but fluid loss and dehydration are moderate. In severe forms there is intense diarrhea and vomiting with significant fluid loss, more than 10 to 20 liters/day. Dry cholera is a rare condition which is characterized by little diarrhea and/or vomiting and a rapid collapse due to severe acute dehydration and a high mortality rate. Death before arrival at the treatment center is frequent [82].

The presentation of cholera differs between endemic and epidemic settings. In endemic settings, rates of asymptomatic *V cholerae* infection ranges from 40% to 80% [3], and cholera can present as a mild diarrhea indistinguishable from infection by other enteropathogens. The most severe cases of cholera in endemic settings are concentrated among young children and previously unexposed individuals. During an epidemic, severe disease occurs in adults as frequently as in children and it is associated with high case-fatality rates [83,84].

The degree of dehydration is graded according to the symptoms and signs that reflect the amount of fluid lost. Hypovolemic shock and death can occur quickly if rehydration therapy is not provided [85]. The degree of dehydration should be systematically evaluated

at admission and re-evaluated during the hospital stay. Patients are classified in three treatment plans according to the degree of dehydration (see table below) [86]:

- Plan A: No dehydration. Because clinical status may deteriorate rapidly, these patients may initially need to be kept under monitoring, especially when they live far from the treatment centre or when correct home treatment cannot be guaranteed.

- Plan B: Moderate dehydration. Patients must be admitted to the treatment centre to receive treatment as indicated below and be monitored until diarrhea/vomiting stops.

- Plan C: Severe dehydration. Patients must be admitted to the treatment centre to receive treatment as indicated below and be monitored until diarrhea/vomiting stops.

After dehydration, hypoglycemia is the most common lethal complication of cholera in children [87]. Hypoglycemia is the result of diminished food intake during acute illness. Acute pulmonary edema is related to over-hydration, due to excessive IV rehydration. It is a common risk among elderly, young children and severely anemic patients. Renal failure (anuria) is a rare complication that can occur when a shock is not rapidly corrected. Urine output normally resumes within 6 to 8 hours after starting rehydration [88,89]. Hypokalemia should be suspected if repeated episodes of painful cramps occur [89].

1.7 Treatment

In an epidemic situation, any patient with diarrhea and/or vomiting is a suspect cholera case. The most important element of cholera treatment is rapid replacement of the water and salts lost through diarrhea and vomiting. Most patients can be treated using oral

rehydration solution (ORS) alone [82,90]. Only severely dehydrated patients need the administration of intravenous fluid (IV). IV fluid might be also required in patients with profuse vomiting. Antibiotic therapy may reduce the volume of diarrhea and carriage time of *V cholerae* in severely ill patients; however antibiotic treatment is not strictly needed in most cholera patients.

Patients without signs of dehydration and with moderate dehydration should be rehydrated orally using the standard WHO-ORS (plans A and B) [91,92], as more potassium, bicarbonate and glucose are available in ORS than in the IV fluids [82,90]. IV is required in patients with severe dehydration, with IV needs around 200mL/Kg (plan C) [86,93]. Ringer Lactate solution is the best option. It provides an adequate concentration of sodium, some potassium and enough lactate, which is metabolized into bicarbonate for the correction of acidosis. Oral rehydration should be started as soon as the patient is able to drink. The patient's condition must be assessed every 30 minutes during the first 2 hours, then every hour for the next 6-12 hours. Monitoring is based on pulse and respiratory rates; and the frequency of urine, stool, and vomiting [86].

In severe cases, antibiotics can reduce the volume of diarrhea and carriage time of Vibrio [94]. Effective antibiotics shorten the duration of diarrhea and reduce the volume of stools losses, while they reduce the duration of shedding bacteria in stools. Most cholera patients are cured by rehydration and do not need antibiotics. Antibiotics are indicated for patients with severe dehydration and/or complications and are given after IV rehydration. Before introducing antibiotics, it is important to check sensitivity and adjust the antibiotic choice accordingly as resistance to different antibiotics has been documented worldwide [95–101].

Zinc supplementation after childhood diarrhea also reduces the incidence of subsequent episodes of diarrhea for several months [102,103]. WHO recommends zinc for children younger than 5 years of age with diarrhea. Children with diarrhea in developing countries also benefit from supplementation with vitamin A [104].

1.8 Prevention

Cholera prevention is based in access to safe water and proper sanitation as well as adhesion to safe food-handling practices [86]. In areas where the safety of water is not ensured, it is recommended to boil the water or treat it with a chlorine product before consumption, to store it in clean, covered containers and to bottle it with unbroken seals.

Hygiene is also a pillar of cholera prevention. It is important to wash hands often with soap and safe water: before eating or preparing food, before feeding or cleaning children, after using the latrine or toilet and after taking care of someone ill with diarrhea.

In order to limit the spread of the diseases it is important to use latrines or bury the feces and not defecate in any body of water. It is also important to clean latrines and surfaces contaminated with feces using a chlorine solution.

Intense information, education and communication activities are required in order to achieve improvements in the quality of the water at the point of use, sanitation and hygiene practices. These activities require strong coordination from partners involved in cholera prevention and controls. Clear and easy to follow messages need to be provided to the population, especially during cholera outbreaks [105].

1.9 Cholera vaccines

Widespread use of oral cholera vaccines (OCV) began in the 1960s. The vaccines then in use were composed of whole *V cholerae* O1 cells, killed using formalin, phenol or heat, and administered by injection. In the 1970s, the interest in these injected whole cell vaccines decreased [106], as it was perceived that they had a low efficacy (around 50%), provided only short-term immunity (3 to 6 months), and had an unacceptable rate of side effects. A Cochrane review, however, found that the duration and efficacy of the whole cell injected vaccines may have been underestimated; it was 54% at seven months (based on 18 trials) and 46% at one year (based on 14 trials). Protection waned by the second year in children under five, but persisted into the third year for those over the age of five years [107,108]. Nevertheless, injected vaccines are no longer in use or available, and attention is now focused on vaccines administered by the oral route.

Vaccines work by stimulating immunity against a pathogen which has been killed, attenuated or otherwise rendered incapable of causing disease, in order to prevent or mitigate the effects of infection with the natural pathogen if it subsequently occurs. The route of administration of a vaccine may influence its immunogenicity and acceptability. Oral vaccines have the potential to stimulate local immunity within the mucosa of the gut, preventing the colonisation and multiplication of *V cholerae*. Since cholera is transmitted orally, oral vaccines may thus have more direct effect than injected vaccines which stimulate immunity in the blood. Oral vaccines are also potentially easier to administer, more acceptable to patients, and have a reduced risk of transmitting blood borne infections [109].

Two main types of oral vaccines have been investigated in clinical trials: inactivated vaccines (containing killed whole cells of *V cholerae*), and live attenuated vaccines (containing genetically modified, non-pathogenic strains of *V cholerae*). In addition, subunit vaccines have been tested which consist only of cell components (antigens). The live

attenuated vaccines are usually given as a single dose, whereas killed whole cell vaccines may require two or three doses at one to two week intervals to produce an adequate immunological response. Three vaccine formulations are currently available [4]:

• WC-rBS (Dukoral): A monovalent inactivated vaccine containing killed whole cells of *V cholerae* O1 plus additional recombinant cholera toxin B subunit. Produced by SBL Vaccine/Crucell, Sweden.

• BivWC (Shanchol): A bivalent inactivated vaccine containing killed whole cells of V cholerae O1 and *V cholerae* O139. Produced by Shantha Biotechnics, India.

• BivWC (mORCVAX): A bivalent inactivated vaccine containing killed whole cells of V cholerae O1 and *V cholerae* O139. Produced by VABIOTECH, Vietnam and only available in Vietnam.

Both WC-rBS (Dukoral) and BivWC (Shanchol) vaccines are prequalified by the WHO.

1.9.1 Dukoral (WC-rBS)

Dukoral was developed in Sweden and first licensed in 1991. It is licensed in more than 60 countries, primarily as a vaccine for travelers to cholera-endemic areas. However, it has also been used in crisis situations in Indonesia, Sudan and Uganda, and in a demonstration project in an endemic area of Mozambique. Dukoral is a monovalent vaccine based on formalin and heat-killed whole cells (WC) of *V cholerae* O1 (classical and El Tor, Inaba and Ogawa) plus recombinant cholera toxin B subunit. The B subunit of cholera toxin was originally produced chemically (WC-BS) but is now produced by recombinant technology (WC-rBS). BS and rBS are practically identical in terms of immune response. To protect the toxin B subunit from being destroyed by gastric acid, the vaccine must be given with a bicarbonate buffer. The vaccine is provided in 3 ml single-dose vials together with the

bicarbonate buffer (effervescent granules in sachets). Vaccine and buffer are mixed in 150 ml of water (chlorinated or not) for persons aged >5 years and in 75 ml of water for children aged 2–5 years. The vaccine has a shelf life of 3 years at 2–8 °C and remains stable for 1 month at 37 °C.

Both prelicensure studies and postmarketing surveillance have demonstrated that Dukoral has a good safety profile, as has also been found safe in pregnant women and in HIV-infected or other immunocompromised individuals. In clinical trials involving around 240,000 participants, adverse events were no more common in vaccinees than in placebo recipients. The adverse events consisted primarily of mild abdominal discomfort, pain or diarrhoea, all of which were mainly attributed to the buffer solution given to both groups. Only 63 adverse reactions were associated with more than 1,000,000 doses of the vaccine sold in Scandinavia during 1992–2003. Dukoral stimulates the production of both antibacterial and antitoxin antibodies, including immunoglobulin A antibodies produced locally in the intestines. The vaccine has been tested in randomized placebo-controlled double-blind prelicensure efficacy trials in both Bangladesh and Peru.

The Matlab trial in Bangladesh [110] involved 62,285 children aged 2–15 years and women aged >16 years. At the time of the trial, El Tor and classical cholera strains co-circulated in the study population. At 4–6 months following WC-BS immunization, the combined protective efficacy against El Tor and classical cholera for vaccinees aged >2 years was 85% (95% confidence interval [CI], 56–95%), dropping to 62% (95% CI, 46–74%) after 1 year of follow up [111]. During the second and third years of follow up, the protective efficacies were 58% (95% CI, 40–71%) and 18% (95% CI, 21–44%), respectively. The cumulative efficacy of the 2 doses over 3 years was 51% (95% CI, 40–60%) against El Tor and classical cholera combined; it was slightly lower against El Tor than against classical cholera [112].

The Matlab results differed considerably among young children and older children and adults. Among children aged 2–5 years, the level of protection against El Tor and classical

cholera combined was 100% (95% CI, 80–100%) at 4–6 months following vaccination; it dropped to 38% (95% CI, 1–62%) at the end of 1 year, to 47% (95% CI, 4–71%) during the second year and to 0% thereafter [111]. The protective efficacy for people aged >5 years was 78% (95% CI, 61–87%) at 1 year and 63% (95% CI, 41–77%) during the second year following immunization. Two doses of the WC-BS vaccine were as protective as 3 doses in people aged >6 years [112,113].During the first year of surveillance in Matlab, recipients of the vaccine made 26% fewer visits to treatment-centres for diarrhoea due to any cause and also had a 26% lower rate of mortality from all causes [113].

Studies of vaccine efficacy similar to the Matlab trials were conducted with Dukoral in Peru during the cholera epidemics in the 1990s. The vaccine conferred 86% protection against El Tor cholera among military aged 16–45 years during the first 4–5 months after vaccination [114]. In a trial in the outskirts of Lima, the vaccine showed no protection during the first year in any age group after 2 doses, but the study was criticized as lacking rigor during the observation period [115]. Following a booster dose given 10 months after the primary series, the vaccine conferred 61% (95% CI, 28–79%) protection in the second year against cholera and 82% (95% CI, 27–96%) against cholera requiring hospitalization [116].

During 2003–2004, the field effectiveness of Dukoral was studied in Beira, Mozambique, in an area where cholera is endemic and there is a high prevalence of HIV [117]. This case-control study included 4 age-matched and sex matched neighbourhood controls for each of the 43 culture-confirmed cases; vaccine effectiveness at 1–6 months after vaccination was 84% (95% CI, 43–95%) among people who received 2 doses; it was 78% (95% CI, 39–92%) among those who received 1 or 2 doses; it was 82% (95% CI, 19–98%) among children aged 2–4 years who received 1 or 2 doses; and it was 67% (95% CI, 16–86%) among people aged ≥5 years who received 1 or 2 doses. Furthermore, since the El Tor strain of *V cholerae* O1 that expresses the classical cholera toxin was responsible for all cases in this setting, the results show that Dukoral protects against this important El Tor variant.

1.9.2 Shanchol and mORCVAX

The closely related bivalent OCV Shanchol and mORCVAX are based on serogroups O1 and O139. Unlike Dukoral, these vaccines do not contain the bacterial toxin B subunit . The original ORCVAX was licensed in Viet Nam in 1997.

From 1998 to 2009, >20 million doses of this vaccine were administered to children in high-risk areas of Vietnam, making ORCVAX the first oral cholera vaccine to be used primarily for endemic populations. In cooperation with the International Vaccine Institute in Korea, ORCVAX was significantly reformulated in 2004 to meet the requirements of WHO and good manufacturing practices. This involved replacing a high toxin-producing strain with the 2 *V cholerae* strains contained in the original Swedish vaccine and doubling the quantities of lipopolysaccharide antigen. Following successful phase II trials in India and Vietnam, this vaccine was licensed in 2009 as mORCVAX in Vietnam and as Shanchol in India; mORCVAX is currently intended for domestic use in Vietnam, whereas Shanchol is produced for Indian and international markets. Shanchol is provided in single-dose vials, mORCVAX in singledose and 5-dose vials. The vaccine has a shelf life of 2 years at 2–8 °C. (Stability tests at ambient temperatures are continuing). According to the manufacturer, Shanchol should be administered orally in 2 liquid doses 14 days apart for individuals aged ≥1 year. A booster dose is recommended after 2 years.

Shanchol and mORCVAX are considered safe and effective vaccines [118,119]. The original WC cholera vaccine included in the Bangladesh trials in the 1980s provided less short-term protection than Dukoral against El Tor and classical cholera, but at 2 years and 3 years of follow up the protection was equal to, or better than with Dukoral [112].

A modification of the original vaccine was evaluated 8–10 months after immunization in an open, controlled trial involving 334 000 residents of the Vietnamese city of Hue during an El Tor outbreak in 1992–1993. The protective efficacy of the vaccine for all ages after 2 doses was 66% (95% CI, 46–79%), and similar results were obtained in children aged 1–5 years and

adults [120]. The overall effectiveness of this vaccine 3–5 years after vaccination was 50% (95% CI, 9–63%) [121]. Following the addition of the O139 strain, the resulting bivalent vaccine was shown in non-inferiority trials to be safe and immunogenic against both O1 and O139 infection. In 2006, a cluster-randomized placebo-controlled double-blind phase III trial of Shanchol that includes 66 900 participants aged >1 year was conducted in slum districts of Kolkata, India. An interim analysis after 2 years of follow up showed an overall protective efficacy of 67% against culture-confirmed cholera among those who received 2 doses [122]. The vaccine was found to be protective in all age groups including in children aged 1–4 years, and the protection showed no decline during the second year of follow up.12 Follow up will continue for 5 years. The cumulative protective efficacy at 5 years was 65% (95% CI, 52-74%), and the study suggested no evidence of decline in protective efficacy over time [123].

1.10 Context: The Republic of Guinea

The Republic of Guinea borders six countries and has a large coastline to the west (Figure 1). This coastal area has two main ports, one in the capital of Conakry, and the second in Kamsar. Guinea is divided into four natural regions: the coastal area (Lower Guinea or Maritime Guinea), the mountainous area of Middle Guinea, the savannah zone (Upper Guinea) and the equatorial forest zone (Forest Guinea). The population was estimated to be 10 million in 2008, with a density of 38.5 inhabitants / km ^{2}. Population density is much higher in and around the capital Conakry, along the coastal strip and in Forest Guinea bordering Liberia and Sierra Leone.

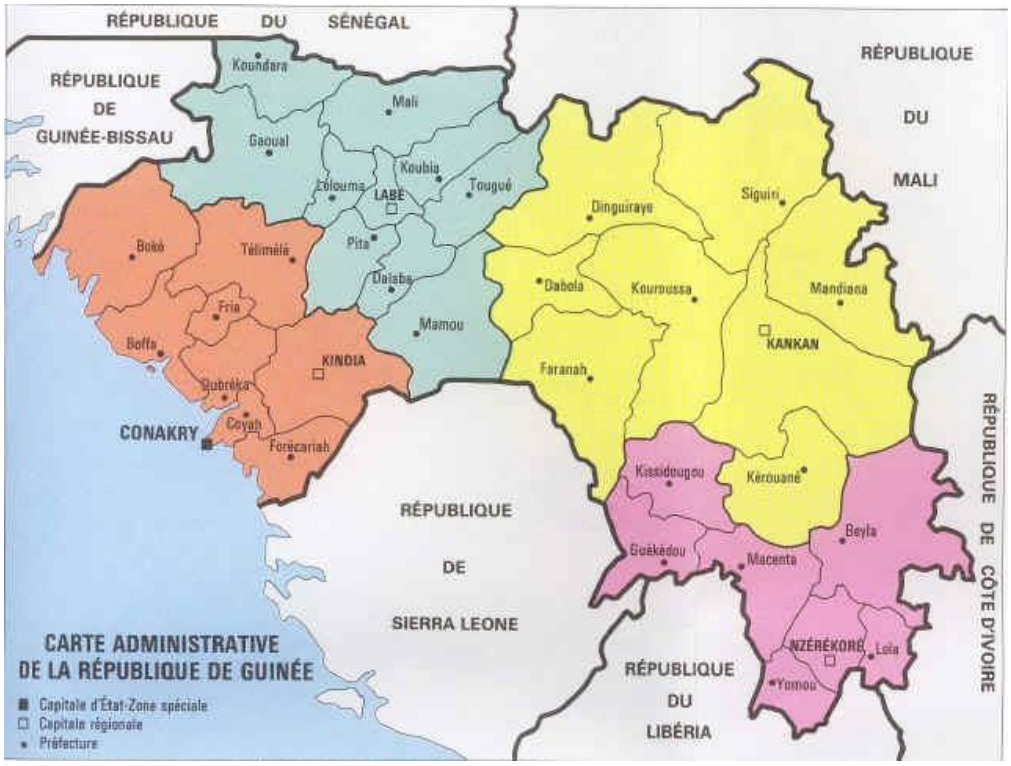

Figure 1. Administrative map of the Republic of Guinea. Orange, Basse Guinée or Guinée Maritime; in blue Moyenne Guinée; in yellow Haute Guinée and in pink Guinée Forestière. Source: http://www.nozay44.com/guinee/

1.11 Cholera in Guinea

1.11.1 Previous cholera outbreaks

The 7th cholera pandemic began in 1961 with the cases reported from Africa in 1970. West Africa was affected including Guinea; where the first epidemic began in the capital city of Conakry and affected only two coastal prefectures, Conakry and Forécariah. Over the past fifty years, Guinea has suffered repeated epidemics interspersed with intervals without cases (Figure 2). In 1978 and 1986, cholera was reported only in the coastal prefectures. The largest reported epidemic occurred in 1994, with more than 30,000 cases and 670 deaths. In this epidemic, the continental (inland) prefectures were affected the first time; although the most affected areas remained the coastal prefectures and the islands [124].

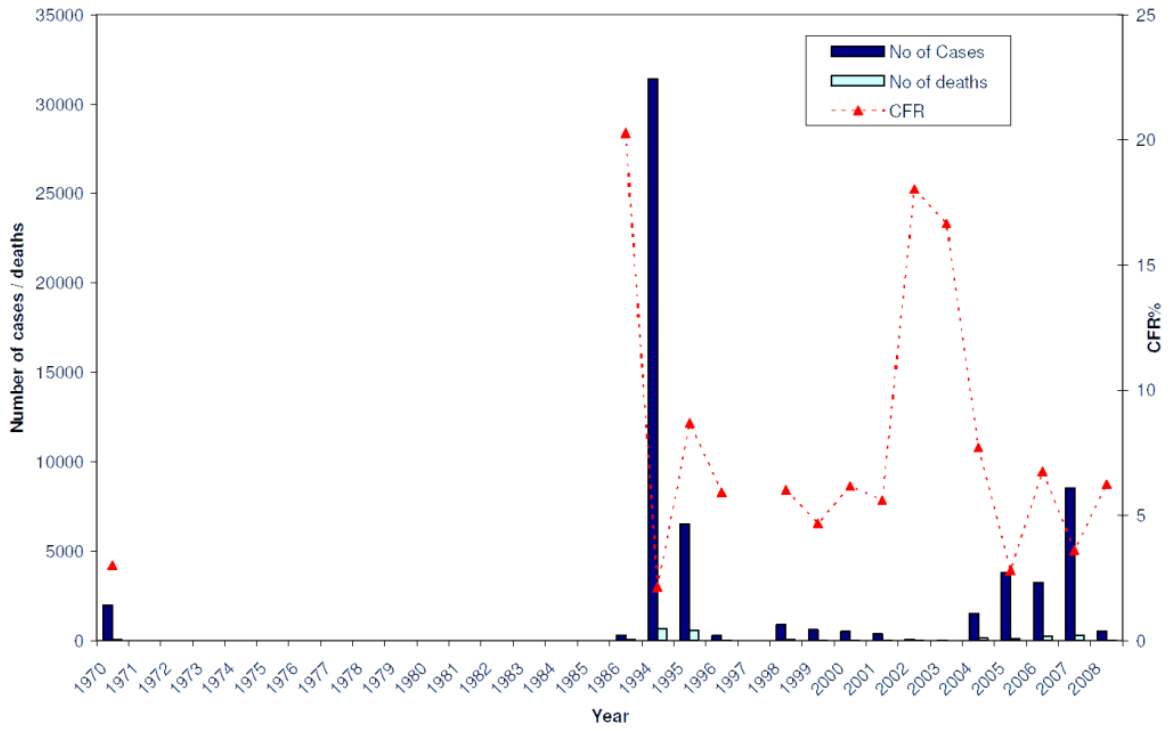

Figure 2. Cholera cases, deaths and case fatality ratio (CFR) reported to the World Health Organization between 1970 and 2008. Source: World Health Organization.

However, from 2003 to 2007, cholera outbreaks were reported each year, with increasing intensity. Maritime Guinea (and mainly the city of Conakry) and Forest Guinea were most affected. There was a clear association between weekly precipitation and number of cholera cases, with all epidemics peaking during the rainy season (July-August). In 2007, the outbreak began early in the year and was associated with a large number of reported cases. From 2008, only sporadic cases were reported, mainly in Kindia region of Maritime Guinea southwest of Conakry [125].

1.11.2 Cholera in Forecariah and Boffa

Forecariah and Boffa prefectures are both located in Maritime Guinea. Forecariah prefecture is located in Kindia region and Boffa prefecture in Boké region (northeast of Conakry).

From 2003 to 2009, Forecariah prefecture reported 5.0% of cholera cases and 6.0% of deaths from Guinea. Forecariah has been affected by a cholera outbreak each year from 2004 to 2007. It is part of a national priority area for cholera prevention and control, in particular the sub-prefectures of Kaback, Kakossa, Kalia and Benti [125].

From 2003 to 2009, Boffa prefecture reported 4.7% of cholera cases and 4.6% of deaths from Guinea. During this period, epidemics were reported in 4 out of 7 years, with and overall attack rate of 36.9 per 10,000 inhabitants and a case fatality rate of 4.4%. In Boffa prefecture, the coastal sub-prefectures were the most affected, with attack rates ranging from 29.3 to 81.6 per 10,000 inhabitants for this period. The coastal sub-prefectures (Tougnifily, Douprou, Boffa-centre et Koba) were considered as national priority areas for cholera prevention and control [125].

1.11.3 Cholera epidemic in 2012

The first cases of cholera were reported in Forecariah in the region of Kindia. A total of 147 cases and 13 deaths were reported from February 2nd to March 8th, 2012, followed by a decrease in the number of cases. On March 3rd, the first case was reported and confirmed in Conakry. After four years without cholera outbreak in Guinea, these local epidemics have emerged well before the rainy season signalling a high risk of large outbreak. A cholera outbreak was also on going in neighbouring Sierra Leone, with 13,934 cases and 232 deaths reported countrywide between January and August 2012 [126].

The regional nature of the epidemic, the early notification of cases before the peak of the rainy season and the long interval without outbreaks, thereby increasing the number of susceptible individuals due to lack of prior exposure, all suggested the possibility of a large epidemic in Guinea in 2012. Case management, water, health education, hygiene and

sanitation interventions were implemented in response to the outbreak. In addition, non-selective mass vaccination campaigns were implemented in the prefectures of Boffa and Forécariah (Figure 3) by Ministry of Health (MoH) of Guinea, with the support of Médecins Sans Frontières (MSF). This was the first cholera outbreak response in Africa using an OCV, and also the first time that Shanchol was used in a mass vaccination campaign on the African continent.

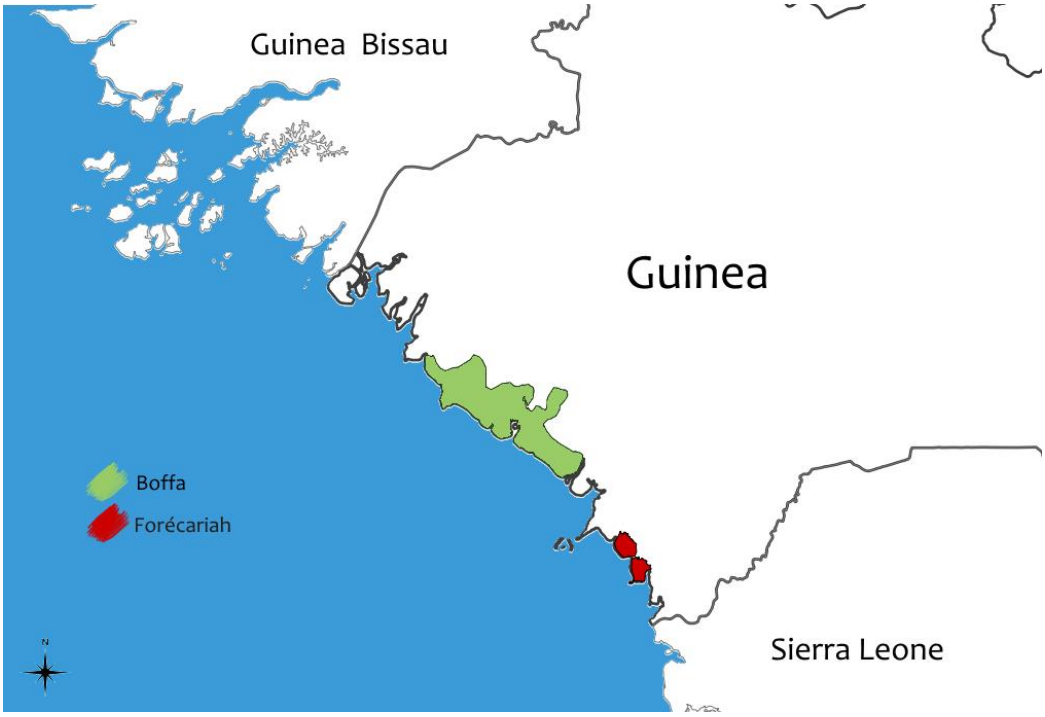

Figure 3: Target areas for the non-selective mass vaccination campaigns, Guinea, 2012.

2. JUSTIFICATION

Provision of safe water and proper sanitation are without doubt the long-term and only solution for cholera control [17,127]. However, controlling cholera globally is far from being achieved; the disease burden is increasing with large-scale outbreaks reported in the past several years, such as those in Haiti and Zimbabwe [7]. Current outbreak response interventions focus on case management and access to health care, as well as the immediate provision of safe water and hygiene promotion [17]. However, current outbreak control activities have proven insufficient to avoid massive numbers of cases and deaths in recent large-scale outbreaks [128]. The adequate treatment of cases for example, although crucial to decrease mortality, has a limited impact in controlling disease spread [7,17].

Oral cholera vaccines, which have the potential to reduce the number of cases and minimize the spread of disease [56,129], could be an important addition to the cholera response arsenal [5,9,17]. The World Health Organization (WHO) prequalifies the OCV Dukoral (SBL Vaccine/Crucell, Sweden) and Shanchol (ShantaBiotechnics, Hyderabad, India). Both are killed whole cell *V cholerae* O1 vaccines; Shanchol also contains *V cholerae* O139 and Dukoral the recombinant cholera toxin B subunit. The two vaccines share a good safety and efficacy profile with an estimated protection of 60–85% for 2–3 years [17]. Although, recommended by WHO (including in response to outbreaks since 2010) [4], their use as public health tools has been limited. Specifically, questions about the acceptability, feasibility, cost and potential diversion of resources have discouraged the use of OCV for outbreak control [130].

Dukoral showed 84% short-term protection (six months) under field conditions, and has been successfully used both in Asia and Africa [110,117]. Conversely, the effectiveness of Shanchol under field conditions needs to be determined as the efficacy of the vaccine has been only measured under experimental conditions in Kolkata, India [122]. Furthermore, as the trial was not designed to evaluate the short-term, but rather long-term protection (at two, three and five years), the protection offered by Shanchol within the first months after vaccination remains unknown [122,131,132].

Shanchol has important differences compared with Dukoral; its price is considerably lower (1.85 vs. 5.25 US$ per dose [133]), it does not require buffer and occupies lower storage volume [134], which reduces the logistic burden to implement mass vaccination campaigns. Evidence about the protection conferred by Shanchol in the first months after administration under field conditions is essential when considering its use for outbreak response. This is especially true at a time when WHO and its partners are in the process of creating a cholera vaccine stockpile for emergency use [135].

It is urgent to identify new tools and approaches for cholera control in Africa. The World Health Organization (WHO) has recommended that demonstration projects using OCV be performed in order to know whether, and in which circumstances, cholera vaccines may assist in the fight against cholera.

This project proposal aimed to gain evidence on the use of OCV to diminish cholera consequences in epidemic situations, trying also to identify critical elements for scaling up its use. Furthermore, it intended to enable the assessment of whether a reactive cholera vaccine intervention in selected, high-risk areas is a feasible, acceptable and effective strategy to reduce morbidity and mortality during future cholera outbreaks.

3. OBJECTIVES

3.1 Overall Objective

To evaluate the feasibility, acceptability and effectiveness of a mass vaccination campaign using the oral cholera vaccine Shanchol in response to an outbreak in order to identify critical elements for scaling up its use in real life situations.

This overall objective was addressed through the following specific objectives:

3.2 Specific Objectives

- Feasibility assessment of the mass vaccination campaign (see Annex 1: related publication 4)
    - To describe the mass vaccination campaign procedures
    - To monitor number of doses administered, the time of administration, the quantity of consumable and non-consumable materials used, the costs, the and vaccine wastage.

- Assessment of the acceptability of the mass vaccination campaign by the population (see Annex 1: related publication 5)
    - To estimate the percentage of people vaccinated in the first and the second round of the mass vaccination campaigns, by age group (1-4 years, 5-14 years and over 15 years old)
    - To estimate the percentage of people who received two doses of vaccine, by age group (1-4 years, 5-14 years and over 15 years old)
    - To estimate the dropout rate between the two rounds
    - To describe the reasons for not being vaccinated during the different vaccination opportunities

  - To describe the acceptability of the oral cholera vaccine used during the mass vaccination campaigns

- Surveillance of adverse events following immunization (see Annex 1: related publication 5)
  - To describe the adverse effects following immunization

- Evaluation of the performance of the cholera RDT Crystal in vaccinated individuals (see Annex 1: related publication 6)
  - To estimate the proportion of positive results of a cholera rapid diagnostic tests in recipients of the cholera vaccine at different time points after vaccination
  - To estimate the mean time to become negative (in those with an initial positive test) after vaccination

- Estimate of the vaccine effectiveness (see Annex 1: related publication 7)
  - To estimate vaccine effectiveness of two complete doses of the oral bivalent cholera vaccine Shachol
  - To determine the presence or absence of bias related with the health seeking behavior that can affect the vaccine effectiveness estimates

4. MATERIAL AND METHODS

4.1 Study oversight and overall design

This study was funded by MSF. The study protocol was approved by the Ethical Review Boards of the Republic of Guinea and MSF. Vaccine and treatment were provided free of charge and participation in the study was voluntary. Written consent was obtained from participants or their parents/guardians for the vaccine effectiveness study and oral informed consent was obtained for the acceptability assessment.

The study design included five components: the feasibility assessment, surveillance of adverse events following immunization, the evaluation of the RDT in vaccinated individuals, a household based survey to assess acceptability and case-control study to estimate the vaccine effectiveness study. Figure 4 shows a summary diagram of the study design.

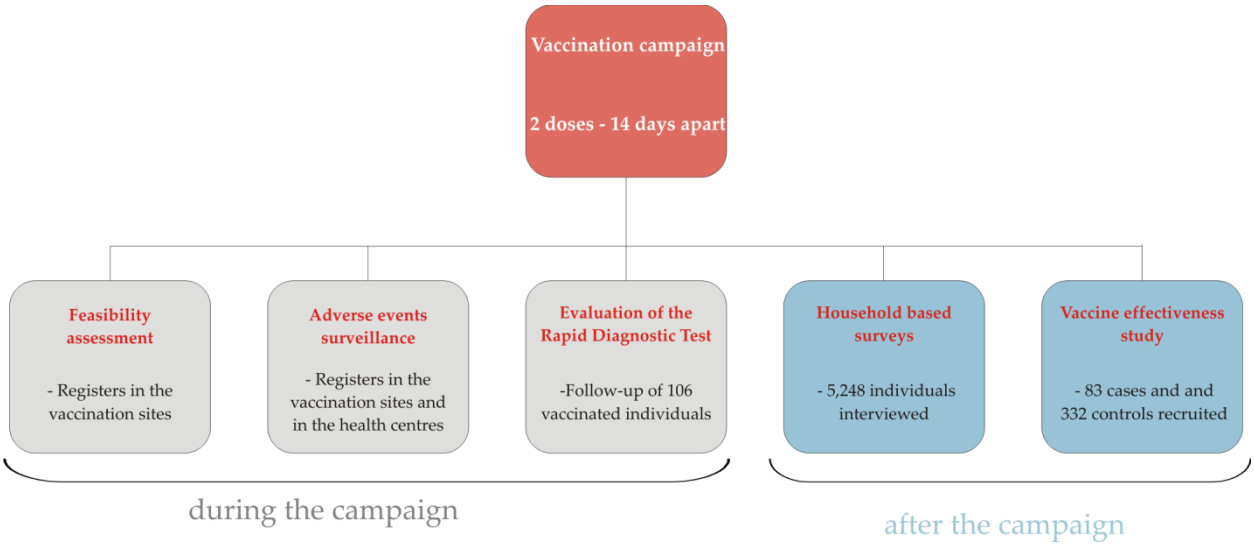

Figure 4: Summary flow chart of the design

4.2 Study population

Non-selective mass vaccination campaigns were implemented in the prefectures of Boffa and Forécariah. In Boffa, the coastal part of the six sub-prefectures bordering the ocean

(Koba, Boffa-centre, Douprou, Tougnifily, and part of Mankountan and Tamita) were vaccinated from April 18 to May 14, and in Forécariah, the sub-prefectures of Kaback and Kakossa were vaccinated from May 27 to June 15 (Figure 3). During the second round, soap and a bottle chlorine solution was distributed by the Red Cross to all women of childbearing age presenting for vaccination.

These campaigns targeted all residents in the targeted areas 1 year of age and older. The campaigns were organized by the MoH of Guinea, with the support of MSF.

4.3 Feasibility assessment of the mass vaccination campaign

A descriptive analysis of mass vaccination procedures was conducted through direct observation and on-site recording of the following information in specific registers (see Annex 2):

- Composition and organization of vaccination teams and other participating personnel
- Number of doses administered
- Vaccine wastage
- Average time of administration and time spent in vaccination sessions
- Logistical problems faced, including transportation and storage of vaccines and water
- Availability of safe water
- Waste managment
- Overall direct costs incurred

4.4 Assessment of the acceptability of the mass vaccination campaign by the population

4.4.1 Study design

A representative sample of the population in each of the survey sites (Boffa and Forecariah) was selected using cluster based sampling with population proportional to size [136]. The survey sample was selected among all resident older than one year at the time of the survey. Residents were defined as a persons living (sleeping and eating) in the area for at least two weeks. Ascertainment of vaccine status was done by examination of individual vaccination cards, as well as oral reporting of the vaccination status.

Definitions

- "Fully vaccinated" person was defined as an individual who received 2 complete doses of cholera vaccine (this was verified either by vaccination card or by interview).

- "Incompletely vaccinated" was defined as an individual who took only one dose or who spitted out or vomited one of the two doses of vaccine (this was verified either by vaccination card or interview).

- "Unvaccinated" was defined as an individual who had no vaccination card and who confirmed on interview that she/he received no dose.

- "Unknown vaccination status" was defined as an individual who had no vaccination card and who was unable to describe their vaccination status.

4.4.2 Sample size calculation

The sample size was calculated to obtain a representative estimate of the proportion of residents who received two doses of the OCV by age group. Children 1-4 years old represent the smallest proportion of the overall population, so the sample size was calculated to ensure a sufficiently precise estimate for this age group.

The following assumptions were considered: 70% of residents received two doses of vaccine, alpha error of 5%, absolute precision of 7% for Boffa and 10% for Forecariah, design effect of 3 for Boffa and 1.5 for Forécariah (since the coverage was expected to be more homogenous in the Forecariah islands).

The sample size required was therefore 494 children aged 1 to 4 years for Boffa and 121 children for Forecariah. Considering a 10% of missing data, it was planned to survey 543 children aged 1 to 4 years in Boffa and 133 children in Forecariah. Taking into account the results of the Demographic and Health Survey of 2005, it was expected 0.7 children 1-4 year old per household in Guinea (6.1 individuals per household and 16% of the population under 5 years old). It is was planned to visit 776 households (60 clusters of 13 households) in Boffa and 180 households (30 clusters of 6 households) in Forecariah.

4.4.3 Sampling procedure

The clusters were allocated proportionally to the population size of the sub-prefecture, district and the sector. Within the selected sectors, the households were numbered. In order to select the first household of the cluster, a random number was chosen among the total number of households in the sector. Subsequent households were selected by proximity (first household to the left).

In urban area of Boffa and in Kaback Island of Forecariah satellite based sampling was used to select the starting point of the cluster. Cluster allocation was also proportional to the population size, but a satellite photo was used to select randomly the starting point [137]. This alternative methodology was use in urban Boffa because of the large number of households to enumerate and in Kaback because of the absence of accurate population data per sector.

A household was defined as a group of people sleeping under the same roof and sharing meals every day for at least the previous 2 weeks. Data concerning all the residents of the selected households eligible for the vaccination were collected.

4.4.4 Recruitment methodology

Prior to the beginning of the survey, all surveyors and supervisors were recruited locally and received a theoretical and practical training of three days. Training consisted of survey and interview methodology and a pilot implementation of the questionnaire.

A total of 22 surveyors divided into 11 teams in Boffa and 4 surveyors in Forecariah conducted the survey. Each team consisted of two interviewers. In Boffa, each supervisor supervised 2 to 3 teams and 2 surveyors in Forecariah.

The teams visited every selected household in each cluster. They conducted face-to-face interviews with the most senior adult household member. If the person was absent, the information was provided by the next most senior adult household member. Survey teams asked for the help of neighbours to trace absentees and re-visit empty (but not abandoned) households later in the day. If during the second visit the occupants could not be found or if they refused to participate, that household was skipped.

4.4.5 Data collection

A standardized pre-piloted questionnaire (Annex 3) was used to collect the following data per each household member:

- Demographic: age, sex, household size

- Vaccination status: verbal and card confirmation

- Reasons for non-vaccination: open question

- Acceptability: side effects, taste and beliefs about the vaccine (only in participants older than 15 years). Acceptability was only collected in Boffa (first site where the mass vaccination campaign was implemented).

Interviews were conducted in the local language (Soussou).

4.4.6 Data entry and analysis

The main outcome was OCV coverage (single and full course) in each of the targeted locations for vaccination. Secondary outcomes included vaccine coverage by age group, and reasons for non-vaccination. Crude vaccination coverage estimates were obtained considering the survey design. The design effect was calculated to estimate the loss of precision due to the cluster based sampling strategy. Sampling weights were calculated at each level to account for the different cluster size.

4.5 Surveillance of adverse events following immunization

4.5.1 Definition of side effects

An adverse events following immunization (AEFI) was defined as a medical occurrence detected by the vaccination site supervisor or a physician with an onset up to 14 days after receipt of a dose of vaccine [138].

4.5.2 Data collection and analysis

AEFI surveillance was implemented in the sites where the mass vaccination campaigns were carried out as well as in the health centres and health posts of the areas targeted by the mass vaccination campaigns for 14 days following each vaccination round.

Data were collected either at the vaccination site by the medical team supervisor if the side effect was observed soon after taking the vaccine. Data were also collected in health posts and health centres if the side effect occurred with a larger delay and the person sought care in the health structure.

The following data were collected using a standardized form (Annex 4): age, sex, pregnancy, history of allergies, vaccination date, consultation date, date of onset of the symptoms, type of symptoms, and clinical outcome (recovery, transfer or death).

Patients who experienced a side effect after vaccination were described in terms of age, sex, time between intake of the vaccine and the onset of the side effect and symptoms.

4.6 Evaluation of the performance of the cholera rapid diagnostic test Crystal in vaccinated individuals

4.6.1 Setting and study design

The study took place in Kabak (Forécariah Prefecture, Guinea) during the second round of the mass vaccination campaign carried out by the MoH and MSF in June 2012. The study population corresponded to the population targeted by the vaccination campaign (all residents of Kabak aged one year and above). Individuals were included if they were vaccinated and accepted to participate. They were excluded if they had watery diarrhea on inclusion (to exclude potential cholera cases) and/or a high probability of not being present for all the follow-up visits. The cohort of vaccinated participants meeting study criteria was followed-up prospectively.

It was estimated that 96 individuals were needed to achieve a minimum precision of 10% around a proportion of 50% of positive RDT, as there were no data on the prevalence of positive tests in the vaccinated population. The sample size was increased to 106 to account for an expected 10% of loss to follow-up. A systematic sampling method (one every 10 individual) was used in every vaccination site.

4.6.2 Recruitment and follow-up procedures

Participants were recruited in 4 of the 31 vaccination sites, selected arbitrarily, as vaccination sites were not thought to have any influence on the study outcomes. Demographic information was collected at inclusion through a face-to-face interview (mainly in Soussou, the local language) and information on stool production and basic clinical symptoms during follow-up visits using an individual standardized case report form (CRF). Participants were asked to collect stool in a pot provided by the study team. Participants' homes were visited daily to collect stool specimens, complete a follow-up form

and to provide them with a new pot for the next stool. The stools were transported to the laboratory and tested them with the RDT. Laboratory technicians completed the information with the RDT results. Follow-up was considered finalized when 2 consecutive negative RDT results were obtained or after 7 days.

4.6.3 Field use of the rapid diagnostic test

The stool samples were tested with the RDT at Kabak Health Center following the manufacturer's instructions by a laboratory technician trained to the use of the test. Crystal VC tests used were manufactured in 2011 and 2012 by Span Diagnostics Ltd., India (catalogue reference number 161C101-10). A small portion of stool was mixed with a buffer and 200 µL (4 drops) of the mix was placed in a test tube. The dipstick test was left in the tube for 20 minutes before reading. If only the control line appeared, the test was negative. If 2 or 3 lines appeared, the test was positive for either *V cholerae* O139, O1, or both. If the control line was absent, the test was considered invalid and repeated once.

4.6.4 Laboratory control of the rapid diagnostic test

Ten by ten dilutions of the Shanchol vaccine were prepared using the dilution buffer provided in the RDT kit. Undiluted and diluted vaccine solutions up to a 10^9-fold dilution were tested with the RDT following the manufacturer's recommendations.

A bacterial suspension adjusted to an optical density at 600 nm (OD600nm) of 0.8 was prepared in the dilution buffer provided in the RDT kit from an overnight culture of *V cholerae* O1 and O139 strains. Such an OD value was previously estimated to correspond to $2x10^8$ *V cholerae* / mL by colony counting of 10-fold serial dilutions spread on agar plates and incubated over night at 37°C. This initial solution was used to prepare solutions at $2x10^7$ and $2x10^6$ bacteria / mL using the dilution buffer provided in the kit, undiluted and diluted solutions were tested with the RDT following the manufacturer's recommendations.

4.6.5 Data analysis

Qualitative variables were described through their frequency and percentages. Continuous variables were described through their mean, median, standard deviation (SD) and percentiles (P25 and P75). The proportion of positive results for O1 or O139 for each day of follow-up was calculated including in the numerator the number of positive results and in the denominator the sum of the total number of tests performed and the number of cases for whom follow-up was stopped after obtaining two consecutive negative results. Missing data (absent or no stool sample) were excluded from this calculation. The 95% exact confidence intervals (95%CI) of the proportion estimate were calculated. To estimate the mean time to obtain a negative RDT result after vaccination (time to become negative), the number of days needed to obtain a first negative result was counted in the group of people who obtained previously a positive result for O1 or O139 after vaccination. Statistically significant differences by gender and age were assessed with a linear regression model. A p value <0.05 was considered significant.

4.7 Vaccine effectiveness study

4.7.1 Surveillance for cholera

Cholera is one of the eight diseases under weekly surveillance in Guinea and included in the Early Warning System. Notifications are done every week from the peripheral health centres to the "Directions Préfectorales de la Santé", from there to the "Directions Régionales de la Santé" and finally every Wednesday to the central level, the "Division de Prévention et de Lutte contre la Maladie".

The epidemiologic surveillance system was reinforced in 2012 that included supplementary training of the medical staff on the case definition, the importance of prompt and systematic notification of all suspected cholera cases and the use of specific registers for cholera. Medical staff was also trained on the use of rapid diagnostic cholera tests (RDT) and on the importance of consistent analysis of surveillance data.

The reinforced surveillance system was established in the following sites:
- In the prefecture of Boffa: in the six sub-prefectures targeted by the mass vaccination campaigns (Koba Tamita, Boffa, Douprou, Tougnifily and Mankountan). These six sub-prefectures include a hospital, six health centres and 23 health posts.
- In the prefecture of Forecariah: in the sub-prefectures targeted by mass vaccination campaigns (Kaback and Kakossa). These sub-prefectures include two health centres, three health posts and a cholera treatment centre. The health centres and health posts received cases arriving from the ports and quays located near the two islands (Mafarenyah health centre, and the health posts of Madinagbé and Mambala).

The WHO cholera case definitions are used in Guinea. In agreement with the Provincial Divisions of Health, in the areas targeted by mass vaccination campaigns the definition of a suspected cholera case in the 2012 epidemic was the following: anyone suffering from acute watery diarrhoea (at least 3 loose stools in 24 hours) with or without vomiting. A confirmed case was defined as any suspected case with a positive stool sample to Vibrio cholera O1 or O139 (by rapid test, culture or PCR). Systematic testing with RDT was done in the health centers and a sub-sample of stools was sent to the Institute Pasteur in Paris for culture and PCR analysis (see laboratory procedures bellow). A specific register was implemented in each surveillance structure beginning on April 14th 2012 in Boffa and May 26th 2012 in Forecariah.

For each suspected case, the following data were collected: date of admission, date of symptom onset, age, sex, town or village of residence, state of hydration at admission,

vaccination status (number of cholera vaccine doses received), discharge date, discharge status (recovered, abandoned, transferred or death), and the result of RDT (negative / positive).

4.7.2 Case–control studies

The case subjects and controls were the residents of study areas. Case subjects with cholera were compared with controls who did not have diarrhoea. In an attempt to assess whether the results with respect to effectiveness could be attributed to bias, case subjects with non-choleric diarrhea (negative result to the RDT) were also compared with controls who did not have diarrhea. Study staff who enrolled the case subjects and controls and who obtained information on vaccination status and other exposure variables were unaware of whether V. cholera was confirmed from the case subject and of how the information on vaccination status was to be used in the analysis.

4.7.3 Definition and Selection of Case Subjects

All suspected cholera cases seeking care in a health center of the study area between one week after the end of the vaccination campaigns and October 31, 2012, were eligible to be included as case-patients if they provided written informed consent (Annex 5) and fulfilled the following criteria: resident in the study area since April 16, 2012; older than 12 months; a positive cholera RDT; and their residence could be located after discharge. Only the first episode of acute watery diarrhea was included.

To assess whether effectiveness results could be attributed to bias, case-patients with non-choleric diarrhea (negative RDT result) were also compared with control-subjects that did not have diarrhea (indicator bias analysis) [117].

4.7.4 Definition and Selection of Controls

A systematic selection procedure was used to recruit four neighbour controls for each case subject. Starting from every third house to the left of the case subject's house, every consecutive house was visited until one eligible control was enrolled. The procedure was then repeated starting from every third house to the left of the control subject's house until four controls were recruited. Only one control was recruited per household. A neighbour of the same sex and within the same age group (1 to 4, 5 to 9, 10 to 19, 20 to 29, 30 to 39 or more than 40 years of age) as the case subject was eligible to be a control if he or she had not sought treatment for diarrhea at the Cholera Treatment Center between January 1, 2012, and the date of onset of the matched case subject's diarrheal illness and if he or she would have sought treatment at the Cholera Treatment Center if severe, watery diarrhea had developed. Eligibility for selection also required the same informed-consent, residency and age criteria as those applied to the case subjects.

4.7.5 Ascertainment of Vaccination and Potentially Confounding Variables

Receipt of the cholera vaccine during the mass immunization program was ascertained in face-to-face home interviews of the case subject and controls. Participants were asked whether they had been vaccinated and, if so, to show the vaccination cards distributed during the campaign. For those who reported that they had been vaccinated but were not in possession of a card, vaccination status and the completeness of dose ingestion were ascertained by oral reporting. Demographic, socioeconomic, and environmental variables were ascertained through special questionnaires administered to case subjects and controls (Annex 6).

4.7.6 Laboratory procedures

For each patient included in the study, a stool sample was collected and used to perform a RDT (Crystal-VC tests, SPAN Diagnostics, India, Lot numbers: 4000007832 and 4000008589).

The doctor/nurse in charge of the health centre performed the test according to the manufacturer's instructions for use, after training by the study team. Results were interpreted according to the manufacturer's instructions. If the control line did not appear, irrespective of other lines, the test was considered invalid and repeated once.

In additional, for a sub-sample of a filter paper disc was dipped into fresh stool and placed into a microtube with 2 to 3 drops of normal saline solution (NaCl 0.9%). Tubes were kept at room temperature and sent to Institut Pasteur, Paris for isolation of *V cholerae* according to standard methods [139]. PCR was systematically performed on all specimens. Detection of the *rfb* was done as described by Hoshino *et al.* [140]. Presence of PCR inhibitors and bacterial DNA were respectively controlled by PCR amplification of an exogenous internal positive control (Applied Biosystems TaqMan) incorporated to each sample and amplification of the 16S rRNA gene.

4.7.7 Statistical analysis

The primary analysis assessed the protection conferred by the receipt of two completely ingested doses of vaccine against confirmed cholera by RDT. It was calculated that 90 cases and 360 controls (ratio 1:4) would be needed assuming 50% vaccine effectiveness, alpha error 5% and 80% power. The secondary analysis assessed the protection conferred by an incomplete course of vaccine (one complete dose or incomplete dose(s) due to spitting or vomiting part of a dose) against confirmed cholera by RDT. A sub-analysis was also conducted considering as case-patients: (i) those with presence of *V cholerae* confirmed by culture and/or PCR and (ii) those with diarrhea but with a negative result to the RDT (indicator bias analysis).

The odds of vaccination between case-patients and control-subjects was compared conditional through logistic regression to account for the matching design; a model with indicator variables was fitted for non-vaccinated, incomplete and complete dosing. The level of vaccine protection was estimated as (1- odds ratio) x 100.

Demographic, environmental, and socioeconomic factors were compared between case-patients and their matched control-subjects in order to assess their potential as confounders of vaccine protection. Variables with a P values <0.2 in the bivariate models were considered as possible confounders. Adjusted estimated of vaccine protection by co-variables that significantly contributed to improve the likelihood of the model were calculated. All P values and 95% confidence intervals were two-sided. Statistical significance was determined as a P value less than 0.05.

4.8 Data entry and data analysis tools

Data entry was performed using EpiData 3.1 (EpiData Association, Odense, Denmark) in specific data entry mask created for each sub-study. Data analysis was performed using Stata 12.0 (College Station, TX, USA) for all the studies. Maps and geographical analysis were conducted using R 2.14 Statistical Package.

5. RESULTS

5.1 Description of the cholera outbreak in Guinea in 2012

In 2012, the first cholera case was reported in Forécariah (Maritime Guinea) on February 2. Both the Microbiology National Laboratory and the Institut Pasteur in Paris confirmed that the circulating strain was *Vibrio cholerae* O1 El Tor-Ogawa. Further studies based on genetic markers analysis showed that it was an hybrid El Tor strain possessing the classical B subunit cholera toxin gene (*ctxB1* genotype) [141]. From February 2 to October 31, a total of 7,350 cases including 133 deaths were reported to the WHO. This number of cases corresponds to an attack rate of 6.4 per 10,000 people. The case fatality ratio (CFR) per 100 cases was 1.8 at country level. The peak of the epidemic was observed in week 34, in which 1,152 cases were reported (MoH data, Figure 5). At country level, the vast majority of cases were reported during the rainy season.

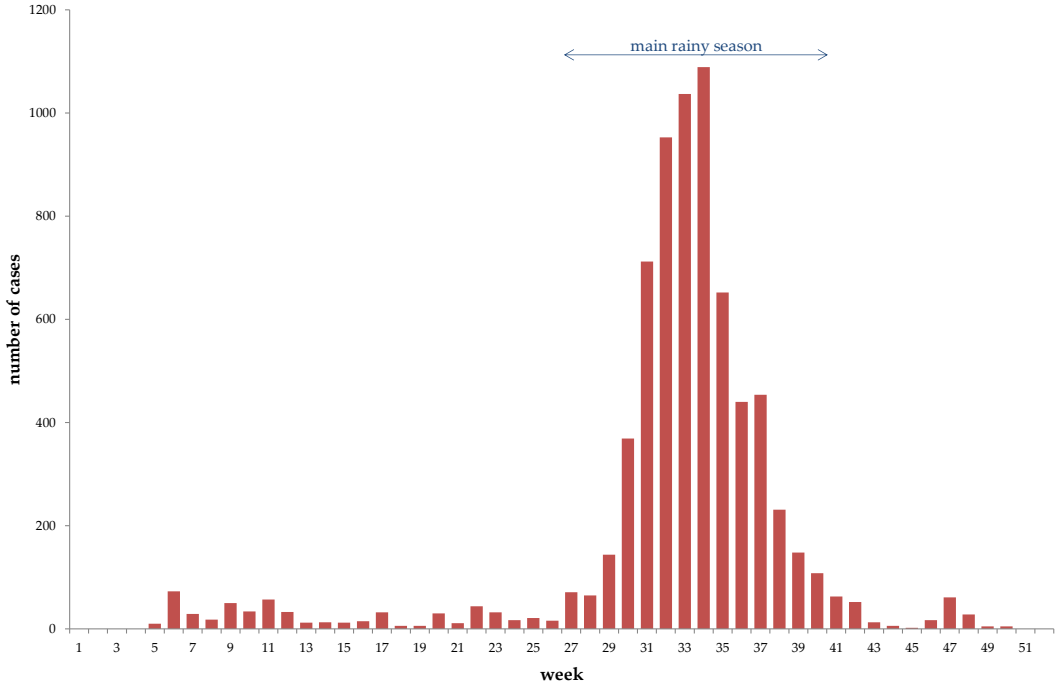

Figure 5: Suspected cholera cases reported in Guinea in 2012 per week.

Figure 6 shows a more detailed description of the geographical distribution of the epidemic. Four prefectures had attack rates over 15 cases per 10,000 individuals (Conakry, Dubréka,

Coyah and Fria); in Fria and Conakry the attack rate reached 27 and 26 cases per 10,000 respectively.

In the city of Conakry, 4,617 cases were reported, which represent 63% of the total number of cases at country level. The first case was declared in Conakry in week 22 (i.e. 17 weeks after the first notification in Forécariah). In Conakry, the peak of the epidemic was observed in epidemiological week 34 in which 727 cases were reported.

Figure 6. Cholera attack rates per sub-prefecture in Guinea 2012.

The median age of the patients was 25 years old (inter quartile range: 16-37). The number of reported cases was similar in men (49%) and women (51%).

The epidemic evolved with a different dynamic in the vaccinated areas compared with the un-vaccinated areas (Figure 7). In the prefectures of Boffa and Forécariah, 283 and 344 cases

were respectively reported in 2012. In the country as a whole, 93% of the cases were reported after week 24, when the implementation of the vaccination campaigns ended. Conversely, in the vaccinated areas of Boffa and Forécariah the percentage of cases reported after the implementation of the vaccination campaigns was respectively 45% and 16% (Figure 7).

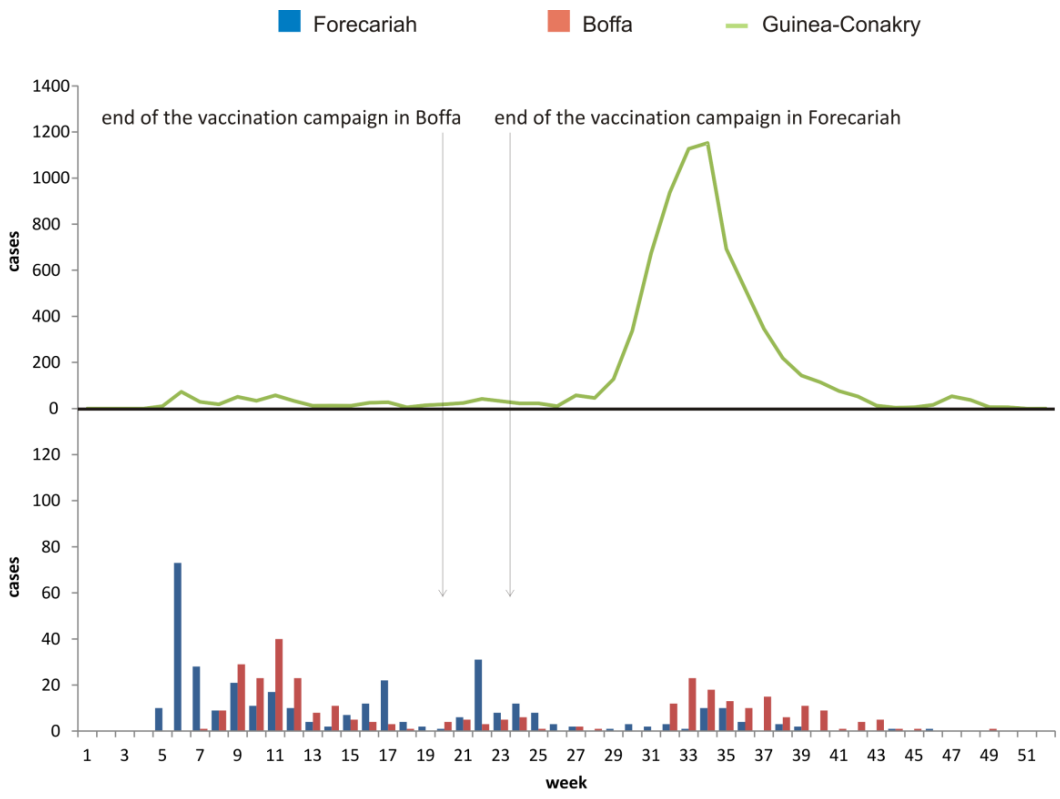

Figure 7. Evolution of the outbreak in the country and in the vaccinated prefectures.

5.2 Feasibility assessment of the mass vaccination campaign

5.2.1 Vaccine procurement, storage, and transport

The bulk of the vaccine supply (320,000 doses) was shipped directly from the manufacturer in India, and 50,000 additional doses from MSF stock in Kampala, Uganda. The volume of the transport containers of vaccine was 29 m^3. Vaccines were transported from Conakry's airport to the district capital in refrigerated trucks and stored in the field in refrigerated trucks or containers. Vaccines reached the field within 2 weeks of the order date.

Vaccine was supplied in individual vials, either in secondary packing of 35 vials or in individual secondary packing inside tertiary packing of 10 vials (Figure 8). One vaccine vial in the 35-vial package had a volume of 13.5 cm^3, about five times greater than a dose of measles vaccine.

Figure 8: Shanchol vaccine in 35 vials packing or in individual secondary packing inside tertiary packing of 10 vials .

5.2.2 Vaccination teams and training

The identification of team members and team composition, as well as identification of each team leader was done by the MoH. Teams and team leaders were identified from each sous-prefecture for the work in their area. Team leaders were MoH medical staff, while the team members could be either medical staff or identified members of community, not

necessarily medical (mostly community health worker, or Red Cross volunteers – people already involved in public health activities).

Each team was composed of 9 fixed team members in Boffa (1 team leader, 2 registrators, 2 vaccinators, 2 preparators, 1 tally sheet, 1 log aid). In addition, community members were hired in Boffa and Forécariah on daily basis to assist at vaccination sites (crier, up to 4 people to fill in vaccination cards, up to 6 people for crowd control). The total number of people working at vaccination sites varied between 9 and 20 in Boffa. In Forécariah, smaller teams of 5 people (1 team leader, 1 registration, 1 preparator, 1 vaccinator and 1 tally sheet) were used and between 2 and 8 daily workers were recruited in addition at each vaccination site. For the second round, Red Cross volunteer joined the team for the distribution of chlorine and soap. In total, 43 teams were created to comple the mass vaccination campaigns in Boffa and Forécariah (Figure 9).

Figure 9: Vaccination team at work.

An initial training was organized before the first vaccination round to explain the basic rational of the intervention and vaccination. Another meeting was organized before the second round to take lessons learned and prepare the second round. Training included small introduction to cholera and cholera vaccination, set-up of vaccination session and role of each team member, as well as practical exercise. Before the second round, a short half day refresher session was organized.

5.2.3 Choosing vaccination sites

Preliminary selection was done together with district medical authorities, then refined in consultation with community leaders. An important criterion was to keep travel distances short so that all family members, including elderly people and mothers with small children, could reach the sites easily. Altogether there were 287 sites, one per village or settlement

5.2.4 Mobilizing the population

Due to the emergency nature of the intervention, the time period for social mobilization was short. The information was transmitted orally; modern media were not used, as local radio or television are not available in the area and the mobile network coverage is low. Public awareness messaging included detailed information about the rationale of the campaign, the vaccine and the importance of two-dose schedule, along with standard cholera control messages regarding the necessity and availability of treatment and prevention measures. Existing material was used to illustrate the standard cholera control messages, but no special material was designed for the vaccination due to the limited amount of time available. Medical, administrative, and traditional authorities were informed in advance. Each community was visited 2 days before vaccination day by a health promoter, who provided educational and awareness information via village leaders (Figure 10). In more populated areas, local outreach workers conducted door-to-door mobilization.

Figure 10: Public awareness campaing.

5.2.5 Vaccination day

Each team had a car (two in Boffa) or boat to reach the vaccination sites. Vaccines were transported and used at ambient temperature on vaccination day. Vaccines leftover at the end of vaccination day were returned to the cold chain and used first on the following day. Before administration, the vaccine vial monitor (VVM) was checked for stability; the vial was shaken, opened, and administered or self-administered under observation (Figure 11). All VVM remained valid during the campaign.

Figure 11: Vaccine administration. Image credit: David Di Lorenzo.

To facilitate ingestion of the vaccine, safe drinking water was provided to each vaccinee (pre-packed 33 cl sachets from a Guinean manufacturer) (Figure 12). Each vaccinee also received a vaccination card during the first round and was asked to bring the card for the second dose. However, during the second round the vaccine was provided to those who had lost their card or were not previously vaccinated.

Figure 12: Provision of safe water. Image credit: David Di Lorenzo.

In Forecariah, the second vaccination round was accompanied by distribution of preventive items (soap and chlorine solution for household water treatment), targeting women of childbearing age.

5.2.6 Number of doses administered and time

A total of 143,039 doses were administered during the first round of vaccination, and 117,139 during the second round in Boffa. The utilisation rate was 99,3%. Few vials were lost during the manipulation of opening procedure, 4 vials were identified as having expired VVM and were removed. Also, few missing vials in the originally packed vaccine box were reported.

A total of 29,505 doses were administered during the first vaccination round and 26,567 during second vaccination round in Forécariah.

Teams vaccinated an average of 703 persons daily, up to 1,830 vaccinations/day/team. They spent several days in the larger villages but covered several smaller sites in one day. The complete campaign took 6 weeks from the decision to proceed until completion of the second round in Boffa (3-week interval between doses) and 5 weeks in Forecariah (2-week interval).

5.2.7 Wate management

All waste management was centralized in two site, one in Boffa and one in In Forecariah, where it was treated.

Figure 13: Waste managment.

Paper and carton was incinerated. Glass was crushed with portative glass reducers and encapsulated.Aluminium covers of the vaccine were encapsulated at the same time in both sites. Maps of encapsulation areas were given to each health structure. Polystyrene, insulation sheets, thermal boxes were stocked in Conakry, in order to be used as building insulation. Thermal boxes were given to Boffa port and to the fishery/ port in Conakry. Waste management of water plastic bags was ensured by the provider who recuperated all plastics and recycle them.

5.2.8 Costs

Cost per dose of vaccine delivered was US$2.89, including $1.85 for the vaccine itself and just over $1 for direct delivery costs (especially transport of teams and material, and payment for teams and other staff). Table 1 lists all costs that were factored into this calculation.

Table 1. Direct costs of mass vaccination campaign. Fixed administrative costs, MSF institutional costs and costs linked to operational research are excluded.

	Total	% Total
Vaccine (1.85 USD/dose)	585,063	64.0%
Water sachets (0.036 USD/sachet)	10,626	1.2%
Airfreight for vaccines	47,719	5.2%
Transit cost for vaccines	9,574	1.0%
Cold chain (truck rental, reparation of container in Boffa)	26,505	2.9%
Vaccination, supervision and sensitisation teams payments	63,308	6.9%
Training for the teams	4,899	0.5%
Small vaccination material and stationary, vaccination cards	13,705	1.5%
Logistic material, site preparation, waste management	13,333	1.5%
Transport cost (cars, trucks, boats and fuel)	139,851	15.3%
Total	914,582	100.0%
Cost per dose delivered	2.89	

5.3 Assessment of the acceptability of the mass vaccination campaign by the population

The surveys were carried out May 20 to 25, 2012 in Boffa and June 16 to 20, 2012 in Forécariah (Figure 14).

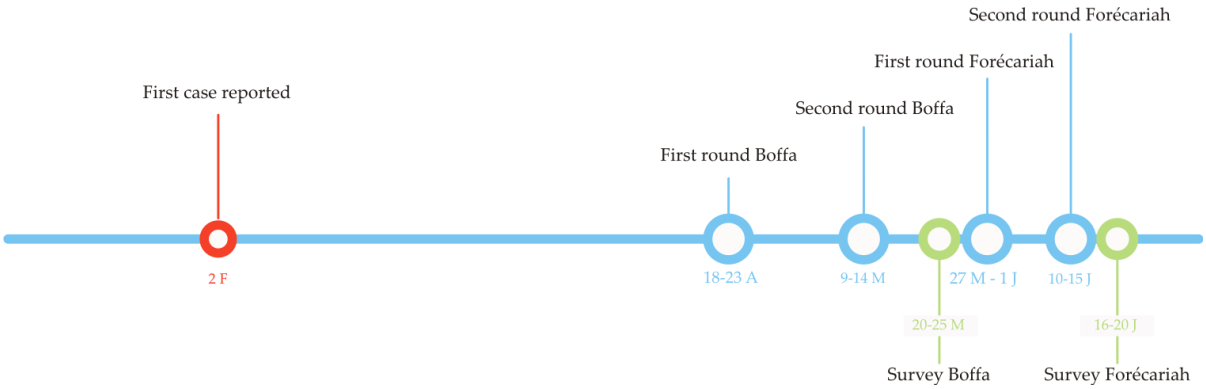

Figure 14: Timeline of the cholera vaccination campaigns and implementation of the field surveys in Guinea in 2012. Months are abbreviated as follows: F = February, A = April, M = May, J = June.

In total, 851 households were visited in Boffa. Of these, 775 (91.1%) were included in the survey, 45 households (5.3%) remained empty after two visits, 3 households (0.4%) refused to participate and 23 (2.7%) were not residents of Boffa. All 180 visited households were included in Forécariah (Figure 15).

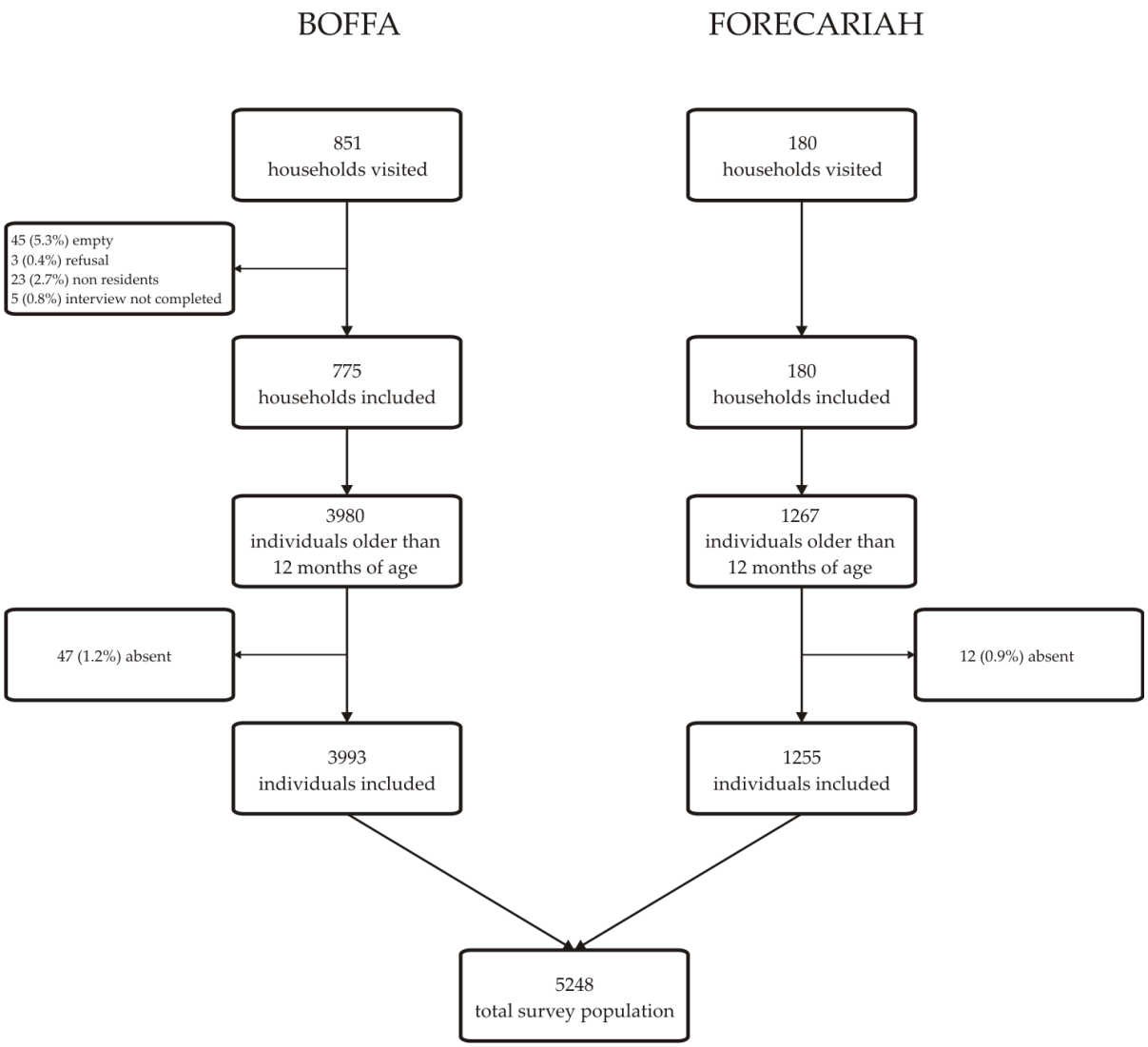

Figure 15: Study flow chart: Number of households visited, number of households included, number of individuals in the targeted age group (older than 12 months of age) residing in the households included in the survey and final number of individuals included in the study.

5.3.1 Household based survey in Boffa

Description of the survey

Overall, 3,993 individuals were included with an average of 5.5 (SD: 5.4) persons included per household. There were slight differences in the number of participants recruited by team; with an average of 327.8 individuals (SD: 114.7) recruited per survey team.

Description of survey participants

The median age of the participants was 15 years old (IQR: 5-30). There were slightly less males (47.6%) than females in the survey sample. The age pyramid shows no important asymmetries in children with an asymmetric shape in the active adult population and a classical shape of demographic expansion with a predominance of the youngest age groups (Figure 16).

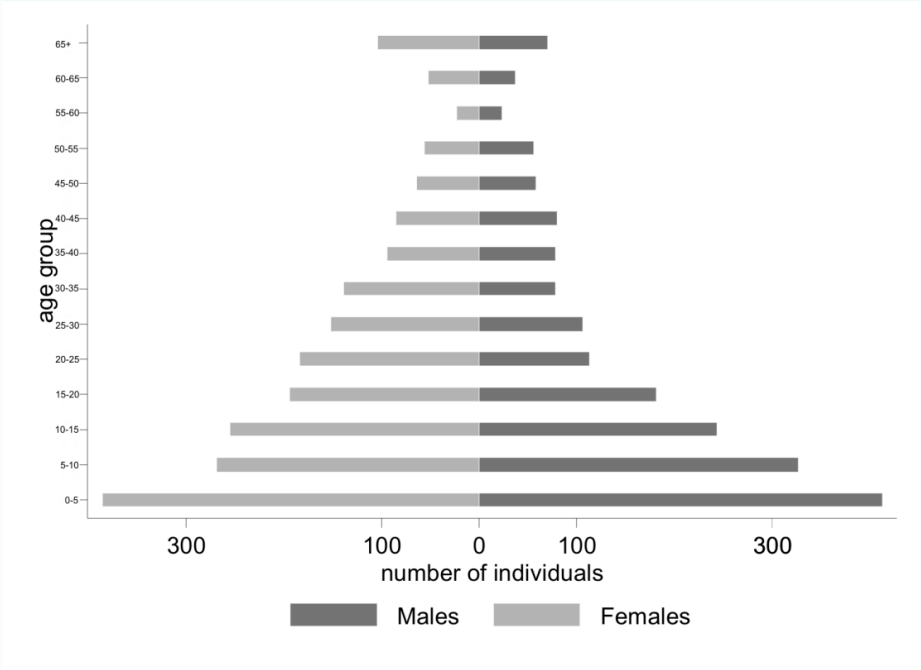

Figure 16. Age pyramid of the person included in the survey, Boffa prefecture, May 2012.

Regarding the sub-prefecture of origin, as expected the most populated areas were more represented in the sample (Table 2).

Table 2. Number of people included in the survey by sub-prefecture. Boffa prefecture, May, 2012.

Sub-prefecture	N	%
Boffa	850	21.7
Douprou	535	13.7
Koba	957	24.5
Tamita	203	5.2
Tougnifili	811	20.7
Mankountan	558	14.3

Cholera vaccine coverage

Overall, the vaccine coverage was 75.8% [95%CI: 71.2-79.9%, deff=10.1] with two doses (fully vaccinated), 17.6% [95%CI: 14.8-20.9%, deff=6.1] received only one dose (incompletely vaccinated) and 93.3% [95%CI: 91.1-95.0%, deff=5.9] either with one or two doses. The dropout rate between the first and second dose was 15.2% [95%CI: 12.2-18.7%, deff=7.0].

Vaccination coverage varied with age (Figure 17). reaching over 80% for two doses (fully vaccinated) among children 1 to 15 years old, while fully vaccinated coverage was lower in adults, especially among males; at 66.0% [95%CI: 59.2-72.2%, deff=1.6].

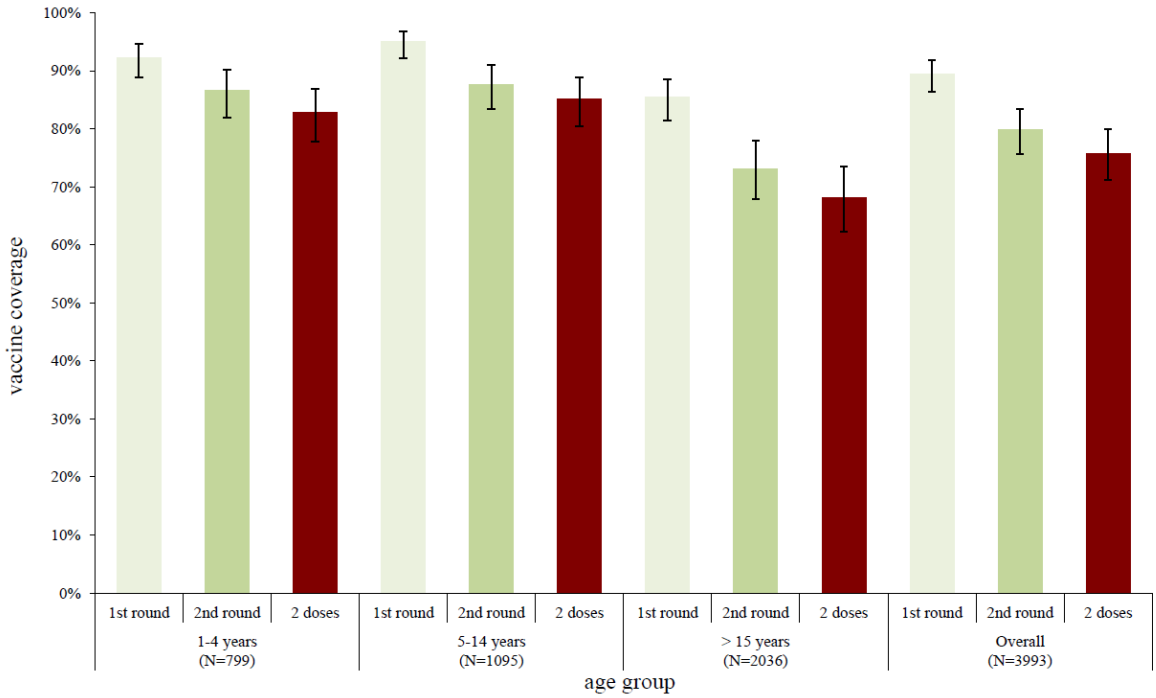

Figure 17. Vaccine coverage by age group of the cholera mass vaccination campaign in Boffa prefecture, first round, second round and two doses (fully vaccinated), April-May 2012.

The overall vaccine coverage was similar in both males and females; 76.6% [95%CI: 71.9-80.7%, deff=5.5] of females and 75.0% [95%CI: 69.8-79.4%, deff=5.8] of males were fully vaccinated. Further details about the distribution of the vaccine coverage by age and sex are provided in Annex 9.

No major differences were observed in the vaccination coverage by sub-prefecture, with the highest estimate in Mankountan and the lowest in Koba (especially in the second round) (Table 3). More details on the geographical variation of the vaccine coverage are shown in Annex 10

Table 3. Vaccine coverage by sub-prefecture of the cholera mass vaccination campaign in Boffa prefecture, first round, second round and two doses (fully vaccinated), April-June 2012.

	First round		Second Round		Full coverage (two doses)	
	n/N (%)*	[95% CI]	n/N (%)*	[95% CI]	n/N (%)*	[95% CI]
Boffa (n=850)	773/847 (91)	[82-96]	692/847 (82)	[74-89]	655/847 (78)	[68-86]
Douprou (n=535)	477/534 (88)	[81-93]	428/534 (79)	[70-86]	411/534 (76)	[67-83]
Koba (n=957)	835/949 (88)	[83-92]	672/947 (71)	[62-80]	645/946 (69)	[59-77]
Mankountan (n=577)	535/577 (93)	[88-96]	506/577 (89)	[82-93]	484/577 (84)	[76-90]
Tamita (n=203)	190/203 (93)	[85-97]	165/202 (80)	[71-87]	160/202 (78)	[66-86]
Tougnifili (n=811)	725/811 (88)	[77-94]	676/811 (83)	[73-89]	636/811 (77)	[64-86]

Overall, 73.9% [95%CI: 69.3-78.0%, deff=9.7] of participants showed vaccination cards. Card retention was higher in children than in adults, and higher in females than males. All groups showed card retention over 70% with the exception of adult males (64.5%).

Among those vaccinated during the first round, 1.4% [95%CI: 0.8-2.2%, deff=2.9] reported spitting out or vomiting the vaccine; the same percentage was observed for the second dose.

Reasons for non-vaccination

Among non-vaccinated individuals (386 during the first and 779 during the second round), the reason for non-vaccination was obtained in 382 and 768 individuals respectively. The main reason for non-vaccination was absence during the campaigns for both the first (77.6% [95%CI: 69.2-84.2%, deff=3.0]) and the second round (71.2% [95%CI: 64.5-77.1%, deff=3.7]). The second most reported reason was "not having the time to go for the vaccination" for both rounds (6% and 10% respectively). The third most reported reason was to be sick during the campaign (4.9% and 4.4% respectively). (Table 4). The percentage of people reporting as the reason for non-vaccination any type of lack of information about the campaign was calculated as a standard quality indicator for campaign; this percentage was 6.5% for the first round and 7.3% for the second round in Boffa prefecture.

Table 4. Reasons for non-vaccination among those not vaccinated. Boffa prefecture, May, 2012.

	1st round		2nd round	
Raison	N=382	%	N=768	%
Absent during the campaign	295	77.2	550	71.6
The person did not have the time to be vaccinated	24	6.3	73	9.5
Sick during the campaign	19	5.0	34	4.4
Not informed about the campaign	12	3.1	22	2.9
Other	9	2.4	22	2.9
The caregiver thought that the child was too young	8	2.1	8	1.0
Vaccination site considered too far	3	0.8	5	0.7
The person did not know the date of the campaign	3	0.8	21	2.7
The person thought that he/she was too old	2	0.5	2	0.3
The person was hospitalized at the time of vaccination	2	0.5	2	0.3
Refusal (link with cultural beliefs)	1	0.3	1	0.1
Bad experience with previous vaccinations	1	0.3	1	0.1
The person did not know the place of vaccination	1	0.3	2	0.3
No explanation	1	0.3	5	0.7
No vaccines available at the vaccination site	0	0.0	8	1.0
Side effects during the first round	0	0.0	7	0.9
The person thought that one dose was enough	0	0.0	2	0.3
The vaccine was considered dangerous	0	0.0	1	0.1
The vaccinator advise the person to not be vaccinated	0	0.0	2	0.3

Side effects (household survey results)

In the retrospective household based surveys, a question about side effects after vaccination was asked to the adults who participated in the survey. Overall, 3.3% [95%CI2.4-4.7%, deff=3.1]) reported being sick after vaccination during the first round and 2.5% [95%CI: 1.7-3.8%, deff=3.0]) during the second round. Among those reporting being sick after vaccination, the most frequently reported symptoms were diarrhea (34.0%) and fever (34.6%). Other symptoms reported included vomiting (8.3%), abdominal pain (10.3%), dizziness (9.0%) and weakness (7.1%).

Among those reporting illness after vaccination 21.7% reported seeking care during the first round and 16.7% during the second round.

Acceptability of the vaccine

Regarding the awareness campaign, 95.7% of survey participants [95%CI: 94.2-96.8%, deff=1.7]) reported being informed about the campaigns. Concerning knowledge about the protection afforded by the vaccine, 94.2% [95%CI 91.3-96.1%, deff=3.8] of participants responded that full protection is obtained after two doses. However, 42.4% [95%CI 34.3-50.9%, deff=10.5] of participants thought that only one dose afforded full protection.

A small percentage of participants considered that the vaccine made them feel sick 3.9% [95%CI2.7-5.8%, deff=2.4]), similar to the proportion of participants reporting side effects. Most participants reported that the taste of the vaccine was bad (77.6% [95%CI 69.5-84.1%, deff=10.7]). However a very high percentage reported that they would be vaccinated again (98.9% [95%CI 97.8-99.5%, deff=2.3]) in a future cholera vaccination campaign.

5.3.2 Household based survey in Forecariah

Description of the survey

In total, 1,255 individuals included with an average of 7.0 (SD: 4.4) persons included per household. There were slight differences in the number of participants recruited by team; in average, 313.8 individuals (SD: 133.8) were recruited per team.

Description of the survey participants

The median age of the participants was 15 years old (IQR: 5-30). There were fewer males (44.1%) than females in the sample. The age pyramid shows no important asymmetries in children with an asymmetric shape in the active adult population and a classical shape of demographic expansion with a predominance of the youngest age groups (Figure 18).

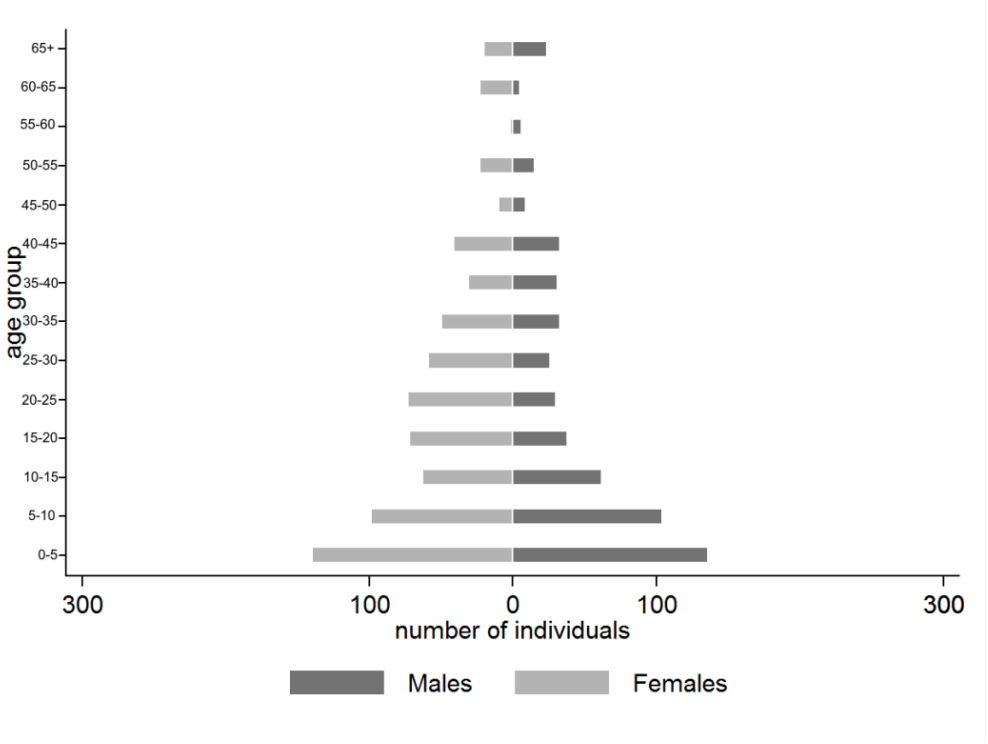

Figure 18. Age pyramid of the person included in the survey, Forecariah prefecture, May 2012.

Regarding the sub-prefecture of origin, as expected more people were recruited from Kaback proportionally to its larger population size (Table 5).

Table 5. Number of people included per Sub-prefecture. Forecariah-prefecture, May, 2012.

Sub-prefecture	N	%
Kabak	754	60.1
Kakosa	501	39.9

Cholera vaccine coverage

Overall, the vaccine coverage was 75.9% [95%CI: 69.8-80.9%, deff=5.0] with two doses (fully vaccinated), 19.3% [95%CI: 15.5-23.8%, deff=3.3] received only one dose (incompletely vaccinated) and 94.9% [95%CI: 91.8-96.9%, deff=3.7] either with one or two doses. The dropout rate between the first and second dose was 13.6% [95%CI: 9.7-18.7%, deff=4.5].

Vaccination coverage varied with age (Figure 19), reaching over 80% for two doses among children 1 to 14 years old. The coverage was lower in adults, especially among males; the coverage for two doses was 56.2% [95%CI: 45.1-66.7%, deff=1] in this group.

The vaccine coverage was higher in females than in males. Overall, 79.4% [95%CI: 74.4-83.6%, deff=2.1] of females and 71.4% [95%CI: 63.3-78.3%, deff=3.7] of males were fully vaccinated. Vaccine coverage among women in childbearing age (15–49 years old) was statistically higher than among men of same age in Forécariah (72.6% [95%CI: 65.4–78.8%] vs. 53.4% [95%CI: 41.6–64.8%], p<0.001). Further details about the distribution of the vaccine coverage by age and sex are provided in the Annex 9.

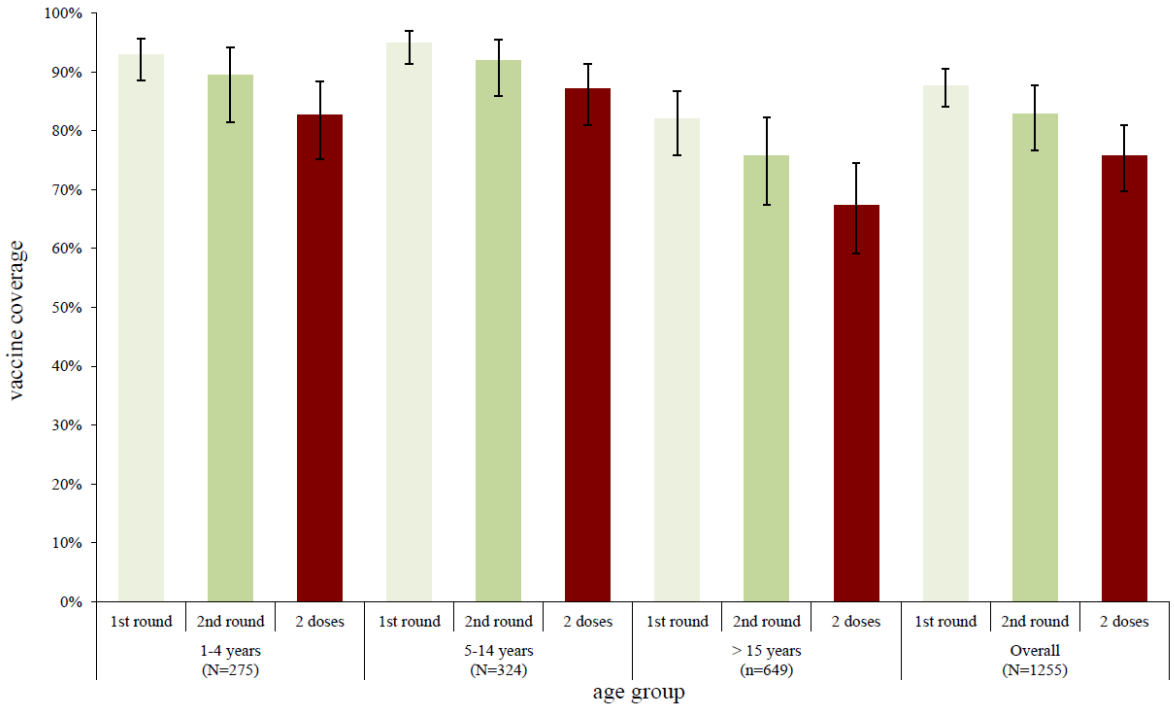

Figure 19. Vaccine coverage by age group of the cholera mass vaccination campaign in Forecariah prefecture, first round, second round and overall, May-June 2012.

No major differences were observed in the vaccination coverage by sub-prefecture (Table 4). More details on the geographical vaccination of the vaccine coverage are shown in Annex 10.

Table 6. Vaccine coverage by sub-prefecture of the cholera mass vaccination campaign in Forécariah prefecture, first round, second round and two doses (fully vaccinated), April-June 2012.

	First round		**Second Round**		**Full coverage (two doses)**	
	n/N (%)*	[95% CI]	n/N (%)*	[95% CI]	n/N (%)*	[95% CI]
Kaback (n=754)	657/744 (87)	[84-90]	605/744 (80)	[72-86]	565/744 (74)	[67-81]
Kakossa (n=501)	447/501 (88)	[80-93]	451/501 (88)	[76-93]	88/501 (78)	[68-86]

Overall, 78.9% [95%CI: 71.8-84.5%, deff=7.2] of the participants showed vaccination cards. The retention of cards was higher in children than in adults, and higher in females than in males. All groups showed card retention over 70% except adults males (65.7%)

Reasons for non-vaccination

Among the not vaccinated individuals (141 during the first and 189 during the second round), the reason for non-vaccination was obtained in 139 and 184 of them respectively. The main reason for non-vaccination was to be absent during the campaigns for both the first (78.4% [95%CI: 68.0-86.1% deff=1.6]) and the second round (70.0% [95%CI: 51.7-79.3%, deff=3.9]). (Table 7). The percentage of people reporting the reason for non-vaccination any type of lack of information about the campaign was 5.0% for the first round and 7.0% for the second round in Forecariah prefecture.

Table 7. Reasons for non-vaccination among those not vaccinated. Forecariah prefecture, May, 2012.

	1st round		**2nd round**	
Raison	N=139	%	N=184	%
Absent during the campaign	115	81.0	122	64.9
The person did not have the time to be vaccinated	6	4.2	8	4.3
Sick during the campaign	5	3.5	8	4.3
Not informed about the campaign	5	3.5	6	3.2
Other	2	1.4	12	6.4
The person did not know the date of the campaign			5	2.7
The person thought that he/she was too old	2	1.4	2	1.1
The person was hospitalized at the time of vaccination	1	0.7	1	0.5
Bad experience with previous vaccinations			7	3.7
No explanation	3	2.1	4	2.1
Waiting time too long			8	4.3
Because of side effects during the first round			1	0.5

5.4 Surveillance of adverse events following immunization

Overall, 48 individuals (28 in Boffa and 20 in Forécariah) spontaneously reported symptoms that were linked with the vaccine by the health personnel and considered as AEFI with 35 after the first round and 13 after the second round. In total, 29 were women (60%) and the median age was 27 years (IQR: 16–36 years); 8 (17%) were children 1 to 4 years. Seven patients reported having a history of allergies (15%). The cause of the allergy was specified for two patients (quinine and chloroquine). The delay between vaccination and symptom onset is shown in Figure 20; the median delay was 7 hours (IQR: 1–24 hours). One quarter reported the symptoms in the following hour after vaccination.

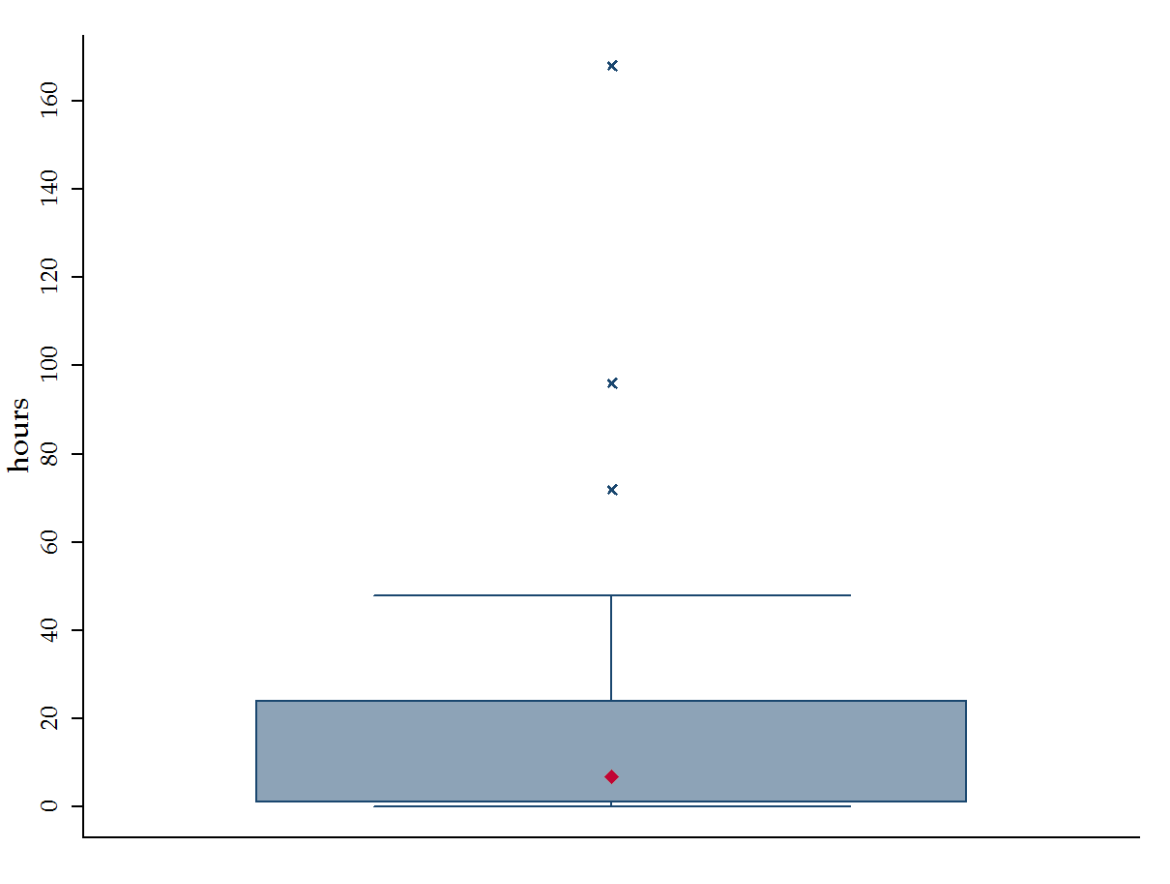

Figure 20. Box-plot of the delay in hours between the vaccine intake and the onset of the AEFI. The median time is represented by a red diamond.

The symptoms reported were mainly gastro-intestinal (Table 8): 28 (20%) diarrhea, 22 (16%) vomiting, 14 (10%) stomach ache and 12 (9%) nausea. In addition, 15 patients (11%) reported fever and general weakness. No patient was transferred to a hospital and no deaths were reported. Most of the patients (n=33, 69%) reported more than one symptom.

Table 8. Symptoms reported by the forty-eight patients reporting adverse events following immunization.

Symptom	**n = 139**	**%**
Diarrhea	28	(20.1)
Vomiting	22	(15.8)
Stomachache	14	(10.1)
Fever	15	(10.8)
Weakness	15	(10.8)
Nausea	12	(8.6)
Dizziness	9	(6.5)
Headache	5	(3.6)
Borgorygms	2	(1.4)
Anorexia	2	(1.4)
Other	15	(10.8)

5.5 Evaluation of the performance of the cholera rapid diagnostic test Crystal in vaccinated individuals

5.5.1 Recruitment and follow-up

A total of 108 individuals were recruited during 2 days in 4 vaccination sites. Two individuals were excluded from the analysis (one was absent during all follow-up visits and for the other, follow-up was stopped accidentally by the study team).

Follow-up of the remaining 106 participants is described in Figure 21. Study participants, exclusions and follow-up results, Kabak, June 2012.. Participants were followed for a median time of 5 days (minimum of 2 and 7 as maximum). Almost half of them (49.1%) were followed for 4 (23.6%) or 5 days (26.4%).

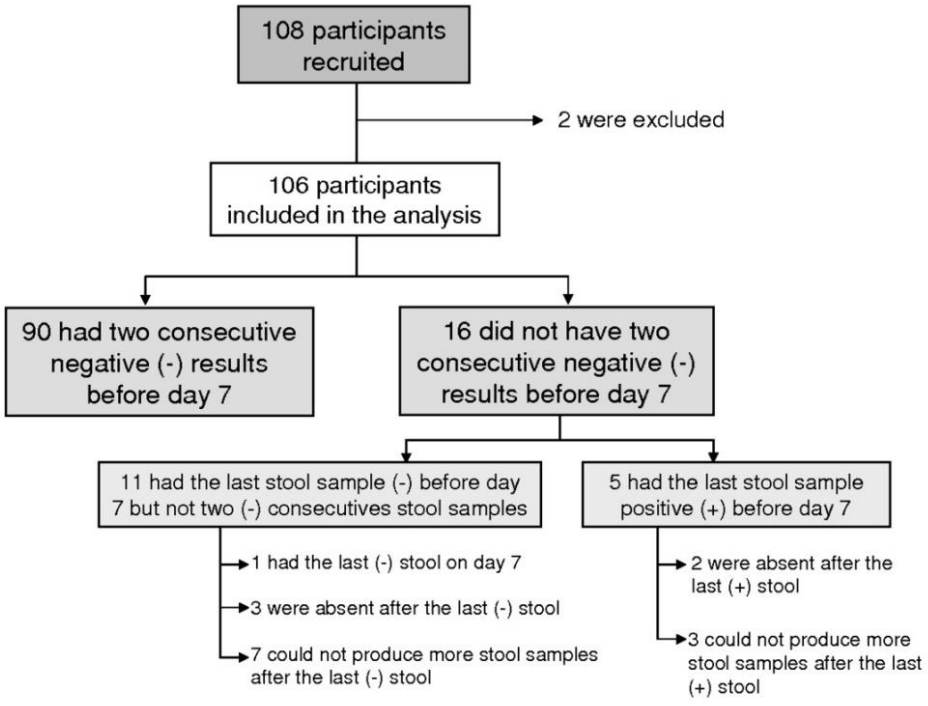

Figure 21. Study participants, exclusions and follow-up results, Kabak, June 2012.

5.5.2 Participant's characteristics, symptoms and delay in stool collection and testing

Among the 106 participants, 79.2% (84) were females and the median age was 25 years (P_{25}-P_{75}=2 – 80). The majority of participants were older than 15 (84.8%) and the proportion of children under five was 5.7%.

In total, 18 participants declared having diarrhea during follow-up, and two reported vomiting. Other symptoms such as constipation, stomachache or headache were declared by 37 participants.

The average delay was 3.9 hours (SD=4.4) between stool production and collection and 6.6 hours (SD=5.9) between stool collection and performance of the RDT (including collection and transport of samples to the laboratory) by the laboratory technicians. As a result, there was an average of 10.5 hours (SD= 6.6) between stool production and performance of the RDT.

5.5.3 Proportion of positive tests after vaccination

Of the 106 participants, 100 (94.3%) became positive with the O139 line after vaccination and 6 never had a positive result. On the first day of follow-up (day 1) 71.1% were positive. On day 3, almost half of the tests remained positive (49.5%) and on day 5 and 6 this percentage decreased below 3% (Table 9).

Table 9. Results of the rapid diagnostic test Crystal VC in participants vaccinated against cholera by day of follow-up, Kabak, June 2012.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day7
A. Tests done	97	97	90	76	46	23	6
a.1. Positive result (+)	69	80	47	20	2	1	0
a.2. Negative result (-)	28	17	43	56	44	22	6
B. Follow-up stopped after 2(-)	0	0	5	17	42	67	85
C. Absent	1	0	0	1	1	4	5
D. No sample available	8	9	11	12	17	12	10
Total[1]	106	106	106	106	106	106	106
Proportion[2] **of positives (%)**	71,1	82,5	49,5	21,5	2,3	1,1	0,0
IC 95% of the proportion	61.5-79.9	73.4-89.4	39.1-59.9	13.7-31.2	0.3-8.1	0.0-6.0	0.0-4.0[3]

[1] The total is the sum of A+B+C+D

[2] The proportion is the result of (a.1/(A+B))*100

[3] 97.5% Confidence Interval, one-sided

Only one participant became positive with the O1 line (together with the O139 line) on the first day of monitoring, and both lines became negative subsequently.

5.5.4 Time to become negative

Of the 100 participants with at least one positive result, five could not be tested on day 7 as they were absent or did not produce stools, although they had a positive result with their last specimen collected (Figure 21). Among these 5 participants, 3 had their last positive stool on day 3, 1 on day 4 and 1 on day 5. For the remaining 95 cases with O139 positive tests, the time to become negative after vaccination was calculated.

For all participants, the mean time to become negative after vaccination was 3.8 days (SD=1.1) and the median time was 4 days (P_{25}-P_{75}= 3 - 5). For males, the mean time to become negative after vaccination was 4.3 days (SD=1.4) and 3.6 (SD=1) for females (p=0.03), with a median of 4 days for both males and females. A linear regression model showed that a longer time to become negative was associated to an older age (p=0.002) and to male sex (p=0.012) (Table 3).

Table 10. Time to become negative by age and sex. Results of the linear regression model, Kabak, June 2012.

	Coefficient	95% Confidence Interval	p
Age	0.020	(0.008- 0.032)	0.002
Sexe	0.669	(0.153- 1.186)	0.012

5.5.5 Laboratory testing of the rapid diagnostic test

The Crystal VC RDT gave positive results for both O1 and O139 when the strip was inserted directly into the vaccine solution prior to ingestion, and remained positive up to 10^4-fold dilutions of the vaccine. At a 10^5-fold dilution, only the O139 line remained positive and none of them were positive at higher dilutions.

The RDT gave a positive signal with the O1 test line at bacterial concentration of $2x10^8$ and $2x10^7$, but was negative at $2x10^6$ bacteria/mL, while all dilutions of *V cholerae* O139 culture tested down to $2x10^6$ bacteria/mL were positive for the O139 line.

Table 11. Results of Crystal VC rapid diagnostic test performed in Shanchol and bacterial suspension dilutions, Pasteur Institute, Paris, November, 2012.

	Control line	Line T1 O139	Line T2 O1
Shanchol dilutions			
Tube 1 (10-fold dilution)	+++	+++	+++
Tube 2 (10^2-fold dilution)	+++	+++	+++
Tube 3 (10^3-fold dilution)	+++	+++	++
Tube 4 (10^4-fold dilution)	+++	++	+
Tube 5 (10^5-fold dilution)	+++	+	-
Tube 6 (10^6-fold dilution)	+++	-	-
Tube 7 (10^7-fold dilution)	+++	-	-
Tube 8 (10^8-fold dilution)	+++	-	-
Tube 9 (10^9-fold dilution)	+++	-	-
O1 and O139 strains dilutions	+++		
O1 - Tube 1 ($2x10^8$bacteria/mL)	+++	-	+++
O1 - Tube 2 ($2x10^7$ bacteria/mL)	+++	-	++
O1 - Tube 3 ($2x10^6$ bacteria/mL)	+++	-	-
O139 - Tube 1 (2×10^8 bacteria/mL)	+++	+++	-
O139 - Tube 2 ($2x10^7$ bacteria/mL)	+++	+++	-
O139 - Tube 3 ($2x10^6$ bacteria/mL)	+++	++	-

Intensity of the positive line: (+) very weak positive; (++) weak positive; (+++) positive

Negative result: (-)

5.6 Estimate of the vaccine effectiveness

5.6.1 Baseline Information

From May 21 to October 31, 2012, 239 patients with acute, non-bloody diarrhea were treated at health centers in the study area (Figure 22); 5 died, yielding a case fatality ratio of 2%. Overall, 40 case-patients and 160 control-subjects were included in the primary analysis (Figure 23). None of the case-patients enrolled in the study died. The median age of participants was 28.0 years (inter-quartile-range: 16.5-39.0). There were fewer females (35.0%) than males (Table 1). Half of the cases sought care on the same day of symptom onset. Dehydration was present in 70% of cases at admission.

Table 12. Characteristics of the case-patients and control-subjects included in the vaccine effectiveness study, Boffa and Forécariah, Guinea, 2012.

	Controls		Cases		
	n	(%)	n	(%)	P value
Total included	160		40		
Males	104	(65.0)	26	(65.0)	
Female	56	(35.0)	14	(35.0)	
Age in years (median and IQR*)	28	16-39	28	18-36	
Profession					0.18
Trader	29	(18.1)	8	(20.0)	
Farmer	37	(23.1)	16	(40.0)	
Pupil / student	29	(18.1)	3	(7.5)	
Fisherman	10	(6.3)	3	(7.5)	
Housewife	10	(6.3)	1	(2.5)	
Unemployed	22	(13.8)	6	(15.0)	
Other	23	(14.4)	3	(7.5)	
Head of household's educational attainment					0.13
None	43	(27.2)	13	(32.5)	
Primary	5	(3.2)	4	(10.0)	

Secondary	21	(13.3)	2	(5.0)	
University	5	(3.2)	0	(0.0)	
Literate	84	(53.2)	21	(52.5)	
Telephone					0.10
No	32	(20.0)	13	(32.5)	
Yes	128	(80.0)	27	(67.5)	
Household size					0.063
0-4 members	34	(21.3)	17	(42.5)	
5-7 members	40	(25.0)	7	(17.5)	
8-12 members	49	(30.6)	9	(22.5)	
>12 members	37	(23.1)	7	(17.5)	
Proportion of children attending school in the household					0.13
None of them	33	(22.9)	14	(37.8)	
Less than half	42	(29.2)	11	(29.7)	
More than half	51	(35.4)	8	(21.6)	
All of them	18	(12.5)	4	(10.8)	
Distance to the closest health center					0.10
Need of transport	107	(66.9)	31	(77.5)	
Walking distance	53	(33.1)	9	(22.5)	
Other cholera cases in the household					0.15
No	155	(97.5)	37	(92.5)	
Yes	4	(2.5)	3	(7.5)	
Treatment of drinking water†					0.15
No	26	(16.3)	11	(28.2)	
Yes	34	(21.3)	5	(12.8)	
Eating food in a public space					0.02
Never	72	(45.0)	11	(28.2)	
Sometimes	49	(30.6)	20	(51.3)	
Everyday	39	(24.4)	8	(20.5)	
Usual place of defecation					0.12

Latrine	81	(50.6)	17	(42.5)	
Pit in the yard	56	(35.0)	14	(35.0)	
In the ground	23	(14.4)	9	(22.5)	
Sharing the latrine with someone suffering from cholera					0.001
No	131	(96.3)	24	(80.0)	
Yes	5	(3.7)	6	(20.0)	

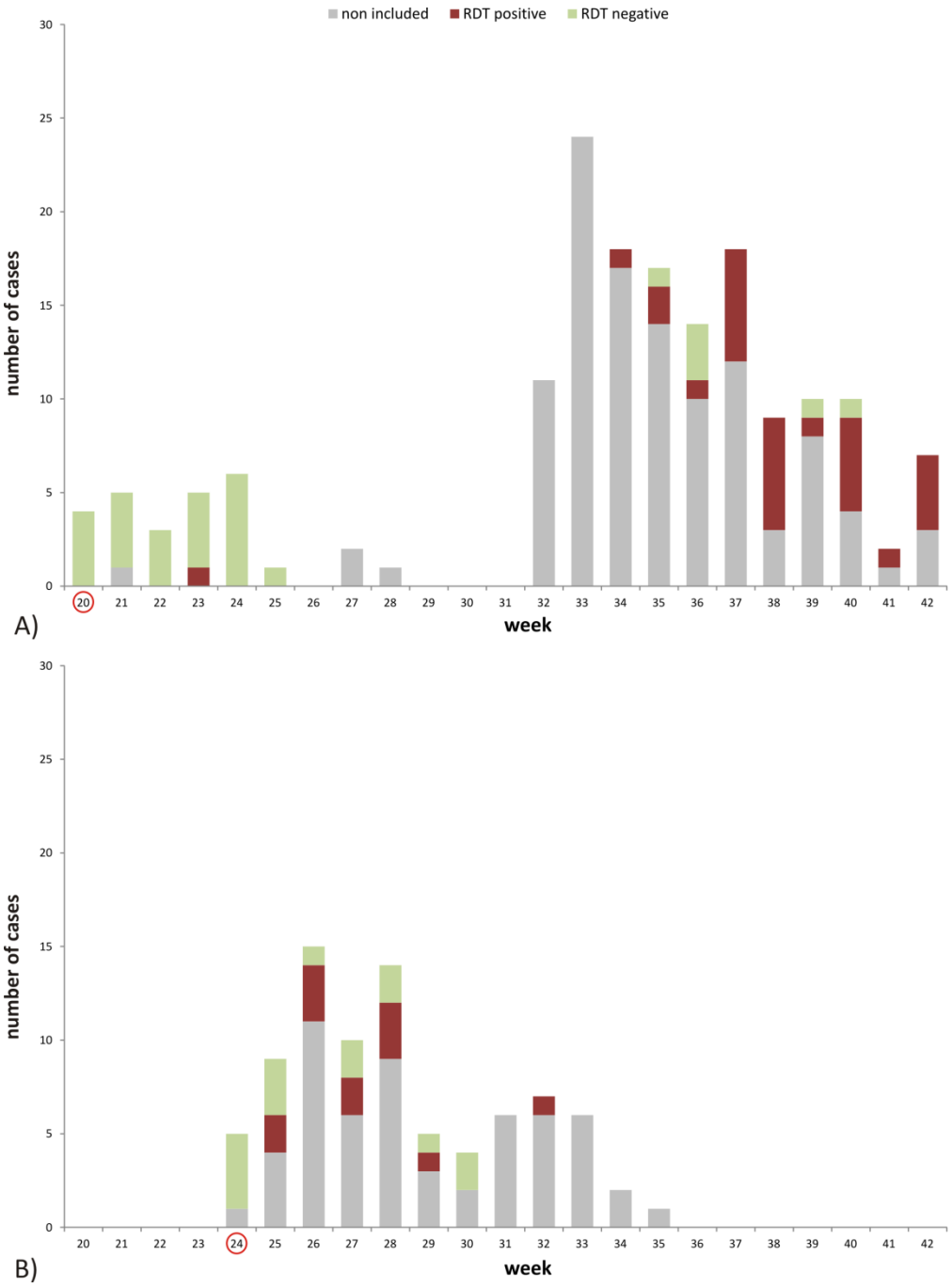

Figure 22. Acute diarrhea cases reported in the study areas after the starting date of the study (week 20 in Boffa prefecture, panel A; and week 24 in Forécariah prefecture, panel B). The cholera cases confirmed by rapid test (RDT) included in the study are represented in red and the non-cholera cases (RDT negatives) included in the indicator bias analysis are represented in green.

Of 36 case-patients included in the primary analysis for whom a specimen was sent for culture and PCR analysis, 18 (50%) were positive for *V cholerae O1*, El Tor-Ogawa; 13 were

positive for culture and PCR and 5 PCR positive but culture negative. All the 36 samples showed a weak amplification signal of the 16S rRNA gene. Among the 18 negative specimens, 5 had an almost undetectable amplification signal.

In addition, 43 watery diarrhea case-patients with a negative RDT result and 172 control-subjects were recruited for the indicator bias analysis (Figure 23).

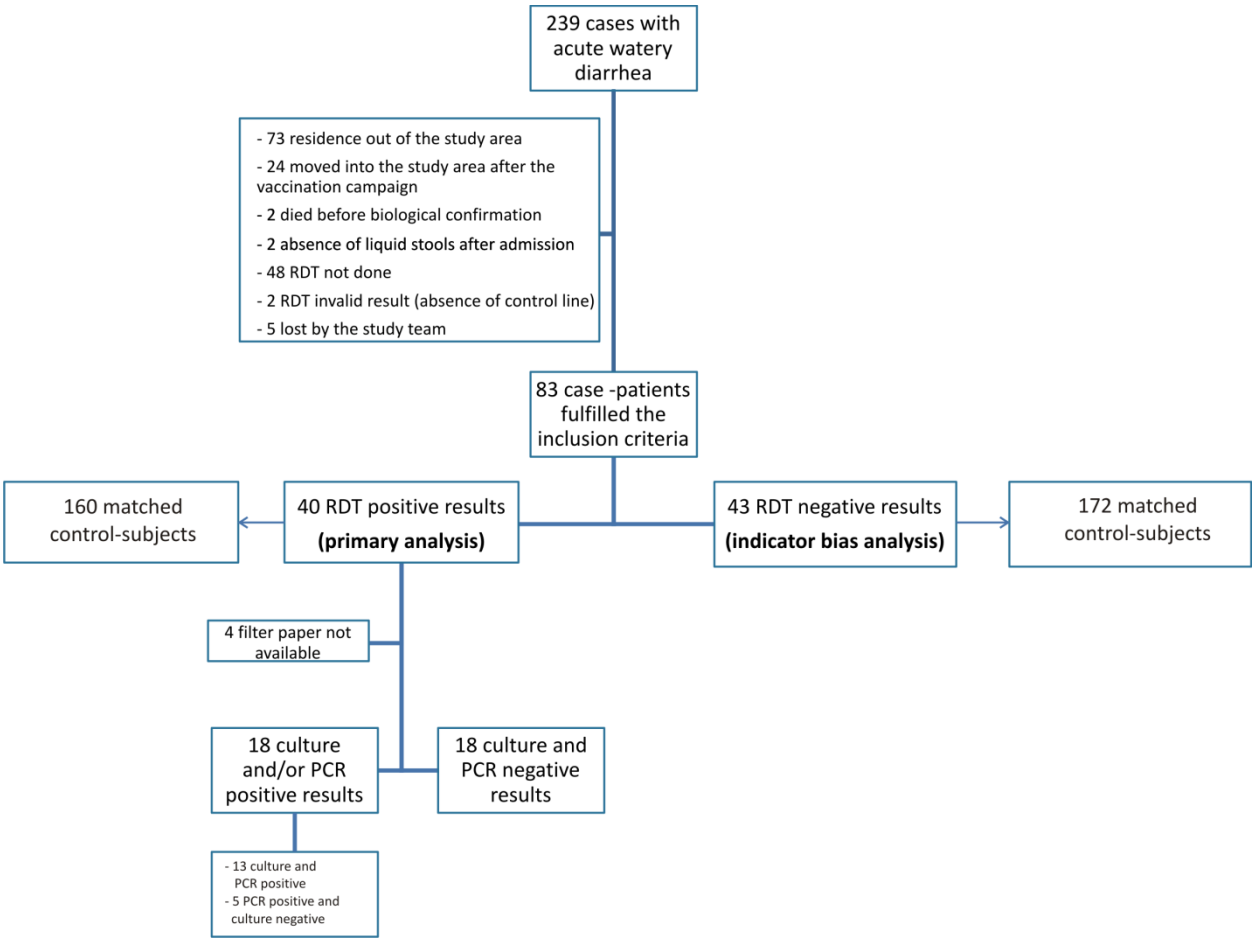

Figure 23. Study flow chart: number of case-patients with a positive result to the rapid diagnostic test (RDT) included in the primary analysis, number of case-patients with a negative result to the RDT included in the indicator bias analysis, and number of case-patients included in the sub-analysis with culture and/or PCR positive cases. Four matched control-subjects were selected for each case-patient.

5.6.2 Analysis of confounders and effect modifiers

Table S1 shows the socio-economic characteristics and the exposure to different risk factors for cholera among case-patients and control individuals. A statistical association was observed between being a case-patient and eating in public places and sharing the latrine with a cholera case. The potential confounding effect of factors with P values lower than 0.2 was assessed in the multivariate conditional logistic regression analysis.

Table S2 shows the socio-economic characteristics and the exposure to different risk factors for cholera among non-cholera watery diarrhea case-patients and control-subjects included in the indicator bias analysis. The non-cholera watery diarrhea case-patients and the matched control-subject showed similar socio-economic characteristics and had similar exposure to different risk factors for cholera infection (Table S2).

5.6.3 Vaccine Effectiveness Analysis

Vaccination with two complete doses was associated with significant protection against cholera, in the crude analysis and after adjustment for potential confounders (86.6%; 95% confidence interval: 56.7 to 95.8%; P value=0.001) (Table 13). The precision of the vaccine effectiveness estimate for an incomplete course of vaccine was inconclusive (42.8%; 95% confidence interval: -83.6 to 82.2%; P value=0.35).

Table 13. Vaccine effectiveness estimates and 95 percent confidence interval (95%CI) of a complete (two doses) and an incomplete vaccine course. Boffa and Forécariah, Guinea, 2012.

Vaccination status	Controls		Cases		VE*	95%CI	P value	aVE†	95%CI	P value
	N	(%)	N	(%)	%			%		
Unvaccinated	23	(14.4)	15	(37.5)	Ref			Ref		
Incomplete course‡	36	(22.5)	14	(35.0)	38.9%	(-55.2% - 76.0%)	0.30	42.8%	(-83.6% - 82.2%)	0.35
Full course (two doses)	101	(63.1)	11	(27.5)	84.0%	(59.7% - 93.6%)	<0.001	86.6%	(56.7% - 95.8%)	0.001
Total	160	(100.0)	40	(100.0)						

* VE: crude vaccine effectiveness estimates, calculated as 1-odds ratio.

† AVE: adjusted vaccine effectiveness. Adjusted by: number of individuals living in the household, treatment of water before consumption and sharing the latrine with a cholera case

‡ Incomplete course: individuals who took only one dose or who spitted out or vomited one of the two doses of vaccine

Table 14. Vaccine effectiveness estimates and 95 percent confidence intervals (95%CI) in the sub-analysis containing only culture and/or PCR confirmed cases and in the sub-analysis using watery diarrhea cases with negative RDT result. Boffa and Forécariah, Guinea, 2012.

Vaccination status	Controls		Cases		VE*	95%CI	P value
	N	(%)	N	(%)	%		
Culture-PCR sub-analysis†							
Unvaccinated	10	(13.9)	8	(44.4)	Ref		
Incomplete course‡	17	(23.6)	6	(33.3)	66.2%	(-53.0% - 92.6%)	0.16
Full course (two doses)	45	(62.5)	4	(22.2)	91.6%	(58.6% - 98.3%)	0.002
Total	72	(100.0)	18	(100.0)			
Indicator bias analysis§							
Unvaccinated	9	(5.2)	4	(9.3)	Ref		
Incomplete course‡	35	(20.4)	7	(16.3)	48.1%	(-177.1% - 90.3%)	0.44
Full course (two doses)	128	(74.4)	32	(74.4)	25.2%	(-225.2% - 82.8%)	0.70
Total	172	(100.0)	43	(100.0)			

* VE: crude vaccine effectiveness estimates, calculated as 1-odds ratio.

† In this sub-analysis only cholera cases confirmed by culture and/or PCR were included in the analysis.

‡Incomplete course: individuals who took only one dose or who spit out or vomited one of the two doses of vaccine.

§ In this sub-analysis case-patients with non-choleric diarrhea (negative RDT result) were also compared with control-subjects that did not have diarrhea in an attempt to assess whether the results with respect to effectiveness could be attributed to bias.

In the sub-analysis including only cases that were culture and/or PCR confirmed, vaccination with two complete doses was also associated with significant protection against cholera (91.6%; 95% confidence interval: 58.6 to 98.3%; P value=0.002) (Table 3).

The odds of vaccination between non-cholera watery diarrhea cases and control-subjects did not vary between these two groups (Table 14).

5.6.4 Sensitivity analysis of the vaccine coverage estimates considering the uncertainty about the vaccination status.

In Scenario 1 of the sensitivity analysis individuals reporting vaccination but without cards are considered as unvaccinated and in Scenario 2 are considered as vaccinated

Table 15. Sensitivity analysis of the vaccine effectiveness (VE) considering the uncertainty of vaccination status among those reporting vaccination but without vaccination cards.

	Controls		Cases		VE	95%CI			P value
	N	(%)	N	(%)	%				
Vaccination status									
Unvaccinated	23	(14.4)	15	(37.5)					
Incomplete course (with card)	22	(13.8)	7	(17.5)					
Incomplete course (without card)	14	(8.8)	7	(17.5)					
Full course (with card)	68	(42.5)	6	(15.0)					
Full course (without card)	33	(20.6)	5	(12.5)					
Scenario 1: those without cards as unvaccinated									
Unvaccinated	70	(43.8)	27	(67.5)	Ref				
Incomplete course (with card only)	22	(13.8)	7	(17.5)	11.8%	(-140.1%	-	67.6%)	0.80
Full course (with card only)	68	(42.5)	6	(15.0)	81.9%	(49.2%	-	93.6%)	0.001
Scenario 2: those without cards as vaccinated									
Unvaccinated	23	(14.4)	15	(37.5)	Ref				
Incomplete course (with and without card)	36	(22.5)	14	(35.0)	38.9%	(-55.2%	-	76.0%)	0.30
Full course (with and without card)	101	(63.1)	11	(27.5)	84.0%	(59.7%	-	93.6%)	<0.001

6. DISCUSSION

6.1 Feasibility of the mass vaccination campaign

A mass vaccination campaign with a 2-dose OCV was successfully conducted at the beginning of a cholera epidemic in a large and remote geographical area in Africa with a highly mobile population and difficult access. Overall, the implementation of the campaign did not differ compared to other mass campaigns used for outbreak response despite the 2-dose schedule. The volume of vaccines and therefore cold chain capacity required was large, but the fact that vaccines could be delivered at ambient temperature on the vaccination day by non-medical staff greatly facilitated the implementation.

The population in the midst of a cholera epidemic was eager to receive the vaccine; despite the short mobilisation and awareness activities, the vaccination coverage was high. Both provision and attention of resources to other preventive interventions remained strong as it was possible to use vaccination sites to deliver other prevention messages and items. While vaccination remains a costly intervention, it allowed for a significant reduction of inputs needed for curative activities in the following weeks. The funding of an OCV stockpile is currently being addressed by WHO and its partners in an attempt to improve the availability of OCV for countries facing outbreaks (12).

The 6 week implementation time lag for finishing the campaign was relatively long, partially due to the 2-dose schedule (given between two to three weeks apart in our case) but should be compared with the average time needed to organize mass vaccination campaigns against meningitis or measles, using single dose vaccines, which may be as long with variations depending on the implementing agency and other constraints. Immunological studies have suggested that partial protection after one dose of vaccine can be obtained (13), but whether the immunological response after one dose is enough to confer clinical protection or not needs to be demonstrated. Similarly, a herd protection effect of OCV has been reported (14, 15), but its extent is still unclear and needs to be confirmed in additional settings.

This experience demonstrated that mass campaigns with a two-dose OCV can be conducted successfully at the beginning of a cholera epidemic, even in a large, difficult-to-access area

in Africa with a highly mobile population, and with little time for preparation of the campaign and social mobilization. Potential obstacles that discouraged earlier campaigns either failed to materialize or were quite manageable; in particular, the population was eager to get vaccinated during the outbreak, and logistical issues were resolved.

In many ways our campaign was "over-resourced," due to the anticipated obstacles. Vaccination teams in Boffa were over-sized (half-sized teams in Forecariah vaccinated the same number of people per day), which increased transportation needs. Transportation of water sachets was logistically challenging; although use of water is not necessary according to the manufacturer, it was provided to facilitate the intake of the salty-tasting vaccine. Vaccination cards were used only to verify vaccination status during the coverage survey. A simplified strategy without use of water and vaccination cards would reduce personnel and transport needs, and therefore related costs.

Another potential simplification relates to vaccine vial presentation and packaging. The vaccine package as single-dose vaccines are voluminous, due partly to bulky secondary packaging. Additionally, the vaccine vial design is not ideal for oral use: single-dose vials are tiny, with metallic caps that are difficult to open.

There may also be potential to reduce cold chain needs. Although the vaccine is equipped with VVM 14 and considered temperature-stable, current labeling requires the vaccine to be stored in the cold chain. Documentation of thermostability is needed for future campaigns to be conducted using vaccines at ambient temperature.

A single-dose vaccine would also greatly simplify OCV campaigns. Studies in India found that partial immune response is achieved after a single dose [14], but whether this response is sufficient to confer clinical protection is not yet known. Similarly, a herd protection effect of Dukoral has been reported [15] [16], but its extent needs to be confirmed for Shanchol in additional settings.

Perhaps the most serious obstacles to wider use of reactive OCV campaigns are cost and limited supply of Shanchol. These constraints led us to drastically limit the target population to a small subset of those at risk; the full at-risk population includes everyone living along the coast of Guinea, including the capital (Conakry) with two million inhabitants, areas that were highly affected once the epidemic began. Funding for an OCV stockpile will be critical for the timely implementation of larger campaigns, an issue currently being addressed by WHO and its partners in an effort to improve OCV access for countries facing cholera outbreaks [17].

6.2 Acceptability of the mass vaccination campaign by the population

The high coverage and good acceptability of the campaigns, conducted in a rural mobile population in Guinea, is encouraging. The percentage of people reporting AEFIs was low and almost all participants reported that they would be vaccinated in a future campaign. However, more evidence is needed about the feasibility of reactive campaigns from densely populated urban scenarios where cholera burden is high and cholera outbreaks evolve faster [52,142–145]. Also the acceptability of target campaigns in such a context should be assessed from a political, public health and community point of view. Determining the short-term protection given by the first dose is a clear priority as an effective one-dose regimen would facilitate the ease and timeliness of reactive campaigns in all contexts.

There are several key limitations of note. Despite the short time span between the vaccination campaign and the data collection for the surveys, it was not possible to card-confirm the vaccination status for 25% of the participants and as a result some information bias may be present. Considering those individuals as not-vaccinated (worst-case scenario), two-dose coverage would decrease to 61% in Boffa and 64% in Forécariah. Second, the precision of estimates was better than expected because the number of participants recruited

was higher (linked with the household size composition) than originally planned. However, population estimates in the surveyed areas are likely to be inaccurate. In most areas, no major differences were observed between administrative and survey coverage, but in Kaback an important deviation was observed. Inaccuracies in the population data could have caused some imbalances in the allocations of clusters; as described, spatial sampling was used in Kaback to avoid this problem.

An additional limitation concerns the use of a quantitative approach to explore campaign acceptability. Although reasons for non-vaccination were specifically collected using an open question, it cannot be excluded the possibility that the population may not have understood certain awareness and education messages. A qualitative assessment would aid in understanding better the reasons for non-vaccination, elucidate possible solutions and provide a better understanding of the perception of the vaccination campaigns by the population.

There are few examples where OCVs have been used as public health tools. Dukoral was used pre-emptively in refugee camps in Uganda and Darfur [146,147] and in endemic areas (Zanzibar and Mozambique) [148,149]. Shanchol has been recently used in Haiti in a pilot campaign [150]. To our knowledge there are only two published examples of reactive campaigns using OCV, and both were conducted in Asia [151,152] using vaccines not prequalified by the WHO. The coverage and acceptability of these campaigns varied depending on the setting and the approach (pre-emptive vs. reactive). High coverage was obtained in Uganda, Darfur and Micronesia [146,147,151] and lower coverage was obtained in Mozambique, Zanzibar and Vietnam [148,149,152]. In Guinea, 76% coverage was obtained for two doses and 93% of the population received at least one dose, which represents, to our knowledge, one of the highest coverage ever reached [146–149,151,152]. The high coverage obtained is a promising outcome considering that this was one of the largest campaigns conducted in terms of number of doses administered, the specificities of the population (rural and mobile), and the short time available for preparation of the campaign, which has been one of the major arguments against outbreak response with OCV

.There are several factors that likely influenced the population to participate in the campaign: first, the campaign was conducted in response to an outbreak and the possibility of even partial protection against a frightening disease was motivating. Second, the population may have been reassured by the involvement of the MoH, public health authorities and MSF; as an example, the vaccination campaign was inaugurated in Boffa with the presence of the Minister of Health. This involvement was also crucial to mobilize human resources and to organize the campaign considering the local specificities. Finally, both the awareness campaign and the vaccination strategy itself (decentralized with sites organized in each village or settlement) involved the communities. This aimed to ensure awareness and provide vaccination opportunities to remote places and difficult to reach population which likely contributed to this high coverage. Vaccination activities started early in the morning and finished late in the afternoon to maximize the opportunities for workers in the main fishing ports. Despite these efforts, the lowest coverage was obtained in adult males.

Significant differences where observed by sex in Forécariah, especially in individuals between 15-49 years old. The vaccination campaign in Forécariah coincided with an intense period in agriculture activities, which was a barrier for the participation in the campaign, especially for the male adults. In addition, the Red Cross Society of Guinea distributed soap and a bottle of chlorine solution to women of childbearing age in Forécariah during the second round of vaccination, which likely increased the coverage in this group. Distribution of soap and chlorine was one of the control measures implemented by the MoH in response to the outbreak in the affected places, but this activity was successfully integrated in Forécariah within the vaccination sites. This suggests that synergies among different preventive approaches is an element to consider in future campaigns both to provide a more comprehensive message on cholera prevention and to improve the vaccine coverage itself.

6.3 Adverse events following immunization

The number of AEFI reported through the surveillance system was low, without severe AEFI reported. Only a small proportion of non-vaccinated individuals during the second round of vaccination reported AEFI as a cause of non-vaccination. This result is coherent with previous publications on vaccine safety where mild symptoms (mostly not requiring medical attention) have been reported [122,153]. The proportion of vaccinated individuals reporting AEFIs was lower in our study than in the cluster randomized clinical trial conducted in Kolkata (15 vs. 76 per 100,000) [122]. This difference is probably explained by: first, our surveillance system was passive compared with the active case finding implemented in Kolkata; and second, access to health care was likely more difficult in the vaccinated area in Guinea (remote rural area) than in the urban context of Kolkata.

With respect to the proportion of vaccinees vomiting or spitting out the vaccine after intake, it was observed a higher percentage than previously documented with Dukoral (no data available for Shanchol) [148]. For administration of Dukoral, the vaccine has to be diluted in water containing a buffer solution. Although administration with water is not necessary for Shanchol, water was offered after vaccine intake. Most vaccinated individuals did not like the taste of the vaccine and offering water may have contributed to fewer incomplete vaccine courses. Additional information should be collected in future campaigns using Shanchol, considering that providing water considerably increased the logistic complexity of the campaign.

6.4 Performance of the cholera rapid diagnostic test crystal in vaccinated individuals

To our knowledge, this is the first study showing that healthy individuals vaccinated with the oral vaccine Shanchol become positive with the cholera rapid test Crystal VC in the first days following vaccination. The proportion of vaccinated individuals positive for the Crystal VC test after vaccination was high (94.3%) for the O139 component of the test, but

low with the O1 component. This proportion of O139-positive tests decreased rapidly to half on the third day after vaccination and to one-fifth on the fourth day of follow-up. The median duration required to have a negative result for those cases presenting a previous positive test was 4 days.

Almost all positive tests (except for one) were positive only for O139 line, despite the fact that the Shanchol vaccines contains the two strains *V cholerae* O1 and O139, with a higher amount of O1 (1500 Elisa units of *V cholerae* O1 LPS and 600 Elisa units of *V cholerae* O139 LPS for a dose of 1.5 mL) [18]. This could be due to a higher sensitivity of the RDT for the O139, as suggested by the results of sensitivity against bacterial cultures showing that the O139 line was reactive with higher bacterial dilutions than the O1 line. Such results were already reported by Nato et al. [7] when evaluating the initial version of the RDT, but are in contradiction with those observed by Mukherjee et al. [13] with the first version of the Crystal VC test, which was reactive at 10^6 bacteria/mL for *V cholerae* O1 and 10^7 bacterial/mL for *V cholerae* O139. These differences of analytical sensitivity between the different versions of the RDT emphasize the need for a proper diagnostic performance evaluation of each new version of the test.

Including pre-vaccination stool status of our study population as well as unvaccinated participants could have provided useful information on the magnitude of potential false positive reactions due to factors unrelated to vaccination, i.e. non-specific reactions, which could have been expected considering the reported moderate specificity of the test [4, 11-13], or positivity due to asymptomatic carriers. The sharp increase and subsequent decrease in the proportion of O139 positive tests after vaccination are not in favour of such assumptions and suggest that the positive results were due to the vaccine alone. Of the 75 tests done after day 5, only three (4%) were positive for O139, and overall only one test was positive for O1 which is lower than the number of false positives that could be expected based on the test specificity. However, it should be noted that this study was conducted in

people without cholera symptoms while the previous evaluations were conducted in suspected cholera cases.

There are several limitations worth noting. First, women and adults were overrepresented in our study sample. Although women were more vaccinated than men were during the vaccination campaign carried out in Kabak, the proportion of women in our study (79.3%) was clearly higher than the vaccinated population (59.5%) [19]. This is likely due to the fact that the majority of men presented early at the vaccination site and were more likely to be excluded given their potential absence for work during the follow-up period. However, although there was a small difference in the mean time to become negative between men and women (4.3 days vs. 3.6), the median was the same for both sexes (4 days) thereby not likely affecting the results presented here. The median age in the study was 25 years compared to 15 for the vaccinated population [19]. Considering that the time to become negative was longer for the older participants, it is likely that the time to become negative was slightly overestimated. Nonetheless, the differences by age were small in magnitude (0.2 days per 10 years of age) and they do not change the interpretation of the results neither our recommendations regarding the use of the cholera RDT in vaccinated areas. Second, it was not possbile to conclude on five cases who had a positive result with their last specimen collected, and for whom further samples could not be collected because they were absent or unable to produce stool samples. When designing the study, it was decided to limit the follow-up period to 7 days, based on the expected time for gastrointestinal transit of the killed bacteria. Although extending the follow-up of participants until they became negative for the rapid test would have been useful for concluding on these 5 individuals, this limit was considered reasonable in the absence of any other data. In addition, even considering that these five people were still positive at day 7, the percentage of positive tests would be still low (5.2%), lower than the expected for non-cholera cases considering the specificity of the test. Third, culture was not performed to exclude participants with possible cholera or asymptomatic carriage of *V cholerae*. Although initially planned in the protocol for participants with diarrhea or with a positive RDT at the end of follow-up (day 7), no culture

was performed since symptoms were found unreliable and none of the specimens tested on the seventh day of follow-up were positive. Finally, specimens were tested on average ten hours after stool production and without the possibility of storage at 4°C due to the lack of electricity in Kabak. This delay seems reasonable given the difficulties to collect the samples immediately after production, although it is unclear the degree to which antigens degrade during this period, which could potentially affect the RDT results.

6.5 Vaccine effectiveness

The results presented here show high effectiveness of two complete doses of Shanchol when administered as part of the response to a cholera epidemic in Africa. Our results represent an estimate of the short-term protection of Shanchol and are in line with previous results with Dukoral [117]. This is highly relevant considering the fact that oral vaccines have shown low levels of protection in low-income African settings in the past [154–156].

This study was carried out under real field conditions during a cholera outbreak with several limitations to note. The outbreak response immunization was part of the control strategy implemented by the Ministry of Health with the support of MSF in response to the epidemic; thus, the exposure in the population was not controlled. Overall, 316,250 vaccines were delivered and 48 non-severe adverse events following immunization were notified; the vaccine coverage was high [157], ranging from 69% in Koba to 84% in Makountan sub-prefectures. High vaccination coverage reduces transmission in vaccinated communities (herd protection) [158–160], thereby directly and indirectly reducing the risk of cholera. In the past, this has limited opportunities to measure effectiveness [161] due to difficulties in recruiting case-patients. In our study, most of the cases were recruited from Koba (Boffa prefecture) where a small local outbreak was reported (August-October 2012). This area showed the lowest vaccination coverage [157] and borders Dubreka where high transmission (overall attack rate 17 per 10,000) was reported. It is important to mention that the small sample size did not translate into low power (99%) as the observed effectiveness

was higher than the assumption considered for the sample size calculation. However, the small sample size limited the possibility of conducting stratified analyses by age and to estimate single dose effectiveness with sufficient precision. This latter figure although not significant, was substantially lower than the protection conferred by two doses, and the point estimate was in line with previous findings with other OCV [117,122,160].

Despite the wide and systematic distribution of vaccination cards and the short time span between the vaccination campaign and the data collection, 25% of the vaccinated people interviewed were not able to provide their vaccination card at the time of the study. As vaccination status for all participants was not confirmed, some information bias may be present. Nevertheless, even if we consider all the individuals who were unable to find their vaccination card as not-vaccinated (worst-case scenario), the vaccine effectiveness would still reach 82%.

Further, case-control studies of vaccine effectiveness may also be prone to bias related to differences in health seeking behavior. In order to measure this potential bias a supplementary analysis was conducted, measuring the odds of vaccination among non-cholera watery diarrhea cases and a sample of matched control-subjects (indicator bias analysis). As the odds of vaccination did not vary significantly between these two groups, this finding was interpreted as absence of large health seeking behavior bias.

Despite difficulties inherent in assessing effectiveness under field conditions, precise estimates of the short-term protection (first six months) of Shanchol were obtained in Africa where the impact of OCV is expected to be the highest in reducing mortality [5,7] and where problems with the protection provided by oral vaccines have been documented in the past [156]. The crude and the adjusted effectiveness estimates were similar after exploring the effect of a large number of well-described possible confounders, as well as when using only PCR or culture confirmed cases. The low proportion of PCR positive samples could be related to the small amount or the poor quality of biological material, as assessed by the weak amplification signal of the 16S rRNA gene. False positive RDT results cannot be

excluded [80,162]; this non-differential misclassification would have underestimated the vaccine effectiveness.

The results presented here suggest that the short-term protection of Shanchol can be higher in the first six months than the protection remaining two years post-vaccination (67% estimate reported by Sur et al.) [122], probably as a result of waning immunity. It is possible that this difference is also partially explained by the fact that some non-vaccinated participants may have naturally acquired immunity, as cholera is endemic in Kolkata. In addition, our estimate might include some indirect protection, although indirect effects were minimized through the matched design [158,159].

An aspect that does not seem to have decreased the short-term protection provided by Shanchol is the cold chain strategy used in Guinea, where vaccines were stored under cold chain, but were transported and used at ambient temperature on the vaccination day. The vial temperature monitor was checked for stability before administration (all remained valid). These results are not surprising considering the good heat stability of Dukoral [4], but this requires more robust documentation which will allow for more flexible delivery strategies in the future. Another aspect that can substantially simplify the use of OCV in outbreak settings is a single dose regimen. Our study was underpowered to provide precise estimates of the one-dose protection. Determining the short-term protection given by one dose is a clear priority towards the implementation of efficient and timely reactive campaigns.

7. CONCLUSIONS AND RECOMMENDATIONS

7.1 Feasibility of the mass vaccination campaign

- Our experience demonstrates the feasibility of implementing OCV mass campaigns at the onset of major epidemics.

- The difficulties found were similar to the campaigns with other vaccines used reactively (e.g., measles).

- The main difficulties were associated to the two doses schedule, cold chain needs and provision of water for vaccine intake

- A simplified strategy without use of water and vaccination cards would reduce personnel and transport needs, and related costs.

7.2 Acceptability of the mass vaccination campaign by the population

- High vaccine coverage can be reached within a few weeks, even in remote rural areas.

- The campaigns were well accepted by the population.

- Good documentation of these interventions is essential to elucidate the strategies leading to successful outcomes as well as key implementation barriers.

- Synergies between different axes in cholera control interventions should be pursued

- Other examples of integrated cholera response than the one presented here should serve also to determine the best use of vaccines for cholera prevention and control.

7.3 Adverse events following immunization

- The safety profile of Shanchol is good when used in reponse to outbreaks

- No severe adverse event following immunization were document

- Few mild adverse event were reported, with mainly ganstrointestinal symtoms associated.

7.4 Performance of the cholera rapid diagnostic test crystal in vaccinated individuals

- The rapid test Crystal VC can become positive in persons recently vaccinated against cholera.

- The rapid test Crystal VC can become positive only with the O139 line, probably linked to its higher analytical sensitivity.

- The tests become negative rapidly and five days after vaccination the proportion of positive tests among vaccinated is less than 3%.

- As the current global pandemic is almost exclusively caused by Vibrio cholerae O1, our results suggest that the current Crystal VC kit can be used normally as soon as 24 h after receiving Shanchol in a context of *V cholerae* O1 epidemic, and after a period of five days in areas where *V cholerae* O139 is present.

- Other cholera rapid diagnostic tests based on the LPS detection are available in the market and could also become positive in recently vaccinated individuals. Thus, an evaluation of other tests or future versions of the Crystal VC test is recommended if they are to be used in the context of oral cholera vaccination campaigns.

- Tthe diagnostic performances of the current modified version of the Crystal VC test should be evaluated with respect to the different sensitivities of the O1 and O139 lines.

7.5 Vaccine effectiveness

- The estimates on the short-term effectiveness of Shanchol is high when used in response to epidemics

- The hihg effectiveness documented here is a key and essential information to improve the current strategies for outbreak prevention and control.

- This evidence supports the current WHO recommendation of exploring the role of OCV in response to cholera outbreaks.

- This evidence should serve to recommend strongly the addition of OCV among the tools to be used in response to epidemics.

8. ACHIEVEMENTS AND WAY FORWARD

This evidence served to strongly recommend the addition of OCV among the tools to be used in response to epidemics, alongside efforts to improve provision of safe water and sanitation and access to cholera treatment. In addition it served to support the creation of an oral cholera vaccine stockpile for emergency use by the WHO and partners. This work has been also considered by the Global Alliance for Vaccines and Immunization (GAVI) in order to include the oral cholera vaccine among the vaccine supported for introduction in the next coming years.

The next research areas were considered as priorities to improve the feasability, acceptability, effectiveness and impact when used in respose to outbreaks:

1. To determine the level of protection of a one dose schedule.
2. To stablished the termostability of the vaccine when used out of the cold chain
3. To determine the safety profile when administered to pregnant women
4. To analyise the possible interference with the co-administration of the oral polio vaccine
5. To assess the the feasibility and acceptability of reactive campaings in urban contexts
6. To determine the overall impact of oral cholera vaccine in response to epidemics in terms of deaths and cases adverted

Five different project are ongoing to adress the above mentioned research questions:

1. A phase II clincial trial that will serve to determine the immune response to one dose schedule and the thermostability of the vaccine

2. A cohort study to measure pregnacy outcomes in vaccinated and unvaccinated pregnant women

3. A phase II clinical trial to analyise the possible interference with the co-administration of the oral polio vaccine

4. A demostration project of the use of cholera vaccine in urban African scenarios

5. Modeling work to estimate the impact of reactive vaccinations

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10. ANNEXES

Annex 1. Related publications

1. **Luquero FJ**, Banga CN, Remartínez D, Palma PP, Baron E, Grais RF. Cholera epidemic in Guinea-Bissau (2008): the importance of "place". PLoS One. 2011 Jan;6(5):e19005.

2. Azman AS, **Luquero FJ**, Rodrigues A, Palma PP, Grais RF, Banga CN, et al. Urban cholera transmission hotspots and their implications for reactive vaccination: evidence from bissau city, Guinea bissau. PLoS Negl Trop Dis. 2012 Nov;6(11):e1901.

3. Grandesso F, Allan M, Jean-Simon PSJ, Boncy J, Blake A, Pierre R, **Luquero FJ**. Risk factors for cholera transmission in Haiti during inter-peak periods: insights to improve current control strategies from two case-control studies. Epidemiol Infect. 2013 Oct 11;1–11.

4. Ciglenecki I, Sakoba K, **Luquero FJ**, Heile M, Itama C, Mengel M, et al. Feasibility of mass vaccination campaign with OCV in response to an outbreak in Guinea. PLoS Med. 2013 Sep;10(9):e1001512.

5. **Luquero FJ**, Grout L, Ciglenecki I, Sakoba K, Traore B, Heile M, et al. First Outbreak Response Using an Oral Cholera Vaccine in Africa: Vaccine Coverage, Acceptability and Surveillance of Adverse Events, Guinea, 2012. PLoS Negl Trop Dis. 2013;

6. Martinez-Pino I, **Luquero FJ**, Sakoba K, Sylla S, Haile M, Grais RF, et al. Use of a cholera rapid diagnostic test during a mass vaccination campaign in response to an epidemic in Guinea, 2012. PLoS Negl Trop Dis. 2013 Jan;7(8):e2366.

7. **Luquero FJ** et al. Epidemic use of killed whole cell *V cholerae* vaccine, Guinea, 2012. NEJM. In press.

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PLoS one

Cholera Epidemic in Guinea-Bissau (2008): The Importance of ''Place''

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Abstract

Background: As resources are limited when responding to cholera outbreaks, knowledge about where to orient interventions is crucial. We describe the cholera epidemic affecting Guinea-Bissau in 2008 focusing on the geographical spread in order to guide prevention and control activities.

Methodology/Principal Findings: We conducted two studies: 1) a descriptive analysis of the cholera epidemic in Guinea-Bissau focusing on its geographical spread (country level and within the capital); and 2) a cross-sectional study to measure the prevalence of houses with at least one cholera case in the most affected neighbourhood of the capital (Bairro Bandim) to detect clustering of households with cases (cluster analysis). All cholera cases attending the cholera treatment centres in Guinea-Bissau who fulfilled a modified World Health Organization clinical case definition during the epidemic were included in the descriptive study. For the cluster analysis, a sample of houses was selected from a satellite photo (Google Earth^{TM}); 140 houses (and the four closest houses) were assessed from the 2,202 identified structures. We applied K-functions and Kernel smoothing to detect clustering. We confirmed the clustering using Kulldorff's spatial scan statistic. A total of 14,222 cases and 225 deaths were reported in the country (AR = 0.94%, CFR = 1.64%). The more affected regions were Biombo, Bijagos and Bissau (the capital). Bairro Bandim was the most affected neighborhood of the capital (AR = 4.0). We found at least one case in 22.7% of the houses (95%CI: 19.5–26.2) in this neighborhood. The cluster analysis identified two areas within Bairro Bandim at highest risk: a market and an intersection where runoff accumulates waste ($p<0.001$).

Conclusions/Significance: Our analysis allowed for the identification of the most affected regions in Guinea-Bissau during the 2008 cholera outbreak, and the most affected areas within the capital. This information was essential for making decisions on where to reinforce treatment and to guide control and prevention activities.

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Introduction

Although cholera has disappeared among the diseases affecting developed countries, it remains one of the main causes of morbidity and mortality in the poorest areas of the world [1,2]. The burden of cholera is underestimated or non-estimated and many countries face recurrent epidemics [1,3]. Sub-Saharan African countries are especially affected, with 95% of reported cholera cases and 98% of deaths [4]. Cholera emerges under poor hygiene and sanitary conditions; thus, the lack of basic services and disorganized urbanization in many Sub-Saharan African countries constitutes the perfect culture medium for cholera [1].

John Snow, one of the founders of modern epidemiology, showed the importance of descriptive epidemiology in cholera epidemics, emphasizing the importance of "place", or the consideration of space, to target prevention and control activities [5]. Today, although resources and tools for mapping are available, the description of place in cholera epidemics remains poor and examples of studies using spatial technologies in the medical literature are limited [6–15].

The objective of this study was to describe the cholera epidemic affecting Guinea-Bissau from May 2008 to January 2009 focusing on place in order to guide prevention and control activities. We also conducted a cluster analysis to obtain more detailed information about the distribution of cases in the most affected area of the capital (Bairro Bandim) with the same aim.

Methods

Context

The Republic of Guinea-Bissau is one of the smallest nations in continental Africa; it is divided into 8 regions (Bafata, Biombo, Bissau, Bolama, Cacheu, Gabu, Oio, Quinara and Tombali) and the capital, Bissau, (Sector Autónomo de Bissau (SAB)). The SAB is the smallest geographical region in the country but the most densely inhabited. Around 27% of the total population of the

country lives in this area. Although the country has experienced several years of stability and development since the end of the civil war in 1998, the life expectancy at birth is 47 years, and 203 children die before the age of five per 1 000 live births [16].

Descriptive epidemiology

Data sources. Daily official data from the Public Health General Direction (DGS) registers was used to describe the epidemic for the entire country in terms of time and place.

For SAB, we put in place a comprehensive data collection system to describe the epidemic from 05/05/2008 to 20/10/2008. These data came from the registers of the Cholera Treatment Center (CTC) in the Hopital Nacional Simao Mendes and the five Cholera Treatment Units (CTU) set up in 5 Health Care Centers (Bandim, Bairro Militar, Ajuda, Antula and Plaque). The collected data included age, sex, place of residence (*bairro* and *area sanitaria*), center where the patient was treated, and clinical outcome (dead or alive).

Population data (denominators) used for rate estimations were obtained from the 1991 census of Guinea-Bissau. A specific growth rate was applied to the different Sanitary Areas (SA) to account for population growth and migration (provided by the Epidemiological Department of the DGS (SE-DGS)). Age and sex distributions were obtained from the annual SE-DGS census projections.

Case definitions, laboratory procedures and statistical analysis. A modified WHO clinical case definition was used for suspected and confirmed cases of cholera [17]. A suspected case was defined as any person suffering from acute watery diarrhea with or without vomiting. A confirmed case was defined as any suspected case with a positive stool sample to *Vibrio cholera O1* or *O139*.

Stool samples were analyzed in the National Laboratory of Microbiology (NLM) in Bissau to confirm the cholera outbreak and to determine the current circulating strain and its antibiotic sensitivity by culture [18]. Additional samples were sent to the Pasteur Laboratory in Dakar for the same purpose.

We describe the cholera outbreak in Guinea Bissau in terms of time and place. For SAB, we describe the epidemic in terms of time, place and person. Central tendency (mean and medians) and dispersion parameters (standard deviation and interquartile range) were calculated for continuous variables, percentage and 95% confidence intervals for categorical variables. To adjust attack rates by age and sex, we used a Poisson regression model.

Survey: cluster analysis

We conducted a cluster analysis to identify areas at high risk of infection in the Bairro Bandim Health District of SAB. Bandim was selected because this neighborhood reported the highest attack rates and the most cases within SAB.

Sample size. To calculate the sample size, we assumed that 20% of the households would have at least one case. We aimed to detect statistical differences for areas with at least 30% of households with one case. Considering a power of 80% and an alpha error of 0.05, the required sample size was 626 households. Assuming 10% of households would be either refusals or absences, the sample size was 678 households. We randomly selected 140 houses from the 2,202 structures identified in the satellite photo obtained from Google Earth™. To do so, we assigned a number to each house. We then used a random number, using the random generator function implemented in R© Statistical Software [19], for selection. For field teams to locate houses, coordinates (WGS 84) of the randomly selected houses were introduced into a handheld GPS (Garmin XL). The team carried out an active search of cases in the selected household and in the four closest households (defined by the field teams) in order to reach the desired sample size in a reasonable time without loosing precision due to the design effect [20]. Thus, the final sample frame was 700 households.

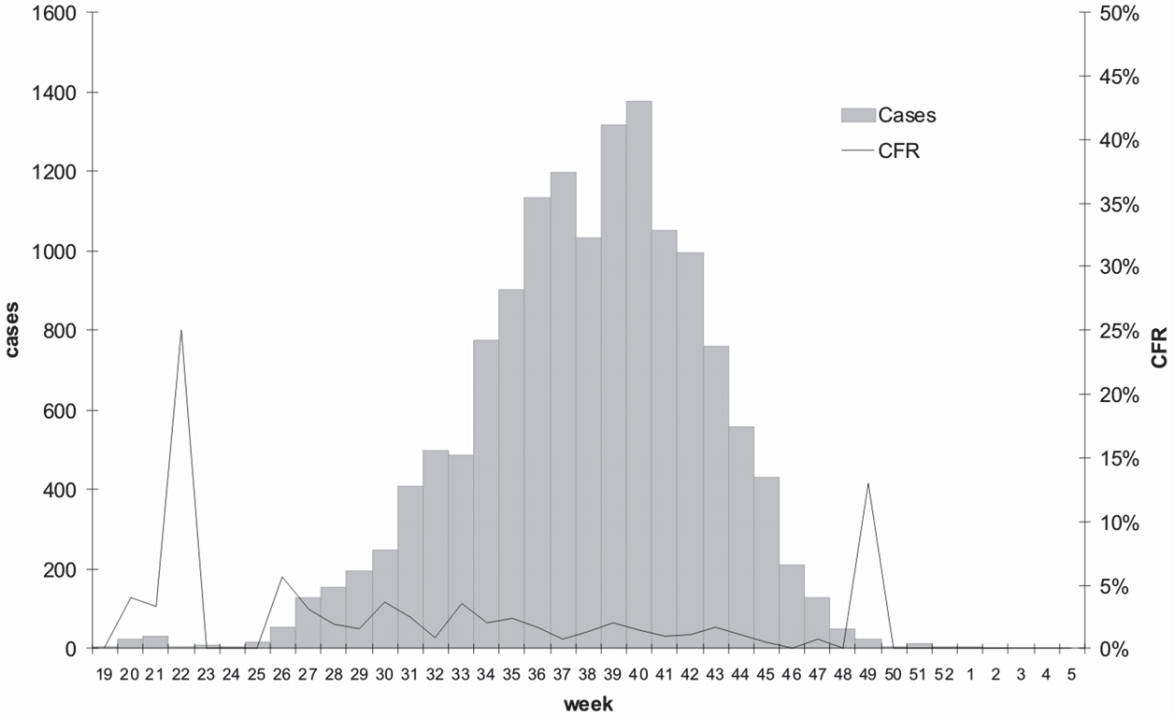

Figure 1. Weekly number of cholera cases and case fatality ratio (CFR%) in Guinea-Bissau 2008–2009.
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Statistical analysis. Our spatial point pattern consisted of locations with at least one cholera case and houses without cases. Thus, the data represented a typical example of a marked point pattern. We analyzed whether or not the observed cholera cases were clustered over and above the level that would be expected under natural environmental heterogeneity. We calculated the K-functions for both the houses without cases and those with at least one case, and the difference was used to detect the extra propensity of the households with cases to cluster. We calculated the standard error for the difference of the K-functions and the 95% confidence intervals in order to know if the observed difference was different from zero, meaning clustering occurred [21]. Next, we computed the probability of finding a house with at least one case using a Kernel smoothing technique [22]. The significance of departure from randomness was assessed by random labeling performing 1000-time simulations to establish upper and lower confidence limits. These analyses were performed using in R© Statistical Software [19]. We confirmed the clustering using Kulldorff's spatial scan statistic using SatScan software [23]. We performed 9999 Monte Carlo replications to obtain estimates of P values and confidence intervals.

Ethical considerations

As this study was conducted during the emergency response to the cholera outbreak and was designed to provide information to orient the public health response, ethical approval was not sought prior to the survey. We sought retrospective approval from the National Ethical Review Board (ERB) of Guinea-Bissau and from the MSF ERB. The MSF ERB considered that since the purpose of the study was to guide prevention and control activities, it could be considered good public health practice rather than research. The National ERB of Guinea-Bissau granted retrospective approval.

Privacy, confidentiality and rights of patients were ensured during and after the conduct of the study. Oral informed consent was obtained in each visited household after detailed explanation of the existence of an outbreak, the objective of study and the planed use of the information. Moreover, health education was carried out in each household regarding cholera transmission and prevention. The information was entered and analyzed anonymously. The study was implemented in collaboration with the Ministry of Health after obtaining authorization to carry out the survey.

Results

Descriptive epidemiology

The first cholera case was declared in Guinea-Bissau on 5 May 2008 (week 19). The epidemic was officially declared in July. Both the NLM and the Pasteur Laboratory in Dakar confirmed that the circulating strain was *Vibrio Cholera O1* El Tor-Ogawa. A total of

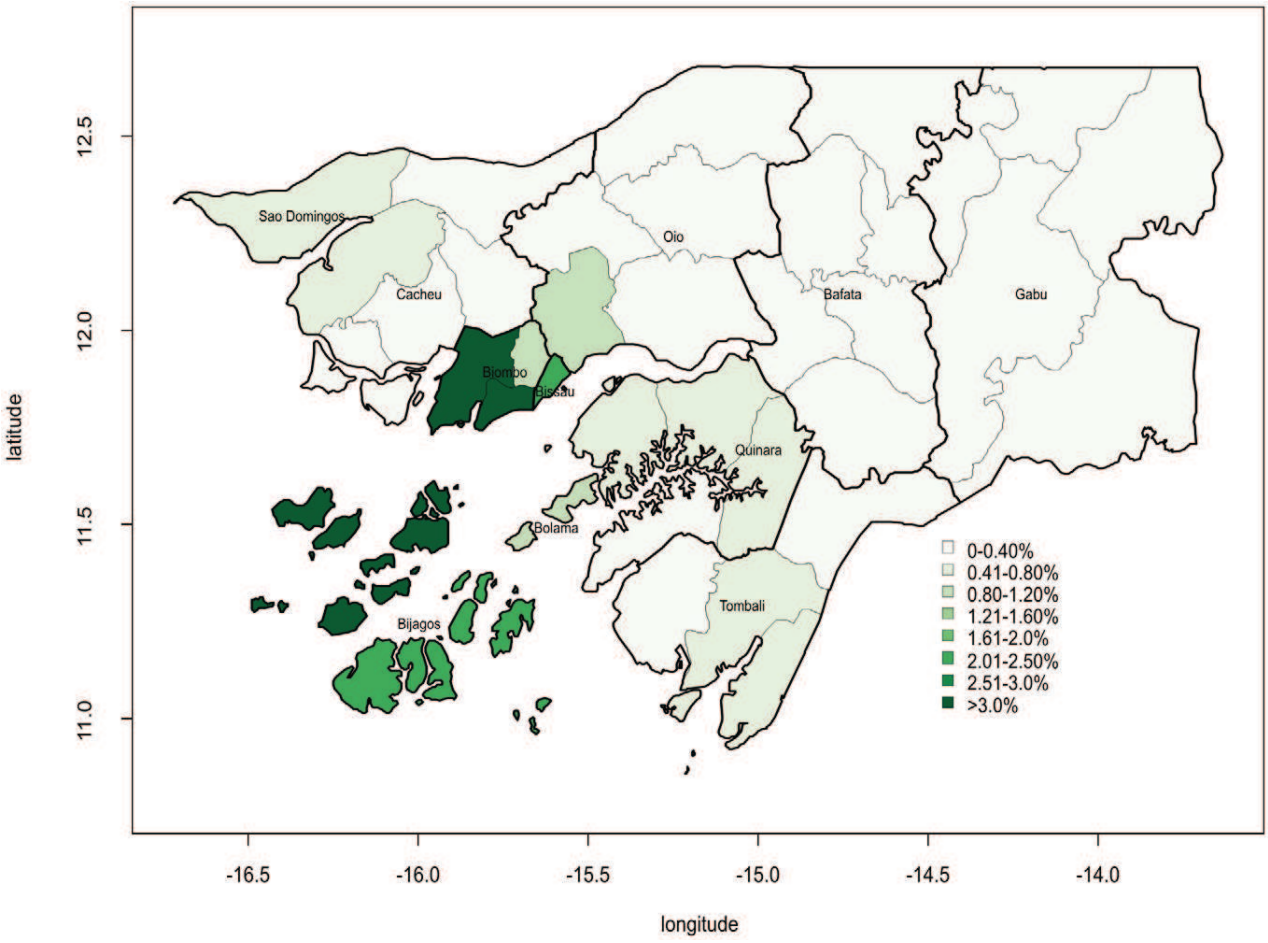

Figure 2. Geographical distribution of the crude cholera attack rate by region and sub-regions in Guinea- Bissau, 2008–2009. Coordinates expressed in sexagesimal degrees.
doi:10.1371/journal.pone.0019005.g002

14,228 suspected cases and 225 deaths were declared in the whole country. This number of cases corresponds to an attack rate of 0.94 (AR) per 100 people. The reported case fatality ratio (CFR) per 100 cases was 1.58. The weekly average number of cases was 395.2 (SD 454.6) and the median 176.0 (IQR = 15.5–764.0). The weekly number of cases varied between a minimum of 2 cases and a maximum of 1,376 cases. The peak of the epidemic was observed after 22 weeks of reported cases, which corresponds to epidemic week 40. After the peak, fourteen consecutive weeks with decreasing numbers of cases were observed (Figure 1).

Figure 2 shows a more detailed description of the geographical distribution of the epidemic. There were several sub-regions with AR over 2% (SAB; Quinhamel and Prabis in Biombo; Bubaque and Uno in Bijagos) and two of them (Ondame in Biombo and Caravelas in Bijagos) reached 4%.

The SAB reported 67% of the total number of reported cases in the country. The first case was declared 5 weeks after the first notification in Tombali. Finally, 9,394 cases and 73 deaths were reported in the SAB, which corresponds to an AR of 2.33%. The reported CFR was 0.78%. The peak of the epidemic was observed in epidemiological week 40. Until the 20 October 2008 (period with individual data collection), a total of 7,749 cases were reported in the CTC and 5 CTUs of SAB. Most cases were treated at the CTC (68.4%, n = 5300/7749). Regarding the CTUs, the Bandim CTU received the most patients (9.8%). Most patients were between 15 and 49 years old (68.6%). This group showed the highest attack rate, almost 3.5 times higher than in the youngest age group. The number of reported cases was similar in men (48.6%) and women, with similar attack rates.

We obtained information about the SA of residence from 7,294 patients (94.1%). Bandim was the SA with the highest number of patients in absolute numbers (24.1%) but also in relative numbers (AR = 3.9%). Compared with Ajuda, people living in Bandim were 2.5 times more affected (Table 1). There was not a clear spatial pattern in the SAB, but all neighborhoods located in the southwest had ARs over 1.5% (Figure 3).

Cluster analysis

From the 140 structures randomly selected, we were able to assess 136. Of the 4 structures not included in the analysis, 2 were not households (one was a cinema and the other a carpentry) and 2 houses were uninhabited. As we also assessed the four closest households to those randomly selected, a total of 616 households were included in the analysis. We found at least one case in 140 households (22.7%; 95%CI: 19.5%–26.2%).

We computed the K-functions for the 476 houses without cases and the 140 houses with at least one case. We also computed the difference between both K-functions and the 95% confidence intervals as explained previously [21]. This comparison showed that the houses with cases were more clustered than houses without cases ($p<0.001$) (Figure 4). Next, we computed the probability of finding a house with at least one case using a Kernel smoothing technique. Two clusters were identified in the study area using both random labeling and the Kulldorff's spatial scan statistic (Figure 5).In the most affected areas (clusters), we estimated that 30% of the houses had at least one case and the least affected only 1% (Figure 5).

Discussion

Our analysis allowed for the identification of the most affected regions in Guinea-Bissau during the 2008 cholera outbreak, and the most affected areas within the capital where 67% of cases were reported. This information was essential for making decisions about where to reinforce treatment and to guide control and prevention activities. As resources are usually limited when responding to cholera outbreaks, knowledge about where to orient interventions is crucial.

Although this analysis provided critical information, this study has limitations. As was the case here, most descriptions of cholera epidemics are *a posteriori*. Comprehensive data collection began one week before the peak of the epidemic and only in the SAB. The description was limited principally due to time and resource constraints as well as the trade-off inherent in emergencies where close concerns and simple analyses are more important than distal and complex analyses [24,25]. We focused only on one affected area of the city and did not establish statistical associations with environmental, social or cultural risk factors. Strengthening local capacity in surveillance of diseases of epidemic potential remains an ongoing need in countries like Guinea Bissau. Further work should also focus on identifying risk factors that may help orient future interventions. Moreover, we simplified the cluster analysis in the sense that we did not count all the cases in a house; we only classified the households as with or without cases. Thus, the cluster analysis captures the spatial distribution of the risk of primary infections (all houses have at least one) but this can limit the identification of clustering due to factors different from the household location (i.e. secondary transmission at household level).

Table 1. Number of cases, population, attack rate per 100 people (AR%), risk ratios (RR) and adjusted risk ratios (ARR) by age, sex and sanitary area.

Variable	Cases	Population	AR%	RR	ARR
Gender					
Female	3 960	203 946	1.94	Ref.	
Male	3 744	199 052	1.88	0.97	0.98
Age (years)					
0–14	1 632	189 409	0.86	Ref.	
15–49	5 312	174 337	3.05	3.54	3.54
>50	798	39 252	2.03	2.36	2.27
Sanitary Area					
Ajuda*	160	10 429	1.53	Ref.	
Antula*	640	30 778	2.08	1.36	1.37
Bandim*	1 756	44 718	3.93	2.56	2.61
Bairro Militar*	932	65 274	1.43	0.93	0.94
Belem	306	17 263	1.77	1.16	1.18
CIM**	154	14 985	1.03	0.67	0.68
Cuntum	857	45 482	1.88	1.23	1.24
Luanda	219	25 236	0.87	0.57	0.58
Missira	516	38 838	1.33	0.87	0.87
Pefine	306	14 808	2.07	1.35	1.37
Plaque*	380	27 633	1.38	0.90	0.89
Quelele	472	28 898	1.63	1.06	1.08
Sintra_Nema	348	21 451	1.62	1.06	1.08
Santa Luzia	248	17 204	1.44	0.94	0.96
Total	7 749	402 998	1.92	-	-

*Cholera treatment unit set up in this area.
**Cholera treatment centre set up in this area.
Sector Autónomo de Bissau, 2008.
doi:10.1371/journal.pone.0019005.t001

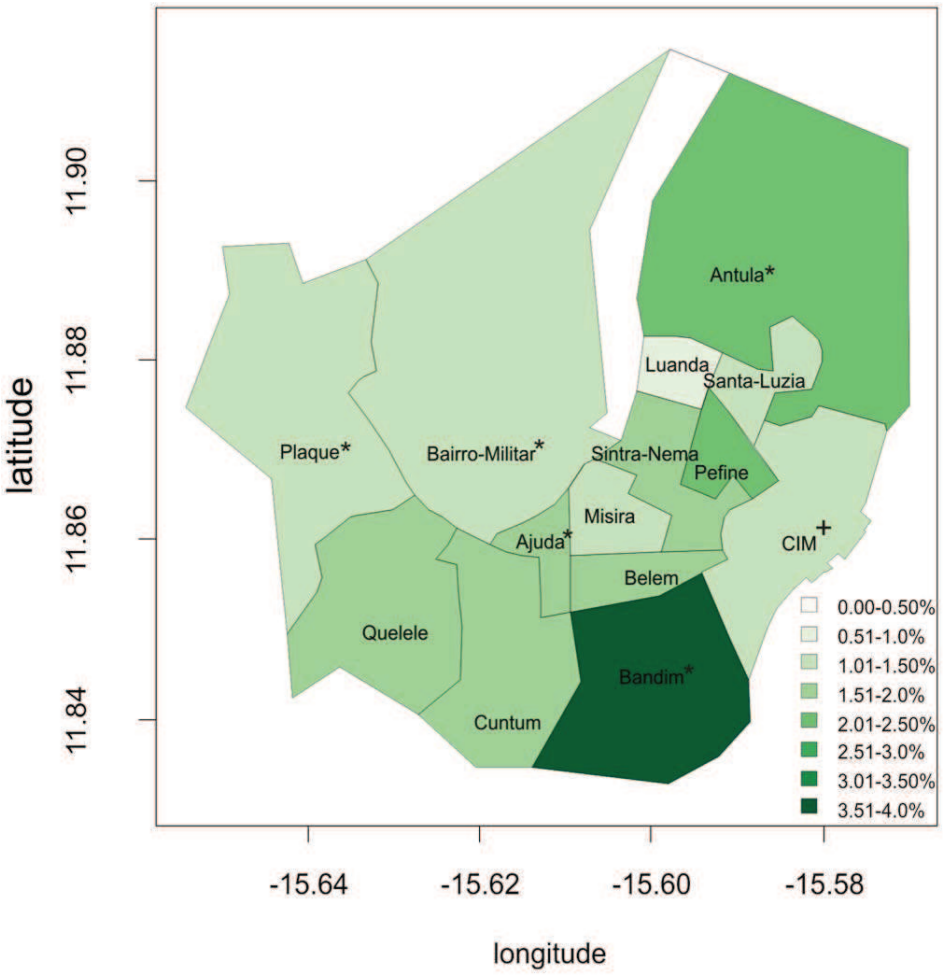

Figure 3. Age and gender adjusted cholera attack rates (%) by Sanitary Area in Sector Autónomo de Bissau, 2008–2009. Coordinates expressed in sexagesimal degrees. * Sanitary area with a cholera treatment centre. + Sanitary area with a cholera treatment unit.
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There are some examples in the literature using spatial techniques to establish associations with environmental variables, but most of these studies are retrospective and come from Bangladesh. Long-term surveillance in Bangladesh, annually affected by cholera, has allowed for research activities regarding the vaccine and etiology of cholera disease. The examples in Africa, where most of the cases occur [4], are scarce and there is a real need for more accurate spatial information. One study in Lusaka, Zambia used a similar methodology to describe the epidemic in one of the most affected neighborhoods of the city [7]. In eastern Democratic Republic of Congo (Kivu provinces) a geographic information system was established, and the authors identified relationships between environmental variables and the number of cholera cases [15]. This analysis allowed the identification of some cities, located on Lake Kivu and Lake Tanganyika, which serve as the main sources of cholera epidemics. Another study in Kumasi, Ghana, analyzed the association of cholera with proximity to refuse dumps [6]. However, in many other countries currently affected by large cholera outbreaks like Zimbabwe, Angola, Mozambique or other west African countries (among those Guinea-Bissau), the spatial epidemiology remains poorly described.

The epidemic prior to 2008 in Guinea-Bissau was in 2005. The same strain (*Vibrio Cholera* O1 El Tor-Ogawa) was circulating during that epidemic and most natural immunity acquired during 2005 had probably vanished during the three-year inter-epidemic period. The AR was higher in 2005 (1.75% vs 0.94%), with a similar CFR. The current outbreak started in May, one month earlier than the outbreak of 2005, but the peak was reached after 22 weeks, thereby doubling the pre-peak period. The first area affected was also different, the current epidemic started in Tombali, but in 2005, the outbreak was first reported in SAB. In both epidemics, the transmission of cholera within SAB facilitated the rapid spread to other regions of the country, and the more affected areas were Bijagos, Biombo and the Sector Autonomo de Bissau in both epidemics.

In all regions, the CFRs were higher at the beginning of the epidemic. This is likely due to the implementation of improved case management and under notification of non-severe cases during the first weeks of the epidemic. Especially high CFRs have been observed in Quinara and Bafata (9.2% and 8.2% respectively), where again the combination of a poor case management and under-notification of non-severe cases likely explain these figures.

The SAB reported 67% of all reported cases. The area with the highest AR was Bandim, playing an important role in the dynamic of the epidemic within the city. Other areas in the southwest of the

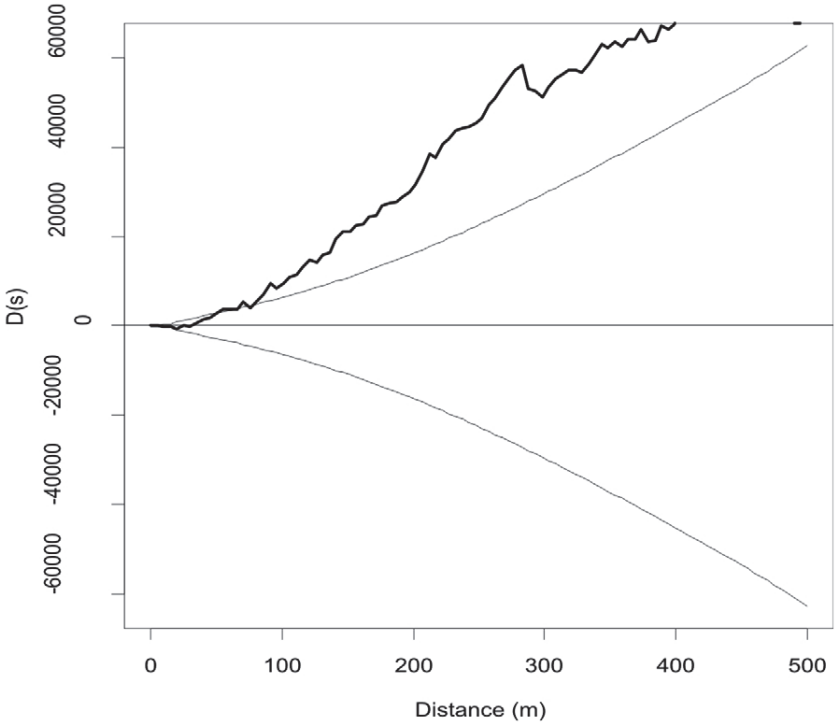

Figure 4. Differences of K-functions and 95% confidence intervals between households with cholera cases and households without cases in Bairro Bandim (Bissau), 2008–2009. A homogeneous set of points in the plane is a set that is distributed such that approximately the same number of points occurs in any circular region of a given area. A set of points that lacks homogeneity is spatially clustered. The k-function is defined as the expected number of points within a distance *s* of an arbitrary point, divided by the overall density of the points. Due to variations in the spatial distribution of the population at risk, a k-function computed only for cases may not be informative. Instead, the k-function calculated for cases can be compared with the one calculated for non-cases, with the difference between the two functions representing a measure of the extra-aggregation of cases over and above the observed for the non-cases. This difference is represented in the figure above, showing extra-aggregation of cases.
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city such as Quelele, Cuntum, Ajuda or Belem also showed higher ARs than other areas. Within big areas like Bairro Militar, it is likely that the distribution of the AR was not homogeneous with some sub-areas more affected, but we could not test this hypothesis because of the lack of smaller spatial scale population data. We focused our investigation in Bandim because of the high AR and the high percentage of total number of cases reported from this neighborhood. This area is close to the markets and the main road, so it may be important not only because of its disease burden but also because of its potential role in the circulation of cholera in the whole city. Nonetheless, it is important to consider that Bandim has been the site of demographic surveillance system with a focus on infectious diseases. It is possible that the extended presence of these activities in the community leads people to seek treatment promptly, and may therefore account in part for the high attack rate in Bandim [26].

The cluster analysis identified two areas within Bandim at higher risk of finding houses with cases. One was the surroundings of the Caracol market. Different factors potentially explain this higher risk. The first is the market itself; people living in this area are more likely to be in contact with other cholera cases because the market has a large inflow of people. There were also plausible foodborne cases originated in the market, which may disporportionaly affect people living in the surrounding area. Another factor is the large amount of waste around the market and garbage in the streets. Moreover, an open drain passed through the market gathering solid waste and dirty water. Within the market, sanitary conditions were inadequate. The number of latrines was insufficient; there were no hand washing points and the control over the food items sold was insufficient. These factors combined undoubtedly facilitate transmission. As a result of this analysis, the authorities cleaned the market and established washing point and installation of additional latrines. In markets, customers tend to touch, taste and/or smell aliments; this reinforced the need for focused behavioral changes together with sanitation measures around markets.

The other affected area also has a high level of crowding and the confluence of two factors that can increase risk: crowding –this area is crossed by of one of the main streets in Bandim– and an area where runoff accumulates waste. Moreover, the altitude of this zone is almost at sea level. It is likely that the freatic (groundwater) level in this area were higher, which implies less filtration and higher probability of contamination under assumption that the source of drinking water is from local boreholes.

General water and sanitation systems and hygiene condition must be improved to avoid further outbreaks. Nonetheless, these improvements take time and investment and preparedness plans must be developed since outbreaks will continue to occur. Our analysis is useful to orient these plans, and we recommend focusing the preparedness activities in three regions: the Sector Autônomo de Bissau, Biombo and Bijagos, as these areas were the most affected in the 2008 epidemic, and in 2005. The early detection of the outbreak and the control plans are especially important in the capital, Bissau, where most cases and deaths occur. One of the

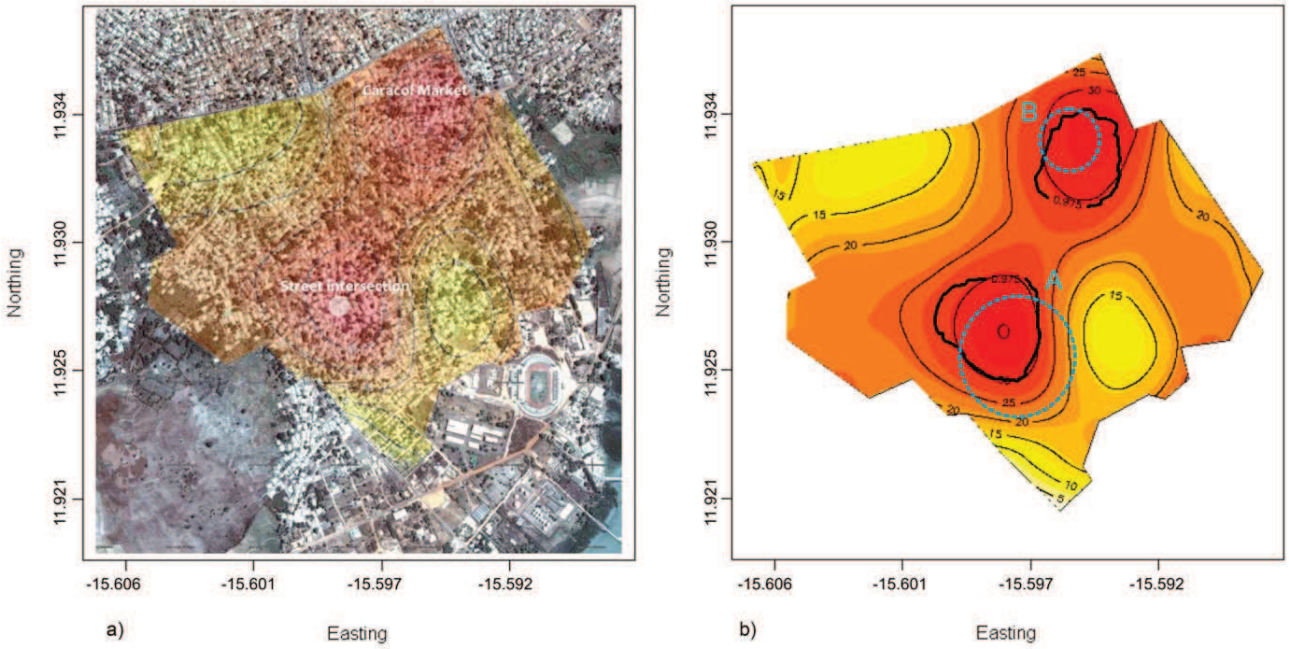

Figure 5. Geographical distribution of the probability of finding a house with at least one cholera case in Bairro Bandim (Bissau) in percentage and areas over the 95% confidence interval. Coordinates expressed in sexagesimal degrees. Figure 5a shows the Google Earth™ picture and the overimposed image of the risk surface (probability of finding a house with at least one cholera case). The figure 5b shows the risk surface and the two areas with statistically significant higher risk (black bold line). The same two clusters were detected (dashed blue circles) using the Kulldorff's spatial scan statistic (cluster A: Log likelihood ratio = 9.95, P = 0.029; cluster B: Log likelihood ratio = 8.81, P = 0.05).
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activities that should be planned in advance is the management of the Caracol market and, depending on resources available, the other markets. A cleaning routine should be established, a food safety assessment implemented and latrines and washing points set up. Education and awareness activities are key points to reinforce in order to reduce the impact of future epidemics. These activities are most important in some neighborhoods of the capital: Bandim, Antula, Quelele, Cuntum and Ajuda. Another point to consider among potential control activities are mass vaccination campaigns with the oral cholera vaccine. Feasibility and effectiveness of mass vaccination campaigns in specifically targeted settlements or populations have been demonstrated in endemic areas [27,28,29] and their use in targeted locations of Guinea-Bissau, like Bandim, should be considered seriously both as a preventive and a reactive strategy. There is an urgent need to identify new strategies, which are feasible, acceptable and cost-effective to prevent or quickly stop epidemics. Use of oral cholera vaccines might be one of the solutions and its role during outbreaks should be explored.

In conclusion, our study shows the importance of the consideration of space for making decisions about where to reinforce treatment and to guide control and prevention activities in cholera outbreaks. The results of this study also highlight the need for geographical descriptions of cholera epidemics in Africa. Available tools for spatial analysis should be integrated into existing surveillance systems in order to improve preparedness and control of cholera epidemics.

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Author Contributions

Conceived and designed the experiments: FJL CNB DR PPP EB RFG. Performed the experiments: FJL CNB DR. Analyzed the data: FJL CNB DR. Contributed reagents/materials/analysis tools: FJL CNB DR. Wrote the paper: FJL CNB DR PPP EB RFG.

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PLOS | NEGLECTED TROPICAL DISEASES

Urban Cholera Transmission Hotspots and Their Implications for Reactive Vaccination: Evidence from Bissau City, Guinea Bissau

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Abstract

Background: Use of cholera vaccines in response to epidemics (reactive vaccination) may provide an effective supplement to traditional control measures. In Haiti, reactive vaccination was considered but, until recently, rejected in part due to limited global supply of vaccine. Using Bissau City, Guinea-Bissau as a case study, we explore neighborhood-level transmission dynamics to understand if, with limited vaccine and likely delays, reactive vaccination can significantly change the course of a cholera epidemic.

Methods and Findings: We fit a spatially explicit meta-population model of cholera transmission within Bissau City to data from 7,551 suspected cholera cases from a 2008 epidemic. We estimated the effect reactive vaccination campaigns would have had on the epidemic under different levels of vaccine coverage and campaign start dates. We compared highly focused and diffuse strategies for distributing vaccine throughout the city. We found wide variation in the efficiency of cholera transmission both within and between areas of the city. "Hotspots", where transmission was most efficient, appear to drive the epidemic. In particular one area, Bandim, was a necessary driver of the 2008 epidemic in Bissau City. If vaccine supply were limited but could have been distributed within the first 80 days of the epidemic, targeting vaccination at Bandim would have averted the most cases both within this area and throughout the city. Regardless of the distribution strategy used, timely distribution of vaccine in response to an ongoing cholera epidemic can prevent cases and save lives.

Conclusions: Reactive vaccination can be a useful tool for controlling cholera epidemics, especially in urban areas like Bissau City. Particular neighborhoods may be responsible for driving a city's cholera epidemic; timely and targeted reactive vaccination at such neighborhoods may be the most effective way to prevent cholera cases both within that neighborhood and throughout the city.

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Introduction

With the introduction of inexpensive, easy to administer, and effective oral vaccines against cholera, vaccination in response to an epidemic (reactive vaccination) may be an effective supplement to conventional control measures. Two safe and internationally licensed oral cholera vaccines are currently available, Dukoral and Shanchol. Both protect against clinical cholera two or more years after vaccination, but neither confers long lasting immunity [1–4]. On an epidemic timescale, these vaccines have efficacies ranging from 66 to 86% [2,5].

Vaccination against cholera has been used preventatively [3,6–8], but before 2012, we know of only two instances, in The Federated States of Micronesia in 2000 and Vietnam in 2008, where vaccination commenced during an epidemic [4,9]. Vaccine efficacy estimates ranged from 76 to 80%, however, no analysis on how vaccination affected the course of the epidemic was reported for either case [4,9].

New data on vaccine performance and the changing epidemiology of cholera prompted the WHO's Strategic Advisory Group to recommend in 2010 that reactive vaccination be considered in specific areas [10]. In order to facilitate rapid procurement and deployment of an oral cholera vaccine, some have proposed the creation of a revolving global stockpile [11,12]. While discussions of the global stockpile proceed, countries that use reactive vaccination must contend with a limited supply that may arrive after a significant delay.

Author Summary

Cholera remains a major public health threat, causing 3–5 million cases and 100,000–120,000 deaths each year. In 2010, data on vaccine performance and the changing epidemiology of cholera prompted the WHO's Strategic Advisory Group to recommend that reactive vaccination be considered in specific areas. We built a spatially explicit stochastic model of cholera transmission and fit it to data from a 2008 epidemic in Bissau City, Guinea Bissau. Using this model we examined the potential effectiveness of reactive vaccination for controlling cholera transmission in Bissau City, comparing strategies for distributing limited vaccine. In simulations, early targeting of a single transmission "hotspot", Bandim, was the most effective strategy, and led to the greatest reduction in cases both within Bandim and in areas where no vaccine was distributed. This finding has implications for cholera control in urban settings in general: public health officials will often know which areas of a city were hotspots of cholera transmission in the past or where conditions promote efficient transmission. When there is limited vaccine, our work suggests that targeting reactive vaccination at these areas will lead to the greatest reduction in cases both in these areas and elsewhere in the city.

Spatial heterogeneities may influence how cholera vaccine can best be distributed in a reactive campaign. The effectiveness of a campaign and optimal allocation strategy will depend upon local cholera transmission dynamics, vaccine supply, and logistical delays [12,13]. Human movement, water and sewerage infrastructure, and natural waterways facilitate cholera transmission across a city. Within neighborhoods, there can be marked variation in the efficiency of transmission.

One country that may benefit from reactive vaccination is Guinea-Bissau, where outbreaks have occurred every three to four years since 1994. Sector Autónomo de Bissau (SAB), or Bissau City, the capital, consistently reports the most cholera cases within the country (unpublished data, Guinea-Bissau Ministry of Health). In 2008, 67% of reported cases occurred in SAB while only 25% of the national population live within its boundaries [14]. Reactive vaccination in SAB may be possible in future epidemics given the concentration of cases within the city and the Ministry of Health's experience with vaccination campaigns.

Here, we explore the possible effectiveness of different reactive vaccination strategies using SAB as a case study. We fit a neighborhood-based meta-population model to the 2008 cholera epidemic. Using this model, we characterize the spatio-temporal dynamics of cholera transmission within the city and estimate the impact that different reactive vaccination strategies could have had on the course of the epidemic.

Methods

Data Sources

During the 2008 epidemic, the Guinea-Bissau Ministry of Health, the WHO, and Mèdecins Sans Frontières implemented a clinic-based cholera surveillance system, which has been described previously [15]. In brief, upon arrival at either the cholera treatment center in the Hospital National Simao Mendes or one of five cholera treatment units (Figure 1C and 1D), health care providers entered patients into a surveillance registry. A patient's age, sex, area of residence, treatment facility, date of presentation, and clinical diagnosis were recorded.

Modified WHO cholera case definitions were used [15]. A suspected case was any person suffering from acute watery diarrhea, and a confirmed case was a suspected case with a positive stool sample containing *Vibrio cholerae* O1 or O139. We included all suspected and confirmed cases with complete information on their presentation date and home sanitary area in this analysis. The population for each sanitary area within the city was extrapolated from 1991 census data using a constant linear growth rate estimated by the Direcção-Geral Saúde. To estimate the population density in each sanitary area we traced the residential areas using Google Earth (v6.0.3.2197), then divided each sanitary area's population by its estimated residential area.

Model of Cholera Spread in SAB

We fit a discrete-time Susceptible-Infectious-Recovered meta-population model to the confirmed and suspected cases reported during the 2008 epidemic with each of 14 sanitary areas in SAB treated as a distinct population. We assume the epidemic follows a first-order Markov process with a fixed generation time of five days. At each time step, the incidence in each area follows a Poisson distribution with a mean determined by the number infected in the last time step in all areas and the proportion of the area's population remaining susceptible. After infection, individuals were assumed to remain immune for the duration of the epidemic (See Text S1 for model details).

We considered models of cholera transmission with and without seasonality assuming (A) equal transmission coefficients between and within all areas of SAB; (B) different transmission coefficients within each area and equal transmission coefficients between all areas; (C) different transmission coefficients within each area and unique symmetric transmission coefficients between each pair of areas; and, (D) different transmission coefficients within each area and unique asymmetric transmission coefficients between each pair of areas in the city. We chose the best model based on Deviance Information Criteria (Text S1). To assess fit we simulated 300,000 epidemics predicting five, fifteen, and fifty days ahead drawing new parameters from the posterior distribution every 1000 simulations.

Posterior distributions were approximated using Markov Chain Monte Carlo methods using JAGS 3.1.0 and R 2.14.0 with non-informative priors [16,17]. We ran 3 chains of 400,000 iterations with a burn in of 50,000, and assessed convergence using the potential scale reduction factor and through visual inspection [18].

Vaccination

We assume every vaccinated individual receives two doses in a vaccine campaign over a 20 day period and that 75% are fully protected ($VE_s = 0.75$ [19]) [3,5,6,20]. In our model vaccinees get no protection until 10 days after the second dose [21,22]. Hence, 75% of the susceptible vaccinees are considered immune starting 30 days after their first dose, with no protection before (Table 1).

We considered campaigns with 50,000, 75,000, or 100,000 doses (i.e. 25,000, 37,500, and 50,000 individuals vaccinated) and targeted vaccination at one, two, three, or all (14) areas (Table 3). When the proposed number of vaccinees in a specific area exceeded the population size, we distributed vaccine to the other vaccination areas or, in the campaigns with one vaccination area, we dispersed the vaccine throughout the city with each person having equal probability of getting vaccinated. We varied the starting time of the vaccination campaign between 20 and 120 days after the first case was detected.

We considered targeted and diffuse (city-wide) campaigns. In diffuse campaigns, vaccine was distributed throughout all areas of SAB. In targeted campaigns, we considered three different

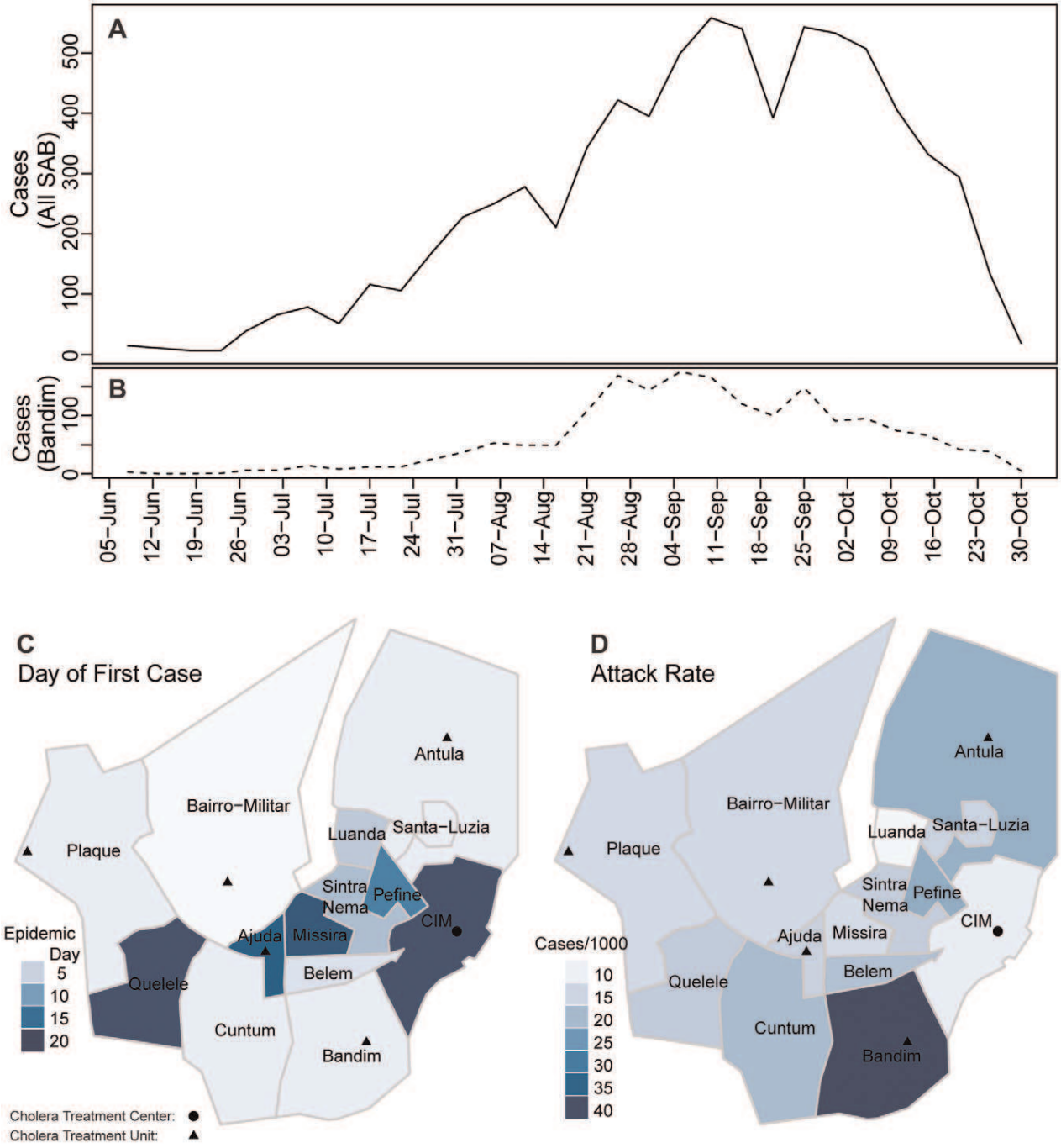

Figure 1. The 2008 cholera epidemic in SAB. Panel A (solid line) shows suspected and confirmed cholera cases reporting to cholera treatment centers/units (shown as circles and triangles) throughout all areas of SAB aggregated in 5-day intervals. The dashed line below (B) shows 5-day aggregated cases from Bandim, the area with the highest attack rate (40.6 per 1000). Panel C illustrates the day of the first reported case for each area. Attack rates (per 1000) for each area are shown in D.
doi:10.1371/journal.pntd.0001901.g001

strategies to select vaccination areas. In the population-based strategy, we selected the areas with the largest population. In the connectivity-based strategy, we vaccinated in areas estimated to be most "connected" to other areas. In the attack rate-based strategy, we chose the areas with the highest attack rate in the 2008 epidemic. We allocated vaccine proportional to population size in all simulations.

Simulation Studies

For each vaccination scenario we ran 5,000 simulations calculating the difference between the final epidemic size with and without vaccination. Epidemics were assumed to follow the observed 2008 epidemic course until 30 days after the first dose. In each simulation we drew new parameters from the joint posterior distribution. As a sensitivity analysis, we ran simulations with

Table 1. Overview of assumptions related to vaccination and immunity.

Vaccine efficacy	75%
Doses per individual	2
Immunity before second vaccine dose	None
Duration of vaccination campaign	20 days
Time from second vaccine dose to complete protection	10 days
Proportion immune after natural infection	100%
Length of immunity from natural infection or successful vaccination	Duration of the epidemic

Main assumptions used in primary analysis related to vaccination and immunity. Additional details are provided in the methods section and Text S1.
doi:10.1371/journal.pntd.0001901.t001

Table 2. Overview of sanitary areas in SAB.

Sanitary Area	Population	Suspected and Confirmed Cases	Attack Rate (per 1,000)
Barrio-Militar	65,274	944	14.5
Bandim	44,718	1,816	40.6
Cuntum	45,482	890	19.6
Missira	38,838	532	13.7
Antula	30,778	662	21.5
Quelele	28,898	493	17.1
Plaque	27,633	396	14.3
Luanda	25,236	229	9.1
Sintra Nema	21,451	355	16.5
Belem	17,263	322	18.7
Santa-Luzia	17,204	261	15.2
CIM	14,985	161	10.7
Pefine	14,808	324	21.9
Ajuda	10,429	164	15.7
All SAB	402,997	7,549	18.7

Estimated 2008 population for each sanitary area projected from 1991 census data (second column). Suspected and confirmed cases with complete location and time data and attack rate during 2008 cholera epidemic (third and fourth columns).
doi:10.1371/journal.pntd.0001901.t002

different generation times (3–10 days) and vaccine efficacies (65%–85%). Additional simulation study details are available in Text S1.

Ethics Statement

Original data collection was approved by the Mèdecins Sans Frontières ERB and the National Ethical Review Board of Guinea-Bissau [15]. The analyses presented in this article were conducted on de-identified data and deemed to be non-human subject research by the Johns Hopkins Bloomberg School of Public Health IRB.

Results

The 2008 Cholera Epidemic

The first case in SAB was reported on June 5, 2008 in Bairro-Militar, the most populated area of the city (Figures 1A, 1B), one month after the first reported case in Guinea-Bissau. Within three weeks, all 14 areas had reported cases (Figure 1C). The Ministry of Health officially declared an epidemic one month after the first case report from SAB. The National Laboratory of Microbiology and the Pasteur Laboratory in Dakar, Senegal identified all positive specimens analyzed as *Vibrio cholerae* O1 El Tor Ogawa.

Nationally, 14,226 suspected cases and 228 deaths were reported with 67% (9,393) of cases and 32% (73) of deaths reported in SAB. The last case in the country was reported in SAB on January 11, 2009. Individual-level data in SAB was collected between June 5, 2008 and October 28, 2008, over which time 8,024 (85%) suspected and confirmed cases were reported. These analyses focus on 7,551 suspected and confirmed cases with complete information on date of presentation, home area, and clinical diagnosis (Figure S1).

In SAB, weekly incidence ranged from 14 to 755. Within-area attack rates ranged from 9.1 to 40.6 per 1,000 (Table 2, Figure 1D), with Bandim having both the most cases (1,816) and the highest attack rate.

Spatial Spread of Cholera in SAB

The final model fit both the overall and area-specific epidemic curves well, even when predicting as far as 50 days (i.e. 10 time steps) ahead (Figures 2A,2B). To understand how transmission varied through time, we calculated the odds that an incident case was caused locally (i.e. attributable to transmission between people in the same area) for each area throughout the course of the epidemic (Figure 3). Only Bandim, Plaque, and Santa-Luzia have an odds consistently greater than 1, suggesting internally driven epidemics in these areas.

Table 3. Vaccination scenarios.

	Vaccination Strategy				
Areas Vaccinated	**Population**	**Connectivity**	**Attack Rate**	**Vaccination Start Day**	**Doses**
1 Area	Bairro Militar (1.00)	Missira (1.00)	Bandim (1.00)	20–120	50,000–100,000
2 Areas	Bairro Militar (0.59)	Missira (0.69)	Bandim (0.75)	20–120	50,000–100,000
	Cuntum (0.41)	Santa-Luzia (0.31)	Pefine (0.25)		
3 Areas	Bairro Militar (0.42)	Missira (0.46)	Bandim (0.50)	20–120	50,000–100,000
	Cuntum (0.29)	Santa-Luzia (0.21)	Pefine (0.16)		
	Bandim (0.29)	Plaque (0.33)	Antula (0.34)		

For each scenario we chose the top 1, 2, and 3 areas that met the vaccination strategy criteria. The number of vaccinees in each area were weighted (shown in parenthesis) to ensure that vaccines were allocated proportional to population size in all simulations.
doi:10.1371/journal.pntd.0001901.t003

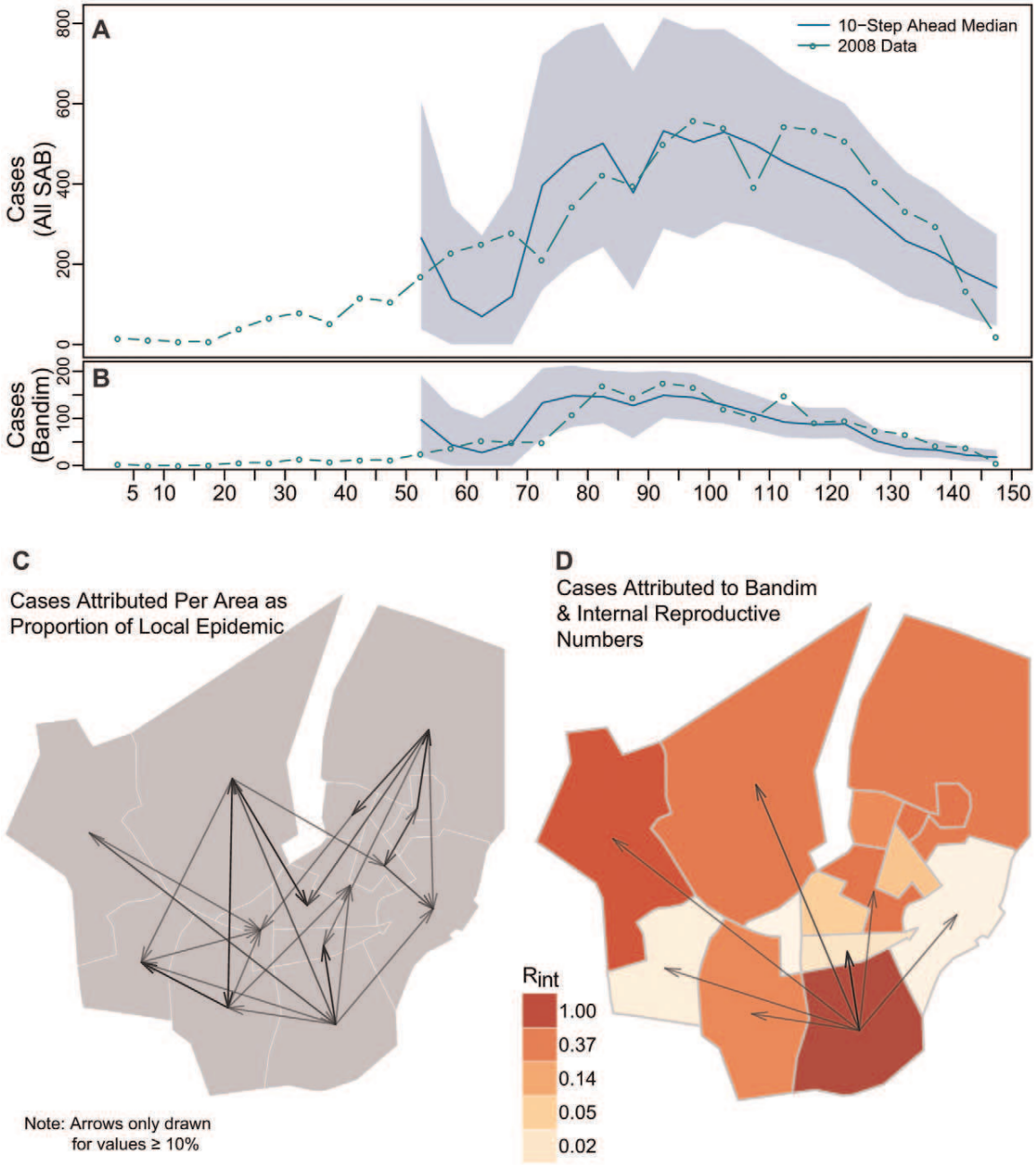

Figure 2. Cholera transmission model overview. 10-step ahead (50 day) predictions for all of SAB (A) and Bandim (B) with 95% predictive interval bands. The arrows in Panel C illustrate the proportion of cases estimated to be caused in each area (head of arrow) by another (tail end of arrow). Panel D illustrates the mean effective internal reproductive number (R_{int}) for each area (colors), and the proportion of each areas epidemic estimated to be caused by Bandim (arrows). Arrow size and transparency are scaled by the magnitude with a minimum of 10% shown.
doi:10.1371/journal.pntd.0001901.g002

We define the effective internal basic reproductive number (R_{int}) as the expected number of cases caused within a given area by one infected individual, within the same area, at the beginning of the epidemic. Only areas with $R_{int} > 1$ can sustain an epidemic absent infections introduced from other areas. The strength of internal epidemics varied with estimates of R_{int} ranging from 0.01 (95% Credible Interval (CI) 0.00–0.07) in Ajuda to 1.17 (95% CI 0.99–1.33) in Bandim (Figure 4). We found no significant correlation between R_{int} and either estimated population size or population density.

Bandim is the only area where we estimate $R_{int} > 1$, and it appears to have played a necessary role in driving the epidemic. With Bandim removed, simulated introductions of cases fail to cause epidemics. In contrast, city-wide epidemics occur with removal of any other single area.

In simulated epidemics based upon our best-fit model, we find that, on average, at least 10% of cases in each area are caused by cases in other areas (Figure 2C, Text S1). External transmission coefficients represent epidemic connectivity between areas, and

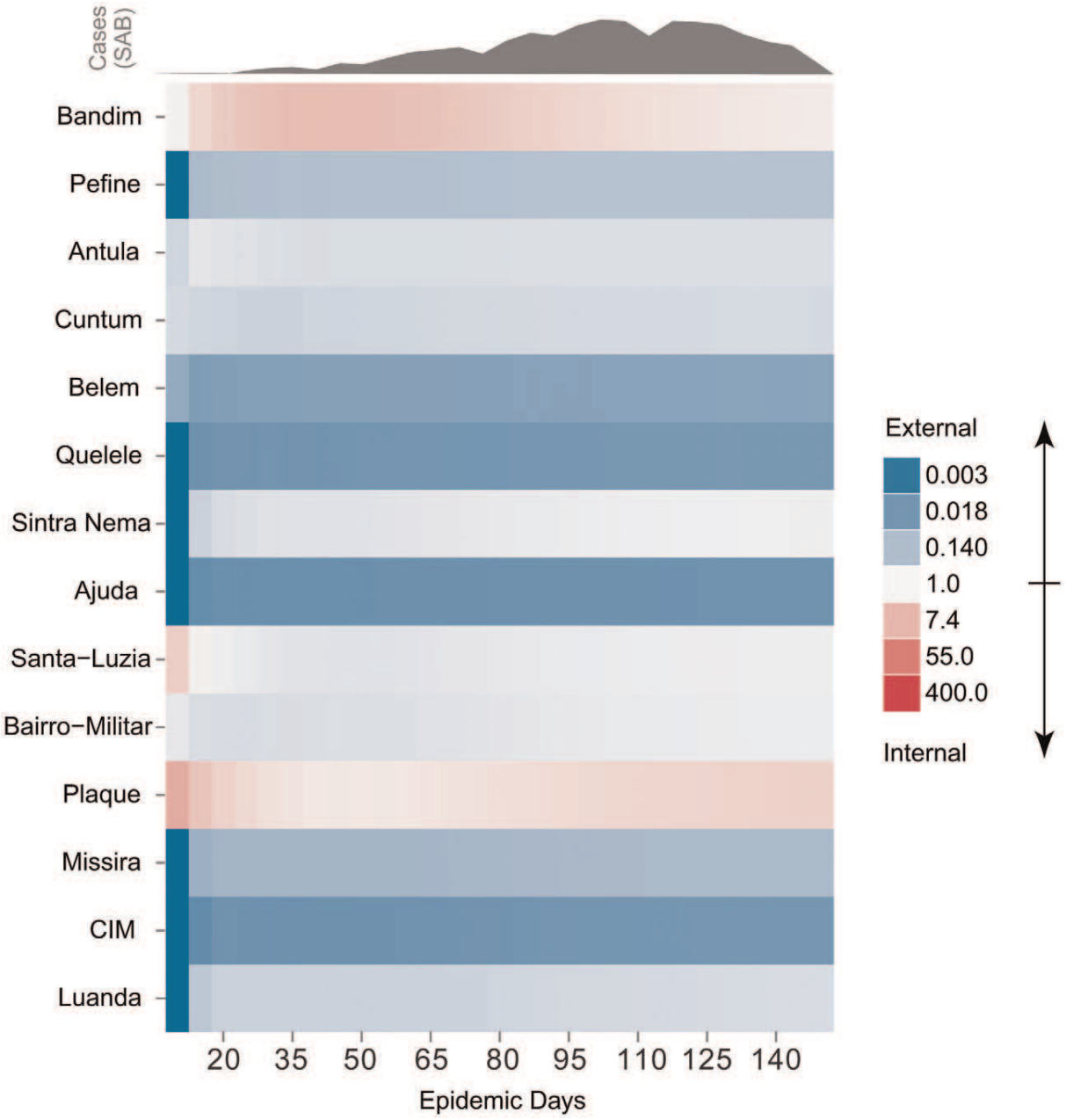

Figure 3. Odds of internally caused case over time by area. Odds of a case being caused internally (i.e. as a result of other cases in that area) vs. externally for all areas throughout the epidemic, sorted by attack rate (top to bottom). Red represents those values in support of an internally driven epidemic and blue represents those supporting an externally driven epidemic. The observed epidemic curve is shown above in grey for reference.
doi:10.1371/journal.pntd.0001901.g003

our estimates suggest heterogeneity in inter-area transmission (Text S1). Based on simulations, we estimate that Bandim contributed over 10% of the cases to over half (7/13) of the other areas (Figure 2D), highlighting the crucial role it played in the epidemic.

The sum of the external transmission coefficients for any area provides an estimate of the effective external basic reproductive number (R_{ext}). This number is the estimated number of cases a single infectious case in that area would cause in all other areas of SAB given the pre-epidemic level of population immunity. Estimates of R_{ext} ranged from 0.37 (95% CI 0.16–0.71) in Belem to 7.32 (95% CI 6.29–8.37) in Missira (Figure 4).

Reactive Vaccination Simulations

Vaccination in the area(s) with the highest attack rate leads to larger reduction in cases than all other targeted and city-wide campaigns at all starting times. Targeting vaccination at Bandim only, the area with the highest attack rate, within the first 80 days of the epidemic averts more cases than other strategies regardless of vaccine quantity (Figure 5). Targeted vaccination in Bandim

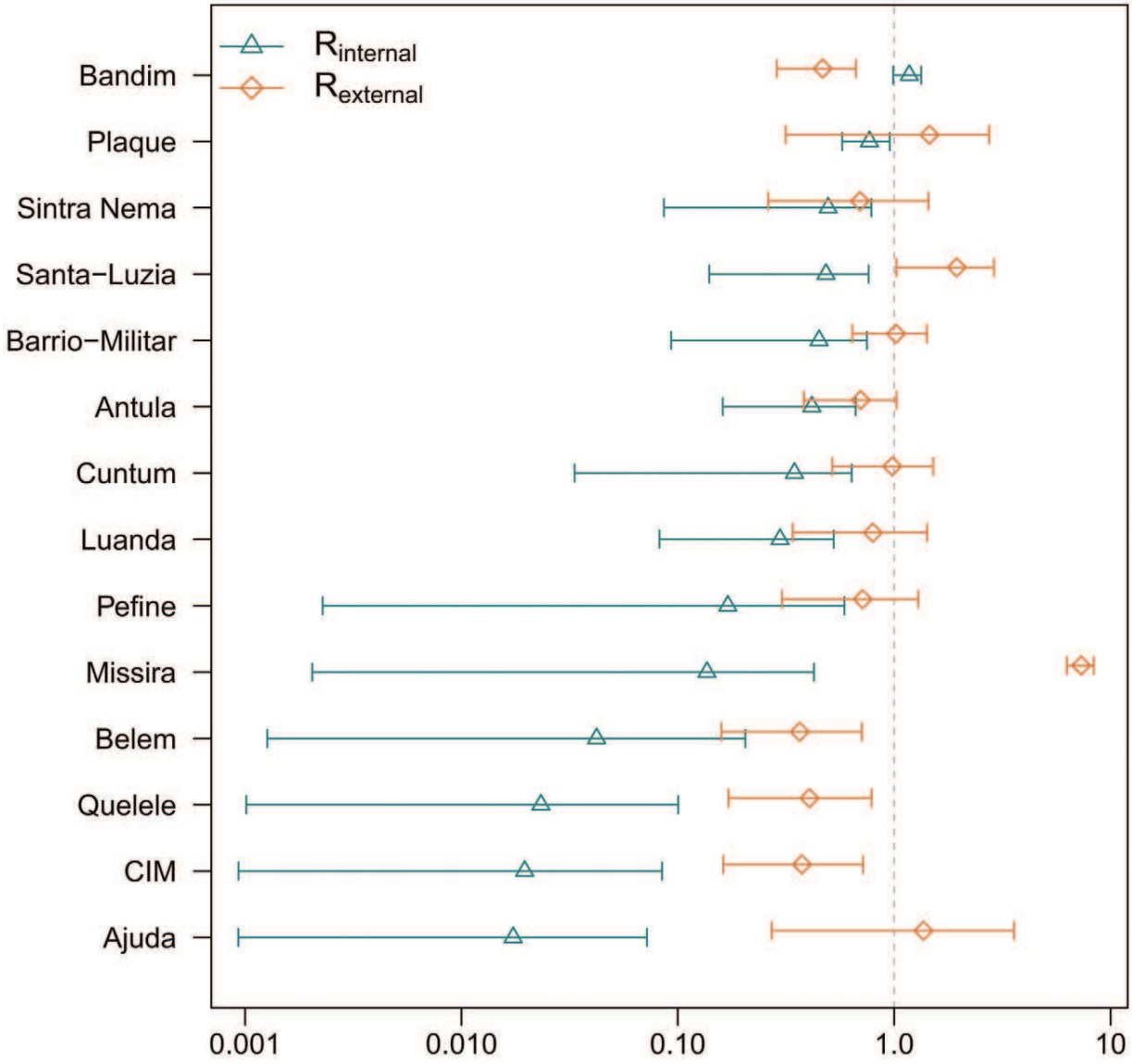

Figure 4. Mean R_{int}, R_{ext} and 95% credible intervals. Sorted from top to bottom by R_{int}.
doi:10.1371/journal.pntd.0001901.g004

starting on day 20 is expected to reduce the final size of the epidemic by 41% (95% Predictive Interval (PI) 0.21–0.69), 56% (95% PI 0.30–0.85), and 67% (95% PI 0.40–0.89) with 25,000, 37,500, and 50,000 vaccinees, respectively. In comparison, a city-wide campaign starting on the same day is expected to reduce the epidemic size by 21% (95% PI 0.07–0.34), 30% (95% PI 0.17–0.44), and 40% (95% PI 0.27–0.55) for 25,000, 37,500, and 50,000 vaccinees (Tables 4,S1,S2).

We found wide variability in the outcomes using different targeting strategies, with the differences diminishing as vaccination is delayed (Figure 5). Under the population-based strategy, only a targeted campaign in the three most populated areas averts more cases than a city-wide campaign (Figure 5, Table 4). Targeting the areas estimated to be most "connected" to others averts fewer cases than city-wide campaigns regardless of vaccination starting time and doses.

Starting day has a profound impact on the effect of all vaccination campaigns: the sooner vaccination begins, the more cases are averted. With 37,500 vaccinees, each day delay in vaccination results in an average of 39.5 (95% CI 37.7–44.2) fewer cases averted when targeting based on attack rate. Increasing the size of a vaccination campaign early on in the epidemic can significantly improve case prevention, however, the marginal benefit of additional vaccine diminishes as vaccination is delayed. On average, each additional person vaccinated as part of a targeted campaign in Bandim starting on day 20 averts 7.5 cases compared to 1.7 cases averted per vaccinee in campaigns starting two months later.

In simulations, early targeted vaccination leads to fewer cases both within the targeted area *and throughout the city* when compared to diffuse campaigns. When starting vaccination on day 20 (Figure 6A), targeting Bandim averts more cases both in Bandim (1,173) and in all the other areas combined (2,265) when compared to a city-wide campaign (341 averted in Bandim and 1,741 in all other areas). As the vaccination campaign is delayed, these differences shrink (Figure 6).

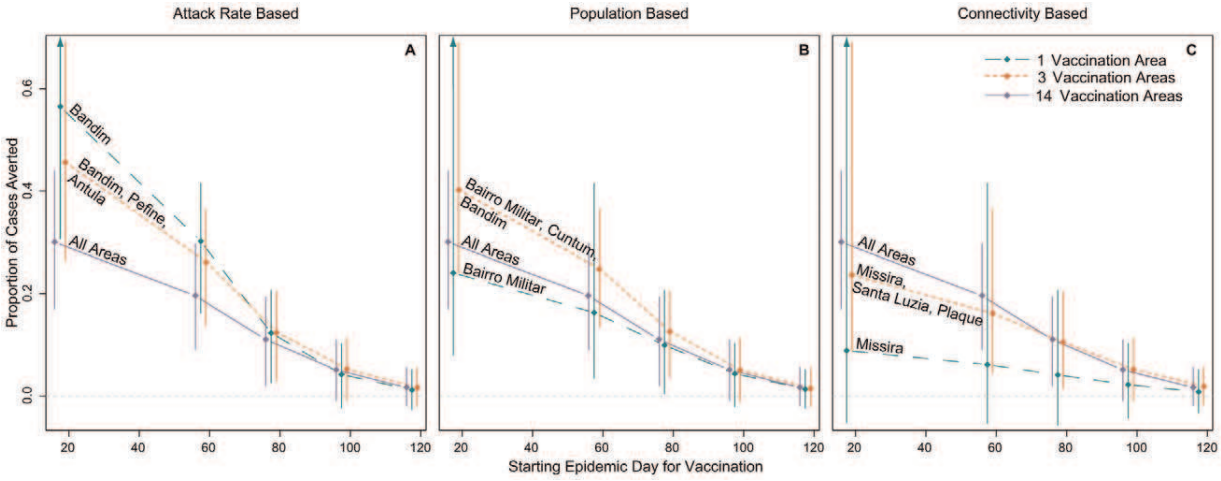

Figure 5. Vaccination results by strategy and start time. Each plot shows the median (diamonds) and 95% predictive interval for the proportion of cases averted by vaccination start time for (A) attack rate-based, (B) population-based, and (C) connectivity-based targeting strategies. The colored lines represent the different number of areas vaccinated. Estimates made from simulations starting at the time of vaccination with 37,500 individuals vaccinated (75,000 doses). Purple lines (14 vaccination areas) are the same in each panel.
doi:10.1371/journal.pntd.0001901.g005

Table 4. Vaccination scenario results summary.

		Vaccination Campaign Start Time							
Distribution Strategy	**# Areas Vacc.**	**Day 20**		**Day 60**		**Day 80**		**Day 100**	
		Cases	**%**	**Cases**	**%**	**Cases**	**%**	**Cases**	**%**
Attack Rate	1 area	4228	0.56	2342	0.30	970	0.12	345	0.04
		2263,6424	0.30,0.85	1195,3392	0.16,0.41	197,1732	0.03,0.21	−186,887	−0.02,0.10
	2 areas	3954	0.53	2266	0.29	986	0.13	379	0.05
		2142,6214	0.29,0.82	1156,3258	0.16,0.40	238,1732	0.03,0.21	−146,928	−0.02,0.11
	3 areas	3422	0.46	2025	0.26	975	0.12	433	0.05
		1903,5174	0.27,0.69	1021,2993	0.14,0.36	222,1708	0.03,0.20	−71,964	−0.01,0.11
Population	1 area	1804	0.24	1272	0.16	777	0.10	359	0.04
		558,3250	0.08,0.41	254,2276	0.03,0.28	27,1565	0,0.19	−166,897	−0.02,0.10
	2 areas	1974	0.26	1405	0.18	859	0.11	396	0.05
		824,3355	0.12,0.42	432,2361	0.06,0.29	102,1633	0.01,0.19	−120,936	−0.02,0.11
	3 areas	3019	0.40	1928	0.25	996	0.13	414	0.05
		1727,4534	0.24,0.59	976,2902	0.13,0.35	269,1739	0.04,0.21	−92,941	−0.01,0.11
Connectivity	1 area	666	0.09	476	0.06	322	0.04	181	0.02
		−363,1742	−0.05,0.22	−404,1372	−0.05,0.17	−436,1102	−0.06,0.13	−349,716	−0.04,0.08
	2 areas	1258	0.17	827	0.11	566	0.07	326	0.04
		154,2375	0.02,0.3	−62,1741	−0.01,0.21	−129,1322	−0.02,0.16	−198,863	−0.03,0.10
	3 areas	1792	0.24	1255	0.16	828	0.10	427	0.05
		603,3032	0.09,0.39	339,2243	0.05,0.27	104,1574	0.01,0.19	−74,967	−0.01,0.11
Diffuse/City-Wide	14 areas	2271	0.30	1521	0.20	872	0.11	421	0.05
		1170,3450	0.17,0.44	658,2464	0.09,0.30	150,1623	0.02,0.19	−71,947	−0.01,0.11

Median count and percent of cases averted by targeting strategy (indicated by left-most column) and vaccination start day (epidemic day) for 75,000 doses (37,500 vaccinees). Values were estimated from simulations starting from the first time period where any vaccinee gained protective immunity. 95% predictive intervals (PIs) are shown below each median value. Differences were calculated from time that the first vaccinated individuals are protected.
doi:10.1371/journal.pntd.0001901.t004

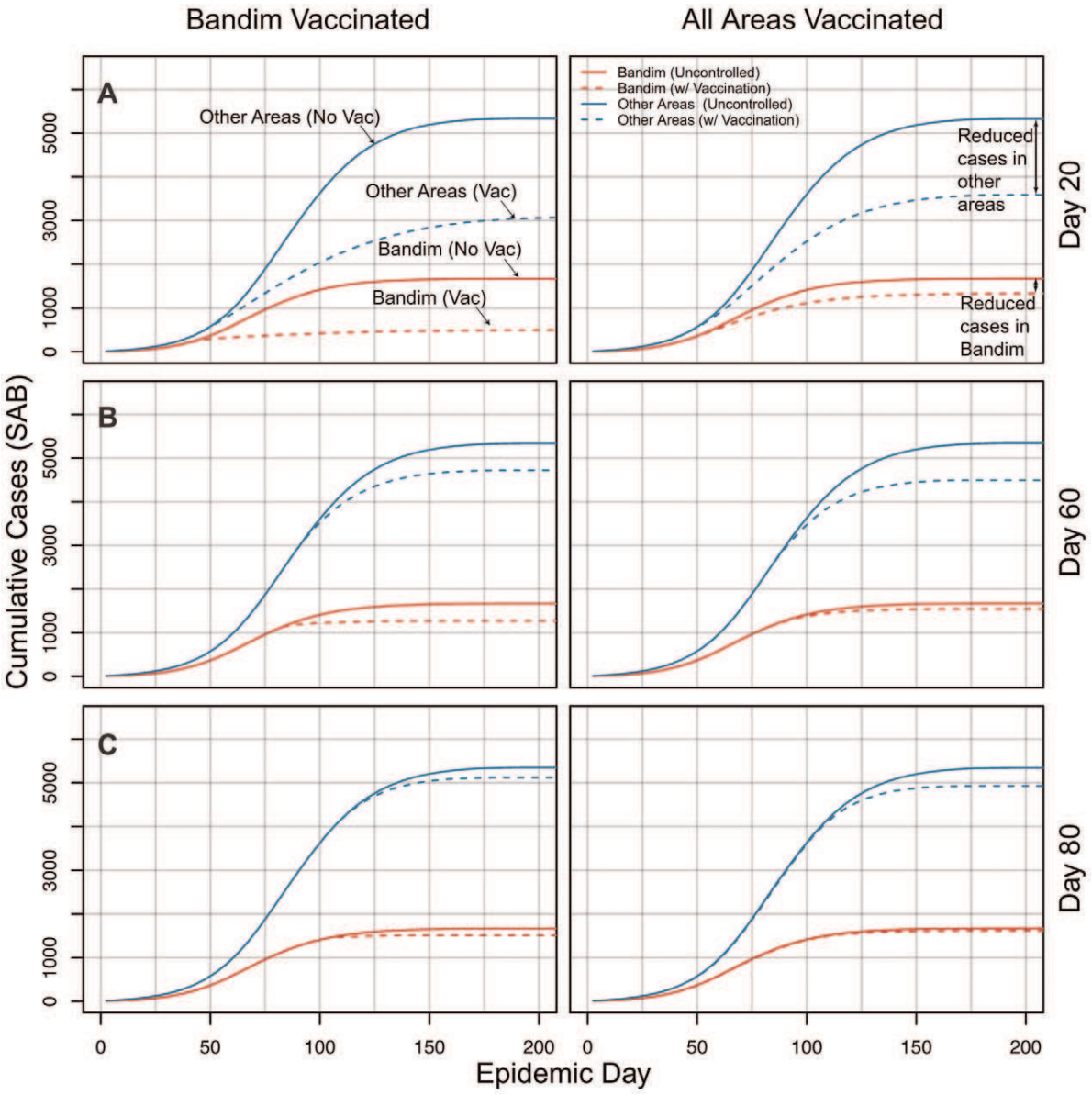

Figure 6. Comparison of cumulative cases within (red) and outside (blue) Bandim under targeted and diffuse vaccination. Dashed lines represent the median number of cases in simulations with vaccination, and the solid lines represent the median number of cases in uncontrolled epidemic simulations (no vaccination). Each row (panels A–C) represents simulations with vaccination started at the epidemic day denoted on the right hand side (e.g. Day 20). Simulations were started from the reported number of cases in the first 5 days of the epidemic.
doi:10.1371/journal.pntd.0001901.g006

Discussion

Using a simple spatially explicit model of cholera transmission, we captured the essential dynamics of the 2008 cholera epidemic in SAB, Guinea-Bissau. This model suggests that there was significant transmission between areas in SAB and that one area, Bandim, drove the epidemic. Our simulations show that early distribution of vaccine is the most important determinant of the number of cases prevented. For example, vaccinating 25,000 individuals in Bandim on epidemic day 20 would have averted more cases (3,109, 95% PI 1,475–5,198) than vaccinating 50,000 in the same area just 40 days later (2,732, 95% PI 1,630–3,738). Our simulations suggest that an early vaccination campaign targeted at Bandim alone would have outperformed distributing the same vaccine quantity throughout the city. Not only are more cases prevented overall, but more are prevented in areas outside of Bandim.

Our results suggest that rapid small-scale vaccination may be more effective than a delayed larger-scale vaccination campaign. For example, on average, each day delay results in an additional 39.5 cases when targeting 37,500 people in the areas with the highest attack rate. Applying the average case fatality ratio from the 2008 epidemic (1.58 per 100 cases [15]) we estimate that each week delay in vaccination would have resulted in an average of 4.4 cholera-related deaths.

Transmission hotspots for other infectious diseases have been exploited to devise novel prevention and control approaches [23,24]. For example, targeted interventions in hotspots may be

key to effective malaria control and elimination [24]. Similarly, cholera hotspots can serve as targets for both reactive and preventative interventions. Identification of hotspots during an epidemic may be challenging. In the case of SAB, Bandim is an area which has had high attack rates in previous epidemics and few improvements in water and sanitation infrastructure. Such historical information may be useful in targeting vaccination; however, more research on combining historical and real-time surveillance data is needed.

In our model, vaccination campaigns lasted 20 days, but in reality the duration will vary by the number of vaccinees targeted and the vaccine used. If Shanchol were used with the recommended inter-dose period of 14 days, the campaign would likely exceed 20 days. While this suggests that our results underestimate the speed by which Shanchol vaccination would occur, these differences would be offset by partial immunity conferred before a second dose [22].

As the time to distribute vaccine doses increases, we expect to avert fewer cases. However, there is some evidence that a single dose of oral cholera vaccine may be sufficient for reactive vaccination [22,25]. If one dose is sufficient to elicit a strong protective response for the time-scale of an epidemic, more people could be vaccinated quickly.

Cholera's generation time is not well characterized and varies widely with the concentration of bacteria in the environment, its survival rate, and the route of transmission [26–28]. We ran analyses with alternate generation times of 3, 7, and 10 days and got the same qualitative results (Figures S3, S4, S5, S6, S7, S8 and Tables S3, S4, S5). We also found that varying the vaccine efficacy to 65% and 85% changed the number of cases averted, but preserved the relative performance of each strategy over time (Figure S2 and Tables S7,S6).

There are a number of limitations to this work. We focus on a single epidemic in Guinea-Bissau. A longer time series would provide insight into variability in transmission across epidemics. The data came from an intensified surveillance effort from both Mèdecins Sans Frontières and the Guinea-Bissau Ministry of Health, however suspected cases that presented after October 28, 2008 were only captured by the national surveillance system without details on timing and home sanitary area.

There are several possible alternative explanations for the elevated attack rate in Bandim. The cholera case definition used is not 100% specific, and some cholera cases may be false positives. People may be more likely to seek care if their neighbors do, hence clinic visits may cluster even if cholera does not. In addition, Bandim has been the location to several surveillance programs and public health interventions through the Bandim Health Project [29], perhaps leading to increased awareness. However, if these phenomena were consistent throughout the epidemic they would not lead to elevated estimates of the local transmission rate under our algorithm.

We found that how rapidly vaccine can be distributed during a cholera epidemic is the most important determinant of the effectiveness of a reactive vaccination program; and that a single area of SAB was an essential driver of the epidemic. Hence, early targeting of this area would have been the most effective way to reactively distribute vaccine. These results may apply to urban cholera epidemics more generally. It seems reasonable that cholera epidemics in other urban settings, particularly in Africa, may be disproportionally driven by specific parts of the city. If these hotspots can be identified, targeted reactive vaccination may be an effective way to prevent cases both within that area and throughout the city, especially when vaccine supply is limited. Regardless of the distribution strategy used, timely distribution of vaccine in response to an ongoing cholera epidemic can prevent cases and save lives.

Supporting Information

Figure S1 5-day aggregated case counts for all sanitary areas during the 2008 epidemic. Data collected from cholera treatment center and cholera treatment units throughout the city from June 5, 2008 to October 28, 2008.
(TIF)

Figure S2 Vaccine efficacy sensitivity analysis. Comparison of proportion of epidemic averted with different 65%, 75% (as in main analysis), and 85% vaccine efficacy over different vaccination starting times. All scenarios shown use attack rate based targeting.
(TIF)

Figure S3 Comparison of transmission parameters with different generation times. Posterior means and standard deviation for transmission coefficients, ($log(\beta)$'s on diagonals and $log(\alpha)$'s on off-diagonals) with 3, 5, and 7 day generation times.
(TIF)

Figure S4 Comparison of internal and external effective reproductive numbers for different generation time aggregations.
(TIF)

Figure S5 Proportion of cases caused in each area by others from 3, 5, 7, and 10-day generation time models. The sum of each row is equal to one, representing 100% of the area's epidemic.
(TIF)

Figure S6 Vaccination simulation results with 3-day generation time, 75% vaccine efficacy, and 75,000 doses.
(TIF)

Figure S7 Vaccination simulation results with 7-day generation time, 75% vaccine efficacy, and 75,000 doses.
(TIF)

Figure S8 Vaccination simulation results with 10-day generation time, 75% vaccine efficacy, and 75,000 doses.
(TIF)

Table S1 Vaccination simulation results with 50,000 doses and 75% vaccine efficacy. Proportion and number of cases averted in 5,000 simulations under different vaccination strategies (Median and 95% Predictive Interval).
(DOCX)

Table S2 Vaccination simulation results with 100,000 doses and 75% vaccine efficacy. Proportion and number of cases averted in 5,000 simulations under different vaccination strategies (Median and 95% Predictive Interval).
(DOCX)

Table S3 Vaccination simulation results from 3-day generation time model, 75,000 doses. Proportion and number of cases averted in 5,000 simulations under different vaccination strategies (Median and 95% Predictive Interval).
(DOCX)

Table S4 Vaccination simulation results from 7-day generation time model, 75,000 doses. Proportion and number of cases averted in 5,000 simulations under different vaccination strategies (Median and 95% Predictive Interval).
(DOCX)

Table S5 Vaccination simulation results from 10-day generation time model. Proportion and number of cases

averted in 5,000 simulations under different vaccination strategies (Median and 95% Predictive Interval).
(DOCX)

Table S6 Vaccination simulation results with 75,000 doses and 65% vaccine efficacy. Proportion and number of cases averted in 5,000 simulations under different vaccination strategies (Median and 95% Predictive Interval).
(DOCX)

Table S7 Vaccination simulation results with 75,000 doses and 85% vaccine efficacy. Proportion and number of cases averted in 5,000 simulations under different vaccination strategies (Median and 95% Predictive Interval).
(DOCX)

Text S1 Details on final model, model selection, and simulations.
(PDF)

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Author Contributions

Conceived and designed the experiments: AA FJL RFG BTG JL. Performed the experiments: FJL AR PPP RFG CNB. Analyzed the data: AA FJL BTG JL. Wrote the paper: AA FJL AR PPP RFG CNB BTG JL.

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Risk factors for cholera transmission in Haiti during inter-peak periods: insights to improve current control strategies from two case-control studies

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SUMMARY

Two community-based density case-control studies were performed to assess risk factors for cholera transmission during inter-peak periods of the ongoing epidemic in two Haitian urban settings, Gonaives and Carrefour. The strongest associations were: close contact with cholera patients (sharing latrines, visiting cholera patients, helping someone with diarrhoea), eating food from street vendors and washing dishes with untreated water. Protective factors were: drinking chlorinated water, receiving prevention messages via television, church or training sessions, and high household socioeconomic level. These findings suggest that, in addition to contaminated water, factors related to direct and indirect inter-human contact play an important role in cholera transmission during inter-peak periods. In order to reduce cholera transmission in Haiti intensive preventive measures such as hygiene promotion and awareness campaigns should be implemented during inter-peak lulls, when prevention activities are typically scaled back.

Key words: Cholera, risk factors, endemic, epidemic, Haiti, prevention, transmission, *Vibrio cholerae*.

INTRODUCTION

Since October 2010 Haiti has been experiencing a cholera epidemic for the first time in over 100 years [1]. As of March 2013, the epidemic has resulted in more than 650 000 cases and 7441 deaths [2]. Immunological naivety of the population to the cholera agent and the contamination of river waters explain most of the high attack rate [3]. Several epidemic peaks have occurred, all during the rainy seasons. The first peak (October–December 2010) was explosive, with very rapid transmission throughout the country; the second peak (May–July 2011) was lower than the first in some places, and higher in others. Since then, peaks have occurred twice a year corresponding to the rainy season. Between peaks a low but persistent number of cholera cases are reported.

Waterborne transmission was clearly identified as the main transmission route during the peak periods [4–6]; however, other factors may increase in

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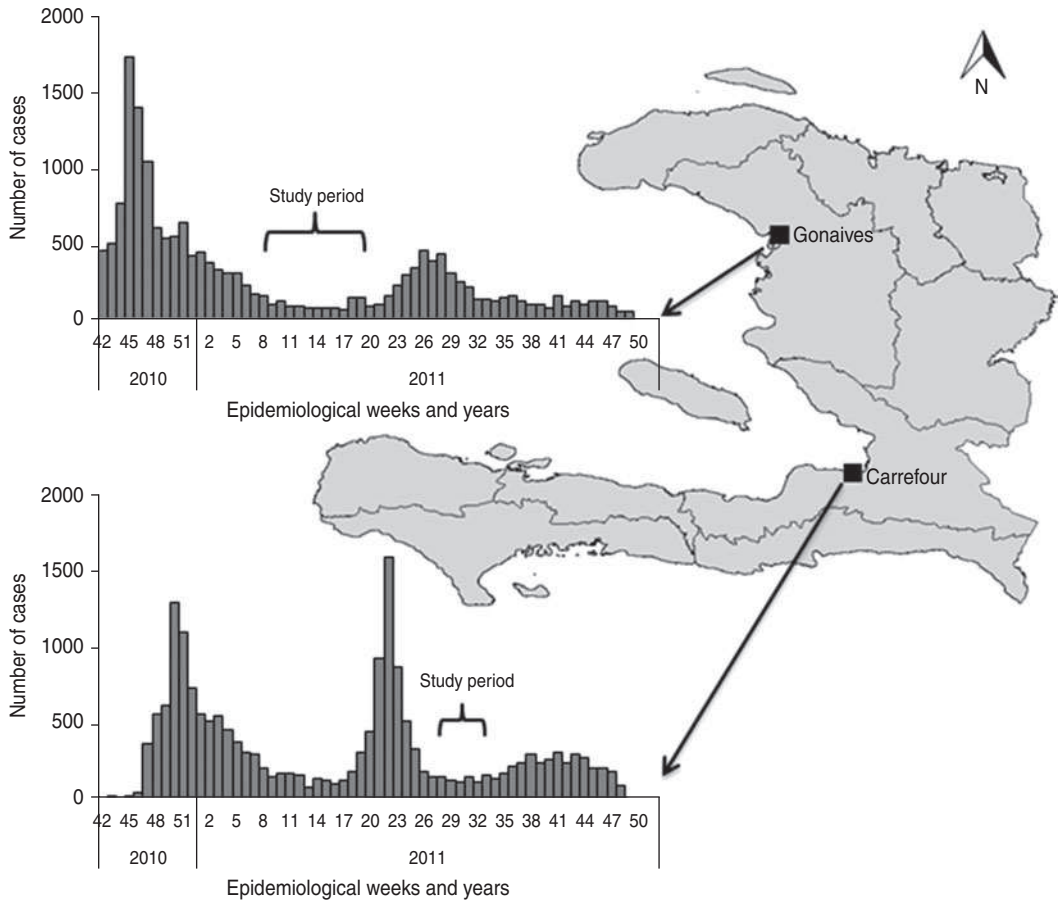

Fig. 1. Location of the towns of Gonaives and Carrefour and periods of participants' interviews in relation to the epidemic curves of the communes, where the towns are located, Haiti, 2011.

importance during the inter-peak periods. Here we present findings from two studies which investigated the risk factors associated with clinical cholera cases that occur during the lull in transmission, factors that therefore may contribute to the maintenance of cholera transmission in urban settings.

METHODS

Study design and settings

The two studies were community-based density case-control surveys, with cases and controls matched by age and gender. The first study was conducted in Gonaives, a city of 230 000 inhabitants [7] and capital of the Artibonite department, from 23 March 2011 to 30 May 2011. The second study was conducted in Carrefour (a suburb of the capital Port-au-Prince; 430 000 inhabitants) [7] from 22 July 2011 to 22 August 2011 (Fig. 1). At the time of the study, Carrefour still sheltered ~40 000 displaced people in camps as a consequence of the January 2010 earthquake [8]. Data were collected from individuals as well as from household observations.

Gonaives was chosen because it was among the first and most affected towns; Carrefour was chosen to explore additional risk factors related to the post-earthquake conditions of the survivors and because of the high incidence reported in previous epidemic waves.

Case and control definitions

A case was defined as a person (1) living in Gonaives or Carrefour since the beginning of the cholera outbreak in October 2010; (2) aged >5 years; (3) presenting with symptoms of acute watery diarrhoea; and (4) with a cholera diagnosis confirmed by a rapid test (Crystal VC® Rapid Dipstick test, Span Diagnostics, India) for *Vibrio cholerae* O1 or O139. In Gonaives, cases were included from the cholera treatment centre (CTC) managed by Médecins Sans

Frontières (MSF). In Carrefour, cases were included from two CTCs: one managed by MSF and another by Save the Children. Participation in the study was proposed to all eligible patients upon admission to the CTC. Written consent was sought after patients tested positive by Crystal VC test and before inclusion in the study.

Two controls of the same sex and age group were selected for each case. Age groups were 5–9, 10–14, 15–19, 20–29, 30–39, 40–49 and $\geqslant 50$ years. A control was defined as a person (1) living in Gonaives or Carrefour since the beginning of the epidemic in October 2010; (2) who had not experienced acute watery diarrhoea since that time; and (3) reported that they would have sought treatment at the CTC if they had developed acute watery diarrhoea.

Controls were selected using spatial random sampling [9]. Two polygons were first drawn to define the urban areas of Gonaives and Carrefour. Points were then drawn randomly within the polygons and superposed onto Google Earth® maps. Points coinciding with a house were retained; two points were randomly attributed to each case as locations to find controls. Investigators located the corresponding houses using GPS devices and verified the presence of a household member eligible for participation as a control. If none was eligible, investigators continued to the nearest house, and so on, until they found a willing control.

Sample size was determined based on the hypothesis that the presence of free chlorine in drinking water stored at home would result in a 2·5-fold decrease in the risk of transmitting cholera. This hypothesis was tested with an alpha risk of 5%, a statistical power of 80% and an estimated loss of 10%, resulting in a sample of 90 cases and 180 controls for each study.

Data collection and management

Trained investigators conducted face-to-face interviews with all cases and controls aged $\geqslant 16$ years; for participants aged <16 years, interviews were conducted with the child's guardian. A locally tailored questionnaire was written in French and translated into Creole, and then back-translated for verification.

Patients who agreed to participate were interviewed either on the day of admission to the CTC or the following day, depending on the severity of their clinical condition. On the day of a case's interview, investigators visited his/her household to assess the hygiene conditions of the latrine (presence of hand washing soap at latrine; overall latrine condition) and to conduct chemical and biological tests of the household's drinking water. The interview and the household assessment of controls were carried out on the same day as, or the day following, the matched case interview.

During the interview, data was collected on the following variables of potential relevance to cholera transmission: origin and quality of food and water, hygiene and sanitation habits, contact with cholera-infected patients, knowledge of transmission and prevention measures, and socioeconomic status.

Evaluating quality of drinking water

The level of free chlorine in households' drinking water was measured with a HANNA HI 701 Checker® HC spectrophotometer (HANNA Instruments®, UK). Properly chlorinated water was defined as being above a threshold of 0·2 mg/l free chlorine [10]. The presence of *Escherichia coli* was assessed using chromogenic medium Aqua-CHROM™ (CHROMagar™, France). After adding a fixed dose of chromogenic medium to a 100 ml water sample, the sample was incubated at room temperature for 24 h. The sample appearance was interpreted as follows: green or blue-green = presence of *E. coli*; yellow = presence of non-*E. coli* coliforms; colourless = absence of *E. coli* and non-*E. coli* coliforms.

Statistical methods

Data were entered using EpiData v. 3.1 (EpiData, Denmark) and analysed using Stata v. 11 (StataCorp, USA).

Matched odds ratios (ORs) were calculated using conditional logistic regression as a measure of cholera risk. Matched ORs, 95% confidence intervals (95% CIs) and P values were estimated with the case/control status as outcome variable and with exposure variables as explanatory variables, and interpreted with a bilateral test. Statistical significance was defined as $P < 0{\cdot}05$.

A score for socioeconomic status was constructed by determining whether or not the family owned specific items (radio, television, refrigerator, oven, washing machine, water storage recipient, car, animals), and by education level of the interviewee and the head of the family (main provider of household

income). Details on how this score was determined are presented in the supplementary online Appendix.

Multivariate conditional logistic regression analysis was performed as described by Hosmer & Lemeshow [11]. Models incorporated those variables that showed a significance level of $P<0{\cdot}2$ in univariate analysis, as well as those generally considered to have public health relevance for cholera (level of free chlorine and presence of *E. coli* in home-stored drinking water). The likelihood-ratio test was used to evaluate the contribution of each variable to the model; relevant first-degree interactions were also analysed.

Ethics

The two studies were implemented in collaboration with the Haitian Ministry of Public Health and Population and they adhered to the principles governing biomedical research involving human subjects, as defined by the Declaration of Helsinki. Protocols were validated by the Haitian Ethics Committee.

Written consent was obtained from participants or a parent/guardian. Privacy and confidentiality of data was ensured during and after conducting the surveys.

RESULTS

Univariate analysis

Direct and indirect contacts with cholera patients

Compared to controls, cases in Carrefour (but not Gonaives) had more frequent exposure to direct contact with cholera patients (living with, visiting or caring for). Sharing latrines with someone suffering from diarrhoea was significantly associated with the risk of getting cholera for both locations (Table 1).

Water and food consumption

Most households in both locations had access to drinking water from protected water sources such as the town water system or private vendors. No significant difference was found in terms of household drinking-water source between cases and controls (Table 1); however, always drinking chlorinated water was protective in both studies (significantly associated in Gonaives and almost significantly associated in Carrefour).

Eating a meal away from home at least once during the week before illness was significantly more frequent on cases than controls in both studies (OR 7·6 and 2·5 in Gonaives and Carrefour, respectively) (Table 1). This was a frequent risk factor as it was reported by 42·2% and 47·8% of cases in Gonaives and Carrefour, respectively. In Gonaives, the most common location of these meals were school, street vendors, and parents'/friends' houses. Investigators collected detailed information about the types of food consumed over the previous week, including fish, seafood, meat, milk, vegetables and fruit, but found no differences in consumption habits between the two groups (data not shown).

Hygiene conditions and hygienic behaviours

A large proportion of households used a latrine in their yard and shared it with other households (more frequently in Carrefour). Soap and water were rarely available at the latrine site, although this was not significantly associated with risk in either location. The use of soap for hand washing and use of individual dishes (rather than a communal serving dish) at meals, was less frequent in cases than controls, although statistical significance was reached only in Gonaives.

In both locations, compared to controls, cases more frequently used non-chlorinated water for washing dishes.

Exposure to information on cholera prevention

Radio was the most common means of receiving information on cholera prevention, but no difference was found between cases and controls in either location. Controls more frequently reported exposure to prevention information from training sessions in Gonaives, at church in Carrefour and via television in both locations (Table 2).

Social and economic status

In both Gonaives and Carrefour, the education levels of the interviewee and his/her head of family were lower in cases than in controls (same trend in both locations, significant only in Carrefour). Cases had fewer household members than controls (same trend in both locations, although significant only in Gonaives), and were less likely to own a television, refrigerator and car (significant in both locations). Socioeconomic score was significantly lower for cases than for controls in both locations (Table 3).

Table 1. *Univariate conditional logistic regression in relation to direct and indirect contact with a cholera patient, quality of drinking water and food consumption by study site, Haiti 2011*

	Gonaives				Carrefour			
	Exposure (%)				Exposure (%)			
	Controls	Cases	OR	95% CI	Controls	Cases	OR	95% CI
Direct and indirect contact								
Cholera case in the household since beginning of the epidemic	17·8	20·0	1·1	0·6–2·1	7·2	17·8	2·7*	1·2–5·8
Visiting someone suffering from cholera	15·6	11·2	0·7	0·3–1·5	8·4	23·0	3·0**	1·5–6·2
Caring for someone suffering from diarrhoea or cholera	13·3	4·4	0·3*	0·1–0·9	4·4	13·5	3·2*	1·3–8·3
Sharing latrine with someone suffering from diarrhoea	16·9	32·5	2·1*	1·2–3·8	13·5	34·2	3·8***	1·8–8·1
Quality of drinking water stored at home								
Residual free chlorine in drinking water >0·2 mg/l	15·6	11·5	0·7	0·3–1·5	60·7	63·2	1·1	0·6–1·9
Presence of non-*E. coli* coliforms	78·2	82·9	1·3	0·6–2·5	33·9	29·9	0·7	0·4–1·4
Presence of *E. coli*	21·2	31·7	1·8	0·9–3·2	20·2	18·2	0·8	0·4–1·7
Always chlorinate water before drinking (self-reported)	48·9	34·4	0·5*	0·3–0·9	75·6	65·6	0·6	0·3–1·1
Ate a meal away from home at least once in week before illness	14·4	42·2	7·6***	3·3–17·4	28·3	47·8	2·5**	1·4–4·5
Places where meal was eaten								
Restaurant	2·8	5·6	2·2	0·6–8·4	4·0	5·6	1·5	0·4–5·6
School†	4·4	14·4	15·6**	2·0–124·3	0·6	0·0	—	—
Street vendor	2·8	8·9	3·2*	1·0–9·8	16·5	25·6	1·7	0·9–3·2
Market	1·7	12·2	19·1**	2·4–149·1	0·6	2·2	4·0	0·4–44·1
Parent's/friend's house	2·8	8·9	6·3*	1·3–30·7	4·0	7·8	2·0	0·7–5·7
Buying *fresco* from street vendor	n.a.	n.a.	—	—	27·4	42·7	2·0*	1·1–3·3

OR, Odds ratio; CI, confidence interval; n.a., not available.
† In Gonaives the odds ratio for this variable could not be calculated due to the absence of pairing with unexposed cases. The odds ratio presented here was calculated by randomly re-coding an exposed case as unexposed.
* $P< 0·05$, ** $P<0·01$, *** $P<0·001$ (two-tailed tests).

Multivariate analysis

In Gonaives the multivariate analysis indicated eating meals outside the home [adjusted OR (aOR) 35·9], owning pigs (aOR 10·3) and sharing latrines (aOR 3·5) to be the strongest and most significant risk factors. The presence of *E. coli* in the family drinking water, which approached the threshold of significance in univariate analysis, became significant in the multivariate analysis. Interactions between the presence of *E. coli* and chlorine levels, between owning pigs and socioeconomic level, and between participant's age and the presence of *E. coli* were explored, but none were statistically significant. Receiving cholera prevention messages either via television (aOR 0·2) or through training sessions (aOR 0·2) was protective (Table 4).

The multivariate analysis in Carrefour confirmed as significant the main factors identified by univariate odds ratios. Three significant variables measuring direct or indirect contact with someone suspected of having cholera (sharing a latrine with someone suffering from diarrhoea, visiting a cholera patient, and caring for someone suffering from diarrhoea or cholera) were highly collinear and were therefore analysed separately in three models with the three variables interchanged. The aORs were 3·2 for sharing a latrine, 3·7 for visiting a cholera patient, and 3·8 for caring for someone suffering from diarrhoea or cholera. Using untreated water for washing dishes (aOR 3·2) remained a significant risk factor, while receiving cholera prevention messages via television or in church was protective in all three models (Table 5).

Table 2. *Univariate conditional logistic regression for hygiene conditions and behaviours by study site, Haiti 2011*

	Gonaives				Carrefour			
	Exposure (%)				Exposure (%)			
	Controls	Cases	OR	95% CI	Controls	Cases	OR	95% CI
Type/location of toilets								
Toilet inside house	4·8	7·2	Ref.		24·9	13·6	Ref.	
Toilet/latrine in garden	1·2	0·0	—	—	1·2	2·5	3·4	0·4, 26·8
Latrine in courtyard	86·7	79·5	0·7	0·2–2·1	67·1	71·6	1·8	0·9–3·8
Latrine belonging to neighbour	7·2	12·0	1·3	0·3–5·2	6·4	6·2	1·6	0·4–5·4
Shallow pit in yard	0·0	0·0	—	—	0·6	4·9	11·9*	1·2–113·9
Other	0·0	1·2	—	—	0·0	1·2	—	—
Persons using the toilet/latrine								
Only household members	74·1	72·8	Ref.		62·3	49·4	Ref.	
Several households	25·9	25·9	1·0	0·5–1·9	36·0	45·6	1·8	1·0–3·3
Anybody	0·0	1·2	—	—	1·7	5·1	5·8	1·0–33·4
Latrines were overflowing	21·7	30·8	1·3	0·7–2·4	4·3	8·8	1·9	0·6–6·3
Water available for hand washing at site of latrines	5·1	4·4	0·8	0·3–2·7	8·6	2·3	0·3	0·1–1·2
Soap available for hand washing at site of latrines	4·5	2·2	0·5	0·1–2·2	9·8	5·8	0·7	0·3–1·8
Use of soap for hand washing	83·1	68·5	0·4**	0·2–0·8	91·5	85·2	0·5	0·2–1·1
Use of individual place setting to eat	84·9	68·9	0·3**	0·1–0·6	91·3	86·7	0·6	0·3–1·4
Using untreated water to wash dishes	38·9	52·2	2·1*	1·2–3·8	19·6	35·6	3·0**	1·5–6·2
Sources of information on cholera prevention								
Television	32·8	20·0	0·4**	0·2–0·8	61·7	42·2	0·4**	0·2–0·7
Radio	75·0	66·7	0·6	0·3–1·1	61·7	51·1	0·6	0·3–1·0
Door-to-door	48·9	54·4	1·4	0·7–2·5	27·8	28·9	1·1	0·6–1·9
Theatre	0·6	2·2	4·0	0·4–44·1	0·6	0·0	—	—
Posters	2·8	2·2	0·8	0·2–4·1	11·7	10·0	0·8	0·3–2·1
Town crier/sound track	3·3	5·6	2·1	0·5–9·5	8·3	10·0	1·3	0·5–3·2
Training session	17·2	5·6	0·3**	0·1–0·7	20·6	16·7	0·7	0·4–1·5
School	19·4	17·8	0·9	0·4–1·9	8·3	10·0	1·3	0·5–3·2
Church	11·7	5·6	0·4	0·1–1·2	11·7	3·3	0·2*	0·1–0·8
Other sources of information	4·4	8·9	1·3	0·4–4·1	2·8	8·9	3·7*	1·1–12·3

OR, Odds ratio; CI, confidence interval.
* $P<0·05$, ** $P<0·01$, *** $P<0·001$ (two-tailed tests).

Statistically significant risk factors common to the two locations were sharing latrines and low socioeconomic level. Information on cholera prevention via television was a common preventive factor.

DISCUSSION

Studies performed in the early phase of the cholera epidemic in Haiti identified contaminated water as a major risk factor in transmission of cholera [4–6]. Waterborne transmission was consistent with the rapid and explosive spread of the epidemic across Haiti and probably with the following peaks which coincided with the rainy seasons. Our findings show that, in addition to contaminated water, other factors related to direct and indirect inter-human contacts may play a major role in continued transmission during the inter-peak periods.

Apart from the association with pig ownership, which requires further investigation and clarification, all other risk factors identified in our studies were already known. Nevertheless, they provide potentially valuable information for decision makers in Haiti. In particular, we stress the importance of control measures during lull periods, when prevention efforts are typically scaled down and the population tends to lose the perception of the risk of getting the disease.

Table 3. *Univariate conditional logistic regression for social and economic status by study site, Haiti 2011*

	Gonaives				Carrefour			
	Exposure (%)				Exposure (%)			
	Controls	Cases	OR	95% CI	Controls	Cases	OR	95% CI
Type of home dwelling								
Concrete	n.a.	n.a.	—	—	80·6	63·3	Ref.	
Wood or iron sheeting	n.a.	n.a.	—	—	13·9	21·1	2·3*	1·1, 5·1
Tent or plastic sheeting	n.a.	n.a.	—	—	5·6	15·6	3·4**	1·5, 8·3
Number of household members†								
1–3	8·9	22·2	Ref.		15·2	21·3	Ref.	
4–5	22·8	26·7	0·5	0·2–1·1	35·4	33·7	0·6	0·3–1·3
6–8	33·9	36·7	0·4*	0·2–1·0	31·5	30·3	0·6	0·3–1·4
⩾9	34·4	14·4	0·2***	0·1–0·4	18·0	14·6	0·5	0·2–1·3
Household owns								
Goats	13·3	15·6	1·2	0·6–2·4	2·8	1·1	0·4	0·0–3·4
Pigs	3·9	16·7	5·5**	2·0–15·1	0·6	2·3	4·0	0·4–44·1
Chickens	25·0	21·1	0·8	0·5–1·5	19·6	18·0	0·9	0·5–1·8
Other animals	19·4	17·8	0·9	0·4–1·8	39·7	26·7	0·5*	0·3–0·9
Household owns at least one								
Radio	77·8	70·0	0·7	0·4–1·2	73·3	60·0	0·5*	0·3–0·9
Television	71·1	46·7	0·4***	0·2–0·7	68·3	53·3	0·5*	0·3–0·9
Refrigerator	21·7	11·1	0·4*	0·2–0·9	31·7	21·1	0·6	0·3–1·1
Oven	6·1	3·3	0·5	0·2–2·0	12·2	6·7	0·5	0·2–1·3
Washing machine	1·1	2·2	2·0	0·3–14·2	1·7	0·0	—	—
Water storage tank	7·8	3·3	0·4	0·1–1·5	3·3	2·3	0·7	0·1–3·3
Car	14·4	3·3	0·2*	0·1–0·7	13·3	3·4	0·2*	0·1–0·8
Socioeconomic score‡ (mean)	1·45	1·13	0·5**	0·3–0·8	1·82	1·32	0·4***	0·3–0·6

OR, Odds ratio; CI, confidence interval; n.a., not available.
† Odds ratios for trend: Gonaives (0·83, 95% CI 0·75–0·92), Carrefour (0·94, 95% CI 0·85–1·04).
‡ Socioeconomic score includes educational level of the interviewee and of the head of the family as well as ownership of radio, television, refrigerator, oven, washing machine, water storage tank, car and animals).
* $P<0·05$, ** $P<0·01$, *** $P<0·001$ (two-tailed tests).

The quality of drinking water was far from optimal in both locations. Self-reported chlorination of drinking water was a protective factor in Gonaives, but adequate chlorine concentration in home-stored drinking water was not. This contradictory result may have multiple explanations. It is possible that the chlorination was incorrectly done, or that the presence of chlorine went undetected due to the delay between chlorination and sample collection (the latter information was not recorded). Alternatively, it might reflect interviewees' reluctance to admit that they had not followed proper hygiene or clean water recommendations. In Carrefour highly chlorinated drinking water was more frequent in households of cases than controls, a finding that may reflect excessive caution by family members after someone in the household falls ill. In either case, it is clear that poor water quality was common, as shown by the high proportion of water samples found to be contaminated with *E. coli* in households of both cases and controls, and that the quality of drinking water needs to be improved.

Direct and indirect contacts, such as helping or visiting a person suffering from diarrhoea [12–14], sharing latrines [15, 16] or a low socioeconomic status [16, 17] are risk factors that have already been described in other cholera epidemic or endemic contexts. In Haiti, it remains unclear whether the risk of cholera via direct contact reflects a lack of means (soap, chlorine, water), insufficient knowledge of essential hygiene measures, or both. The Haitian Ministry of Public Health and Population, together

Table 4. *Multivariate analysis of risk factors associated with cholera illness in Gonaives, Haiti 2011*

	Univariate analysis			Multivariate analysis		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Ate a meal away from home at least once in week before illness	7·6***	3·3–17·4	<0·001	35·9***	7·9–163·4	<0·001
Household owns pigs	5·5**	2·0–15·1	0·001	10·3**	2·3–46·6	0·002
Sharing latrine with someone suffering from diarrhoea	2·1*	1·2–3·8	0·013	3·5*	1·3–9·5	0·016
Presence of *E. coli* in drinking water stored at home	1·8	0·9–3·2	0·074	3·5*	1·2–10·0	0·021
Chlorine level >0·2 mg/l in drinking water stored at home	0·7	0·3–1·5	0·376	0·5	0·2–1·9	0·341
Always chlorinate water before drinking	0·5*	0·3–0·9	0·019	0·3	0·1–1·0	0·060
Receiving information on cholera prevention via television	0·4*	0·2–0·8	0·015	0·2*	0·1–0·8	0·021
Receiving information on cholera prevention in training session	0·3**	0·1–0·7	0·009	0·2*	0·0–0·9	0·035
Number of member in household (ref. 1–3 members)						
4–5	0·5	0·2–1·1	0·099	0·5	0·2–1·7	0·291
6–8	0·4	0·2–1·0	0·044	0·7	0·2–2·6	0·595
⩾9	0·2***	0·1–0·4	<0·001	0·1**	0·0–0·5	0·004
Socioeconomic score	0·5**	0·3–0·8	0·001	0·5*	0·3–1·0	0·036

OR, Odds ratio; CI, confidence interval.
* $P<0·05$, ** $P<0·01$, *** $P<0·001$ (two-tailed tests)

Table 5. *Multivariate analysis of risk factors associated with cholera illness in Carrefour, Haiti 2011*

	Univariate analysis			Multivariate analysis		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Sharing latrine with someone suffering from diarrhoea†	3·8***	1·8–8·1	<0·001	3·2*	1·3–7·7	0·011
Using untreated water for washing dishes	3·0**	1·5–6·2	0·002	3·2**	1·4–7·3	0·006
Ate a meal away from home at least once in week before illness	2·5**	1·4–4·5	0·001	1·8	0·9–3·7	0·117
Presence of *E. coli* in drinking water stored at home	0·8	0·4–1·7	0·582	1·5	0·5–4·3	0·489
Chlorine level >0·2 mg/l in drinking water stored at home	1·1	0·6–1·9	0·845	1·0	0·5–2·4	0·920
Receiving information on cholera prevention via television	0·4**	0·2–0·7	0·002	0·4**	0·2–0·9	0·027
Receiving information on cholera prevention at church	0·2*	0·1–0·8	0·027	0·1**	0·0–0·5	0·003
Socioeconomic score	0·4***	0·3–0·6	<0·001	0·5**	0·3–0·8	0·002

OR, Odds ratio; CI, confidence interval.
† Two other variables measuring contacts with suspected cholera cases (visiting someone suffering from cholera and caring for someone suffering from diarrhoea or cholera) were collinear with sharing the latrines with someone suffering from diarrhoea. We built separate models replacing sharing the latrines with these two variables; the odds ratios were 3·7 (95% CI 1·2–11·9) for visiting someone suffering from cholera and 3·8 (95% CI 1·5–9·5) for caring someone suffering from diarrhoea or cholera.
* $P<0·05$; ** $P<0·01$; *** $P<0·001$ (two-tailed tests).

with other interested parties, distributed cleaning kits to caregivers of patients admitted to CTCs to limit transmission within patients' homes; although well-intentioned, this effort may have little impact since most intra-household transmission would have already occurred by the time of the distribution.

In our studies the investigation of household latrines did not go beyond whether the latrine was overflowing and whether soap and water were present, so it remains unclear whether the observed elevated risk was directly linked with contaminated latrines or, again, with insufficient knowledge of essential hygiene measures. Nevertheless, as most households lacked soap for hand washing, prevention efforts should focus on making soap and chlorine available. Considering that sharing a latrine with neighbours is common in Haiti, outreach campaigns should specifically address this issue by reinforcing the importance

of cleaning latrines after use and of providing decontamination of shared latrines.

Selling food and beverages in the streets and markets, a common activity in developing countries has also been identified as a key factor in cholera transmission in other contexts [18–21]. In Gonaives this factor was notable for both the strength of the association and the high proportion of associated cases suggesting that food consumed in the market or at school was highly implicated in cholera transmission. Since these studies were conducted, street vendors in several Haitian cities (Dessalines, Gros Morne) have been given information about cholera transmission, along with supplies of chlorine, soap and hand washing buckets, which were well-received by both vendors and customers. These and other preventive measures should be strongly encouraged until more permanent hygiene and sanitation measures are in place.

In Carrefour, conditions specific to post-earthquake victims, such as living in a tent or a dwelling made of plastic sheeting, were associated with increased risk. Early in the epidemic, displaced populations had relatively sufficient access to clean water and improved sanitation. However, since then, some displaced people have been relocated and aid agencies have reduced their services inside the camps. Two surveys by the Dinepa (National Water Board) Observatory [22, 23] showed that already by the end of 2011 there had been an alarming decrease in access to safe drinking water, and that there was poor maintenance of latrines and hand-washing facilities in the surveyed camps.

The association with owning pigs was highly unexpected. Although *V. cholerae* has been detected in stool samples of animals, including pigs [24], to our knowledge this is the first time that owning animals has been associated with risk of contracting cholera. Pig ownership may be a proxy indicator for a risk factor we did not investigate and merits further investigation. It may be worth including this potential risk factor in further studies on cholera transmission.

Both studies show that insufficient practice of essential hygiene measures is an important issue to tackle in Haiti, but also that targeted information campaigns can help reduce cholera incidence. Visual messages on television, the persuasive appeal of a church leader, and the personal motivation required to attend a training session, may enhance the likelihood that people will implement the suggested hygiene measures. Prevention information through various means was widespread in Haiti during acute transmission phases, but gradually decreased as the peak subsided. Prevention campaigns can effectively make an impact to reduce cholera incidence and should remain active during low transmission periods.

These studies involve some limitations. One is the low specificity of the Crystal VC test [25], leading to inadvertent inclusion of some non-cholera patients among cases. Another is that the selection of controls was based on self-reports of no prior history of cholera. The two misclassifications above, however, would only have weakened the results, i.e. hidden weak associations such as using soap, a protective factor demonstrated by other studies [26, 27]. In addition, we cannot exclude that some controls had an asymptomatic form of cholera, which occurs frequently [28, 29] and is potentially transmissible [30]. However, the risk factors we evaluated apply only to symptomatic cholera.

We have presented evidence that in addition to contaminated water, human-to-human and mediated transmission through food handling or sharing latrines, may play a substantial role in the maintenance of *V. cholerae* during the lull between periods of peak caseloads in Haiti. Reinforcing efforts to raise public awareness of risk reduction measures and to improve hygiene, clean food and safe water practices are effective interventions for cholera control that should be implemented also during lull periods. Such interventions are, however, difficult to implement and maintain especially when the perception of the risk of getting the disease decreases. Specific plans for low transmission periods should be also foreseen as a promising approach to reducing or eliminating circulating *V. cholerae*, thereby averting the occurrence of future outbreaks in Haiti.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0950268813002562.

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DECLARATION OF INTEREST

None.

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Health in Action

Feasibility of Mass Vaccination Campaign with Oral Cholera Vaccines in Response to an Outbreak in Guinea

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Background

The number of reported cholera cases worldwide, as well as the frequency and scale of cholera epidemics, are increasing [1]. Traditional prevention measures, which focus on provision of safe water and proper sanitation, are undoubtedly the long-term solution for cholera control. But for populations in many low-income countries these measures remain out of reach: in Africa, 40% of families cannot access safe water and 60% have no access to appropriate sanitation [2]. Furthermore, once a cholera outbreak has started, these solutions are unlikely to be implemented fast enough or on a large enough scale to help control the spread. Nationwide epidemics, such as the recent one in Haiti—with over 600,000 cases and 7,000 deaths reported within the first 2 years [3]—highlight the urgent need for new tools and strategies.

Two oral cholera vaccines (OCVs) are currently licensed and prequalified by WHO: Dukoral (Crucell, Leiden, Netherlands), and Shanchol (ShanthaBiotechnics Ltd., Basheerbagh, Hyderabad, India). Both are given as a two-dose regimen and were shown to be safe and to provide sustained protection over several years [4]; Shanchol showed 66% efficacy over 3 years [5]. WHO recently updated its guidelines on cholera outbreak response to recommend considering OCV use in epidemic situations (as well as in endemic settings) [4].

However, ongoing questions and debate about the feasibility, cost, timeliness, and acceptability of reactive OCV campaigns have discouraged their use [6,7]. Arguments against using OCV during epidemics have included: limited availability of vaccine; logistical challenges of rapidly transporting and delivering high volumes of cold-chain–requiring vaccines in resource-limited settings; difficulty achieving sufficient coverage with a two-dose regimen; acceptance of vaccination by the population; high vaccine cost; and fear of diverting limited resources from other control measures [6,7]. Practical experience with OCV during epidemics has therefore remained limited to small-scale interventions in Asia [8–11].

Here we describe the implementation of the first large-scale reactive OCV campaign, conducted in Guinea between April and June 2012, and the first use of OCV Shanchol in Africa.

Summary Points

- Oral cholera vaccines are safe and effective, and in 2010 were added to WHO recommendations for cholera outbreak control. However, doubts about feasibility, timeliness, and acceptability by the population, and the fear of diverting resources from other preventive interventions, have discouraged their use during epidemics.
- We report on the first large-scale use of oral cholera vaccine as an outbreak control measure in Africa; this was also the first time Shanchol vaccine was used in Africa.
- We administered 312,650 doses of vaccine during two vaccination rounds in two coastal districts in Guinea. The feasibility, timeliness of implementation, and delivery cost were similar to those of other mass vaccination campaigns.
- The campaign was well accepted by the population, and high vaccination coverage was achieved despite the short time available for preparation, the two-dose schedule, the remote rural setting, and the highly mobile population.
- Oral cholera vaccines are a promising new tool in the arsenal of cholera control measures, alongside efforts to improve provision of safe water and sanitation and access to cholera treatment.

Cholera Context in Guinea

Guinea, a country on the West African coast, regularly experiences cholera epi-

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Abbreviations: OCV, oral cholera vaccine; MSF, Médecins sans Frontières; VVM, vaccine vial monitor.

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demics, with peaks occurring during the rainy season in July–August. The last major epidemic was in 2007, with 8,289 cases and 295 deaths [12]. However, in 2012 the first cholera cases were reported in February, long before the rainy season. As in previous epidemics, cases were first reported from the islands north and south of the capital, Conakry, in the Boffa and Forecariah districts. These islands are characterized by intense fishing activities and trade, a highly mobile population, limited access to health care, and poor access to safe water or basic sanitation.

The early start of the outbreak, together with a long inter-epidemic period and an ongoing cholera epidemic in neighboring Sierra Leone [13], suggested that a major epidemic was imminent. Considering these factors, the Ministry of Health of Guinea, with support of Médecins sans Frontières (MSF) decided in April 2012 to use OCV alongside already-implemented treatment and prevention strategies (health education; distribution of soap and chlorine for household water treatment).

Implementation of the Vaccination Campaign

Target population. The campaign focused on the coastal and island populations of the above-mentioned districts, which extend over about half the length of the Guinean coast: first, a population of 163,000 people in Boffa district, and 46,000 people in parts of Forecariah district (Kaback and Kakossa islands, and some neighboring ports on the mainland). Everyone older than 12 months presenting at vaccination sites was eligible to receive the vaccine during both vaccination rounds, which were spaced 2–3 weeks apart.

Vaccine procurement, storage, and transport. The bulk of the vaccine supply (320,000 doses) was shipped directly from the manufacturer in India, and 50,000 additional doses from MSF stock in Kampala, Uganda. The volume of the transport containers of vaccine was 29 m^3. Vaccines were transported from Conakry's airport to the district capital in refrigerated trucks and stored in the field in refrigerated trucks or containers. Vaccines reached the field within 2 weeks of the order date.

Vaccine was supplied in individual vials, either in secondary packing of 35 vials or in individual secondary packing inside tertiary packing of 10 vials. One vaccine vial in the 35-vial package had a volume of 13.5 cm^3, about five times greater than a dose of measles vaccine.

Vaccination teams. Forty-three teams composed of community members (community health workers, Guinean Red Cross volunteers, etc.) were assembled. Each team had a medical or paramedical leader and four to eight members, plus up to 12 helpers. Training for team leaders and members included a practice vaccination session.

Choosing vaccination sites. Preliminary selection was done together with district medical authorities, then refined in consultation with community leaders. An important criterion was to keep travel distances short so that all family members, including elderly people and mothers with small children, could reach the sites easily. Altogether there were 287 sites, one per village or settlement (Figure 1).

Mobilizing the population. Due to the emergency nature of the intervention, the time period for social mobilization was short. The information was transmitted orally as described below; modern media were not used, as local radio or television are not available in the area and the mobile network coverage is low. Public awareness messaging included detailed information about the rationale of the campaign, the vaccine and the importance of two-dose schedule, along with standard cholera control messages regarding the necessity and availability of treatment and prevention measures. Existing material was used to illustrate the standard cholera control messages, but no special material was designed for the vaccination due to the limited amount of time available. Medical, administrative, and traditional authorities were informed in advance. Each community was visited 2 days before vaccination day by a health promoter, who provided educational and awareness information via village leaders. In more populated areas, local outreach workers conducted door-to-door mobilization.

Vaccination day. Each team had a car (two in Boffa) or boat to reach the vaccination sites. Vaccines were transported and used at ambient temperature on vaccination day. Vaccines leftover at the end of vaccination day were returned to the cold chain and used first on the following day. Before administration, the vaccine vial monitor (VVM) was checked for stability; the vial was shaken, opened, and administered or self-administered under observation (Figure 2). All VVM remained valid during the campaign.

To facilitate ingestion of the vaccine, we provided safe drinking water to each vaccinee (pre-packed 33 cl sachets from a Guinean manufacturer). Each vaccinee also received a vaccination card during the first round and was asked to bring the card for the second dose. However, during the second round we provided the vaccine to those who had lost their card or were not previously vaccinated.

In Forecariah, the second vaccination round was accompanied by distribution of preventive items (soap and chlorine solution for household water treatment), targeting women of childbearing age.

Teams vaccinated an average of 703 persons daily, up to 1,830 vaccinations/day/team. They spent several days in the larger villages but covered several smaller sites in one day. The vaccine wastage rate was below 1%. A total of 46 non-severe

Figure 1. Vaccination team at work. *Image credit: David Di Lorenzo.*
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Figure 2. Administration of the vaccine. *Image credit: David Di Lorenzo.*
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adverse effects were reported (mainly diarrhea and vomiting).

Vaccination Coverage

Altogether 172,544 doses of vaccine were administered during the first round and 143,706 during the second. Based on administrative population figures, coverage with at least one dose (either first or second dose) was 92% in Boffa and 71% in Forecariah, and with the complete two-dose regimen was 68% in Boffa and 51% in Forecariah. However, a household survey conducted immediately after the campaign (Francisco Luquero, personal communication) found two-dose coverage in both areas to be about 76%, and one-dose coverage >90%. These differences are likely to be due to overestimation of actual population size by official figures.

Time and costs. The complete campaign took 6 weeks from the decision to proceed until completion of the second round in Boffa (3-week interval between doses) and 5 weeks in Forecariah (2-week interval).

Cost per dose of vaccine delivered was US$2.89, including $1.85 for the vaccine itself and just over $1 for direct delivery costs (especially transport of teams and material, and payment for teams and other staff). Table 1 lists all costs that were factored into this calculation.

Evolution of the epidemic. We were able to complete the vaccinations in two affected areas before the start of the seasonal cholera peak (Figure 3). The campaign's final outcomes will not be known until ongoing vaccine effectiveness and impact studies are completed; however, while the number of cholera cases peaked in other parts of Guinea during the rainy season, it remained at low levels in vaccinated districts (Ministry of Health, Cholera situation update, December 2012).

Lessons for the Future

This experience demonstrated that mass campaigns with a two-dose OCV can be conducted successfully at the beginning of a cholera epidemic, even in a large, difficult-to-access area in Africa with a highly mobile population, and with little time for preparation of the campaign and social mobilization. Potential obstacles that discouraged earlier campaigns either failed to materialize or were quite manageable; in particular, the population was eager to get vaccinated during the outbreak, and logistical issues were resolved.

Ironically, in many ways our campaign was "over-resourced," due to the anticipated obstacles. Vaccination teams in Boffa were over-sized (half-sized teams in Forecariah vaccinated the same number of people per day), which increased transportation needs. Transportation of water sachets was logistically challenging; although use of water is not necessary according to the manufacturer, we provided it to facilitate the intake of the salty-tasting vaccine. Vaccination cards were used only to verify vaccination status during the coverage survey. A simplified strategy without use of water and vaccination cards would reduce personnel and transport needs, and related costs.

Another potential simplification relates to vaccine vial presentation and packaging. The single-dose vaccines are voluminous, due partly to bulky secondary packaging. Additionally, the vaccine vial design is not ideal for oral use: single-dose vials are tiny, with metallic caps that are difficult to open.

There may also be potential to reduce cold chain needs. Although the vaccine is equipped with VVM 14 and considered temperature-stable, current labeling requires the vaccine to be stored in the cold chain. Documentation of thermostability is needed for future campaigns to be conducted using vaccines at ambient temperature.

Table 1. Direct costs of mass vaccination campaign.

Item	Total (US$)	% Total
Vaccine ($1.85/dose)	585,063	64.0%
Water sachets ($0.036/sachet)	11,385	1.2%
Airfreight for vaccines	47,719	5.2%
Transit cost for vaccines	9,574	1.0%
Cold chain (truck rental, reparation of container in Boffa)	26,505	2.9%
Vaccination, supervision and sensitisation teams payments	63,308	6.9%
Training for the teams	4,899	0.5%
Small vaccination material and stationary, vaccination cards	13,705	1.5%
Logistic material, site preparation, waste management	13,333	1.5%
Transport cost (cars, trucks, boats and fuel)	139,851	15.3%
Total	915,341	100.0%
Cost per dose delivered	2.89	

Fixed administrative costs, MSF institutional costs, and costs linked to operational research are excluded.
doi:10.1371/journal.pmed.1001512.t001

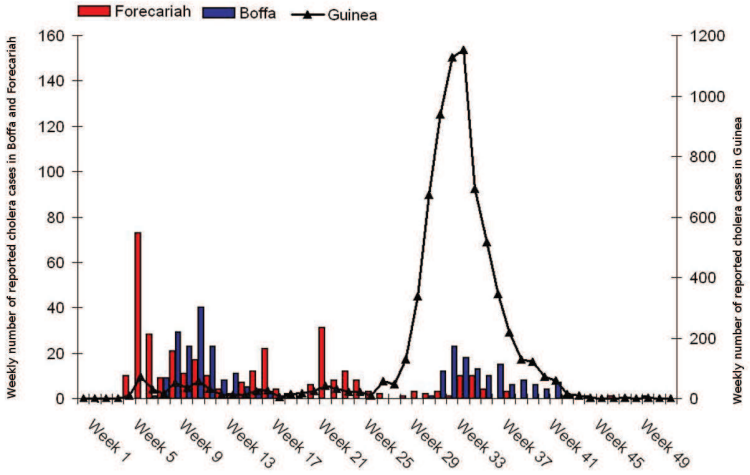

Figure 3. Weekly number of reported cholera cases in Guinea, and Boffa and Forecariah districts, Guinea, 2012. The vaccination campaign in Boffa took place on epidemiological weeks 13 and 16 and in Forecariah on weeks 22 and 24. *Source: Ministry of Health, Guinea.* doi:10.1371/journal.pmed.1001512.g003

A single-dose vaccine would also greatly simplify OCV campaigns. Studies in India found that partial immune response is achieved after a single dose [14], but whether this response is sufficient to confer clinical protection is not yet known. Similarly, a herd protection effect of Dukoral has been reported [15,16], but its extent needs to be confirmed for Shanchol in additional settings.

Perhaps the most serious obstacles to wider use of reactive OCV campaigns are cost and limited supply of Shanchol. These constraints led us to drastically limit our target population to a small subset of those at risk; the full at-risk population includes everyone living along the coast of Guinea, including the capital (Conakry) with two million inhabitants, areas that were highly affected once the epidemic began. Funding for an OCV stockpile will be critical for the timely implementation of larger campaigns, an issue currently being addressed by WHO and its partners in an effort to improve OCV access for countries facing cholera outbreaks [17].

Conclusion

Our experience demonstrates the feasibility of implementing OCV mass campaigns at the onset of major epidemics, similar to the campaigns with other vaccines used reactively (e.g., measles). OCVs are a promising additional tool for controlling cholera epidemics and should help prevent many illnesses and deaths, especially in settings with limited access to health care and where immediate improvements in sanitary conditions are improbable. In the near future, experience implementing OCV campaigns should be carefully documented, to provide future guidance for its most effective use.

Supporting Information

Alternative Language Text S1 Article translated into French by Hélène Joguet. (DOC)

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Author Contributions

Analyzed the data: IC SK FJL MH RFG DL. Wrote the first draft of the manuscript: IC. Contributed to the writing of the manuscript: IC SK FJL MH CI MM RFG FV DL. ICMJE criteria for authorship read and met: IC SK FJL MH CI MM RFG FV DL. Agree with manuscript results and conclusions: IC SK FJL MH CI MM RFG FV DL.

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PLOS | NEGLECTED TROPICAL DISEASES

First Outbreak Response Using an Oral Cholera Vaccine in Africa: Vaccine Coverage, Acceptability and Surveillance of Adverse Events, Guinea, 2012

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Abstract

Background: Despite World Health Organization (WHO) prequalification of two safe and effective oral cholera vaccines (OCV), concerns about the acceptability, potential diversion of resources, cost and feasibility of implementing timely campaigns has discouraged their use. In 2012, the Ministry of Health of Guinea, with the support of Médecins Sans Frontières organized the first mass vaccination campaign using a two-dose OCV (Shanchol) as an additional control measure to respond to the on-going nationwide epidemic. Overall, 316,250 vaccines were delivered. Here, we present the results of vaccination coverage, acceptability and surveillance of adverse events.

Methodology/Principal Findings: We performed a cross-sectional cluster survey and implemented adverse event surveillance. The study population included individuals older than 12 months, eligible for vaccination, and residing in the areas targeted for vaccination (Forécariah and Boffa, Guinea). Data sources were household interviews with verification by vaccination card and notifications of adverse events from surveillance at vaccination posts and health centres. In total 5,248 people were included in the survey, 3,993 in Boffa and 1,255 in Forécariah. Overall, 89.4% [95%CI:86.4–91.8%] and 87.7% [95%CI:84.2–90.6%] were vaccinated during the first round and 79.8% [95%CI:75.6–83.4%] and 82.9% [95%CI:76.6–87.7%] during the second round in Boffa and Forécariah respectively. The two dose vaccine coverage (including card and oral reporting) was 75.8% [95%CI: 71.2–75.9%] in Boffa and 75.9% [95%CI: 69.8–80.9%] in Forécariah respectively. Vaccination coverage was higher in children. The main reason for non-vaccination was absence. No severe adverse events were notified.

Conclusions/Significance: The well-accepted mass vaccination campaign reached high coverage in a remote area with a mobile population. Although OCV should not be foreseen as the long-term solution for global cholera control, they should be integrated as an additional tool into the response.

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Introduction

Provision of safe water and proper sanitation are without doubt the long-term and only solution for cholera control [1,2]. However, controlling cholera globally is far from being achieved; the disease burden is increasing with large-scale outbreaks reported in the past several years, such as those in Haiti and Zimbabwe [3]. Current outbreak response interventions focus on case management and access to health care, as well as the immediate provision of safe water and hygiene promotion [1]. However, current outbreak control activities have proven insufficient to avoid massive numbers of cases and deaths in recent large-scale outbreaks. The adequate treatment of cases for example, although crucial to decrease mortality, has a limited impact in controlling disease spread [1,3]. Oral cholera vaccines (OCV), which have the potential to reduce the number of cases and minimize the spread of disease [4,5], could be an important addition to the cholera response arsenal [1,6,7].

The World Health Organization (WHO) prequalifies the OCV Dukoral (SBL Vaccine/Crucell, Sweden) and Shanchol (Shanta-Biotechnics, Hyderabad, India). Both are killed whole cell *V. cholerae* O1 vaccines; Shanchol also contains *V. cholerae* O139 and Dukoral the recombinant cholera toxin B subunit. The two vaccines share a good safety and efficacy profile with an estimated protection of 60–85% for 2–3 years [1]. Although, recommended by WHO (including in response to outbreaks since 2010) [8], their use as public health tools has been limited. Specifically, questions about the acceptability, feasibility, cost and potential diversion of resources have discouraged the use of OCV for outbreak control [9].

Author Summary

Two safe and effective oral cholera vaccines are recommended by the World Health Organization for cholera prevention and control; however, concerns about the acceptability, potential diversion of resources, cost and feasibility of implementing timely campaigns has discouraged their use. In 2012, the Ministry of Health of Guinea, with the support of Médecins Sans Frontières, organized the first mass vaccination campaign using a two-dose oral cholera vaccine (Shanchol) as an additional control measure to respond to an on-going nationwide epidemic. This was also the first time that Shanchol was used in a mass vaccination campaign on the African continent. High coverage was reached within a few weeks, and the campaigns were well accepted by the population. Synergies between different axes in cholera control interventions should be pursued as described here, and although oral cholera vaccines should not be foreseen as the long-term solution for global cholera control, they should be integrated as an additional tool into the outbreak response strategies.

In 2012, the Ministry of Health (MoH) of Guinea, with the support of Médecins Sans Frontières-Operational Centre Geneva (MSF) organized the first cholera outbreak response in Africa using an OCV in the Republic of Guinea (Guinea). This was also the first time that Shanchol was used in a mass vaccination campaign on the African continent. Cholera has been reported in Guinea since 1970. The largest outbreak was in 1994 with more than 30,000 cases and 670 deaths reported. The most affected areas were the coastal prefectures and the islands (Maritime Guinea, where the capital Conakry is located) [10]. From 2003 to 2007, cholera outbreaks were reported each year during the rainy season (July–August) throughout the country with Maritime Guinea remaining the most affected area. From 2008 to 2011, only sporadic cases were reported [11].

In 2012, the first cholera cases were reported in Forécariah (Maritime Guinea) before the rainy season. From February 2 to March 8, a total of 147 cases and 13 deaths were reported. On March 3, the first case was reported and confirmed in Conakry. A cholera outbreak was also on going in neighbouring Sierra Leone, with 13,934 cases and 232 deaths reported countrywide between January and August 2012 [12]. The regional nature of the epidemic, the early notification of cases before the peak of the rainy season and the long interval without outbreaks, thereby increasing the number of susceptible individuals due to lack of prior exposure, all suggested the possibility of a large epidemic in Guinea in 2012.

Case management, water, health education, hygiene and sanitation interventions were implemented in response to the outbreak. Non-selective mass vaccination campaigns were implemented in the prefectures of Boffa and Forécariah (Figure 1). Two doses of Shanchol, two weeks apart were offered from April 18 to May 14, 2012 in Boffa and from May 27 to June 15, 2012 in Forécariah (Figure 2). Overall, 316,250 vaccines were delivered by 43 teams (of 9 members in Boffa and 5 in Forécariah) in 287 vaccination sites (one per village or settlement). All individuals

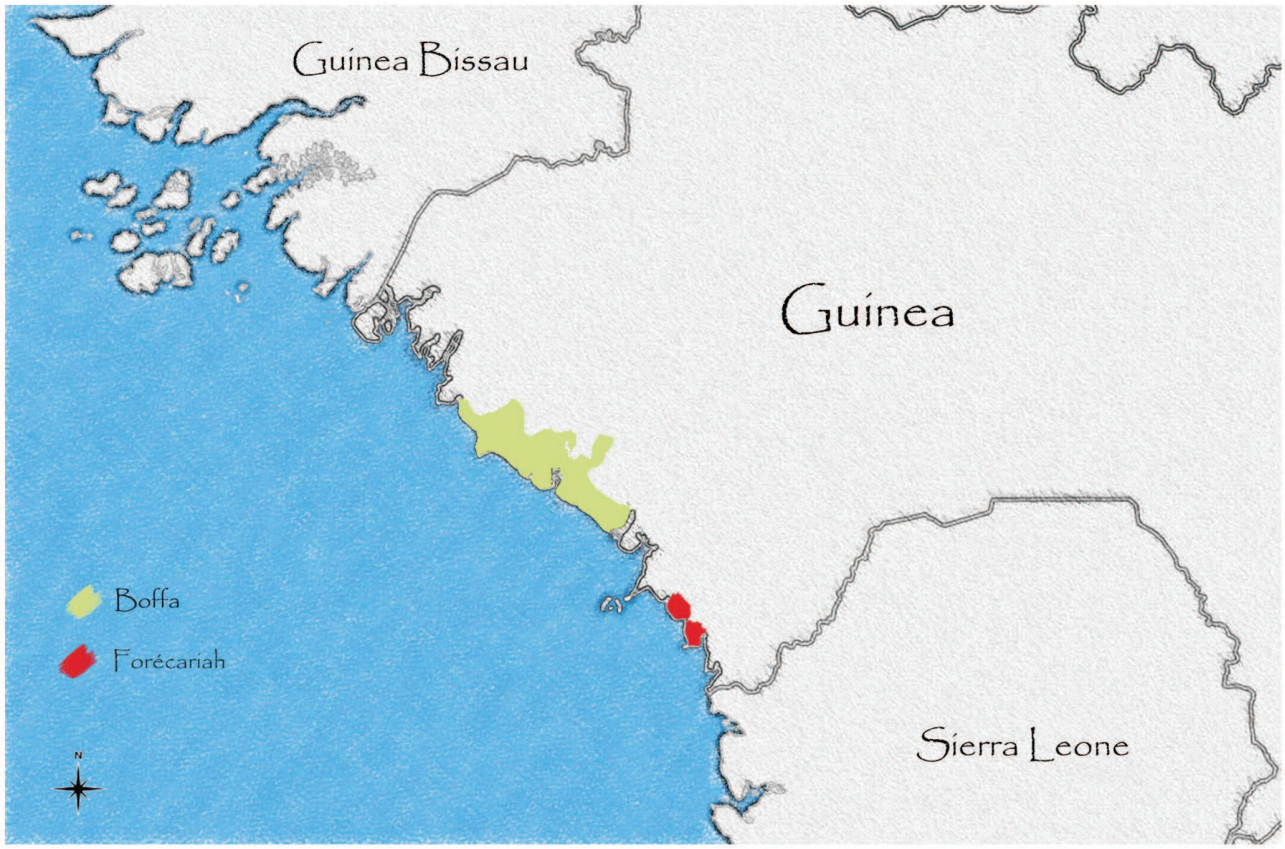

Figure 1. Target areas by the non-selective mass vaccination campaigns, Guinea, 2012.
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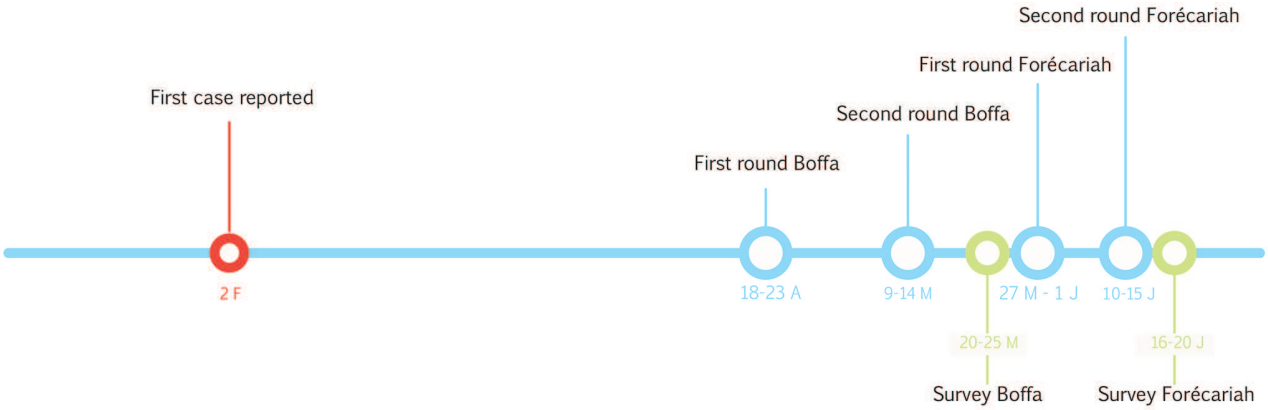

Figure 2. Timeline of the cholera vaccination campaigns and implementation of the field surveys in Guinea in 2012. Months are abbreviated as follows: F = February, A = April, M = May, J = June.
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older than 12 months were eligible for vaccination in both rounds. Pregnant women were offered vaccine after a careful examination of the risk and benefits (an on-going outbreak in a remote rural place with limited access to health care and high cholera associated mortality in the past) following the manufacture and WHO recommendations [8]. Vaccines were stored under cold chain, but were transported and used at ambient temperature on vaccination days. Before administration, vaccine vial temperature monitor was checked for stability and all remained valid.

Here, we present the results of household-based vaccination coverage and acceptability surveys and surveillance of adverse events.

Methods

Cross-Sectional Survey

All individuals older than 12 months, resident in the six sub-prefectures bordering the sea in Boffa prefecture (Koba, Boffa-centre, Douprou, Tougnifily, and part of Mankountan and Tamita) and in the sub-prefectures of Kaback and Kakossa in Forécariah prefecture were targeted for vaccination and were eligible for inclusion in the survey (Figure 1).The coastal area of Boffa combines both inland areas and several islands. Kaback and Kakossa are two separate islands. Residents were defined as persons living (sleeping and eating) in the area for at least the previous two weeks. The adult population is mobile with men in particular, leaving and returning to the area for fishing, agriculture and trade.

A representative sample of the population in each survey site (Boffa and Forécariah) was selected using cluster-based sampling with population proportional to size [13]. To sample households within the selected sectors, all households were enumerated. The first household was selected with the aid of a random number table and subsequent households were selected by proximity (first household to the left). In the urban area of Boffa and in Kaback Island in Forécariah, satellite-map based sampling was used to select randomly the starting point of the cluster [14]. This methodology was used in urban Boffa because of the large number of households to enumerate and in Kaback Island because of the absence of accurate population data per sector.

The sample size was calculated to obtain a representative estimate of the proportion of residents who received two doses of OCV by age group (1–4, 5–14, 15 years and older). Sample size was calculated to ensure a sufficiently precise estimate for children aged 1 to 4 years as this group was the smallest. We considered the following assumptions: 70% of children would receive two doses of vaccine, alpha error of 5%, absolute precision of 7% for Boffa and 10% for Forécariah, design effect (deff) of 3.0 for Boffa and 1.5 for Forécariah (coverage was expected to be more homogenous in the islands). Taking into account the results of the 2005 Demographic and Health Survey [15], we expected 0.7 children 1–4 year old per household (average of 6.1 individuals per household and 12% of the population between 1 and 4 years). Assuming 10% of missing data, we planned to visit 780 households (60 clusters of 13 households) in Boffa and 180 households (30 clusters of 6 households) in Forécariah. A household was defined as a group of people sleeping under the same roof and sharing meals every day for at least the previous two weeks.

Training and Data Collection

All surveyors and supervisors were recruited locally and received a theoretical and practical training. Training consisted of survey and interview methodology and a pilot implementation of the questionnaire.

Teams conducted face-to-face interviews after consent. Survey teams asked for the help of neighbours to trace absentees and re-visit empty (but not abandoned) households later in the day. If during the second visit the occupants could not be found or if they refused to participate, that household was skipped.

A standardized pre-piloted questionnaire was used to collect the following information: demographic data (age, sex, and household size), vaccination status (card-confirmed and orally reported), reasons for non-vaccination (open question), and acceptability data (adverse events, taste and beliefs about the vaccine). Questions concerning acceptability were only collected in Boffa (first site of vaccination) in participants older than 15 years. Interviews were conducted in the local language.

Surveillance of Adverse Events following Immunization

Surveillance of adverse events following immunization (AEFI) was implemented at vaccination sites, health centres and health posts in the target areas. An AEFI was defined as a medical occurrence detected by the vaccination site supervisor or a physician with an onset up to 14 days after receipt of a dose of vaccine. During the awareness campaign and at the time of vaccination, participants were told to report to a vaccination site or a health centre if they felt ill after receiving the vaccine. The following data were collected using a standardized form: age, sex,

pregnancy, history of allergies, vaccination date, consultation date, date of onset of the symptoms, type of symptoms, and clinical outcome (recovery, transfer or death).

Data Entry and Analysis

Our main outcome was the OCV coverage (single dose and full course) in each of the target locations. Vaccine coverage was calculated dividing the number of individuals reporting being vaccinated by the survey population and expressed as a percentage. Vaccination coverage estimates include both card-confirmed and oral reporting. Secondary outcomes included vaccine coverage by age group, sex and reasons for non-vaccination. Crude vaccination coverage estimates and 95% confidence intervals (95% CI) were obtained considering the survey design. The design effect was calculated to estimate the loss of precision due to the cluster based sampling strategy. Sampling weights were calculated to account for differences in the cluster size.

Data entry was performed using EpiData 3.1 (EpiData Association, Denmark) and data analysis was performed using Stata 12.0 (College Station, USA).

Ethical Considerations

The Ethical Review Board of Guinea and the MSF Ethical Review Board approved the study protocol. Oral informed consent was obtained from participants in all instances. All children had consent given from a parent/guardian and all adult participants provided their own consent. Oral informed consent was requested since the study did not present any risk of harm to subjects and did not involve procedures for which written consent is normally required outside the research context. The procedure was approved by the ethical review boards. The request of consent was registered in a log-book. Privacy and confidentiality of the data collected from participants was ensured both during and after the conduct of the surveys. All treatment was provided free of charge and participation was voluntary.

Results

The surveys were carried out May 20 to 25, 2012 in Boffa and June 16 to 20, 2012 in Forécariah (Figure 2). In total, 851 households were visited in Boffa. Of these, 775 (91.1%) were included in the survey, 45 households (5.3%) remained empty after two visits, 3 households (0.4%) refused to participate and 23 (2.7%) were not residents of Boffa. All 180 visited households were included in Forécariah. Overall, 3,993 individuals were included in Boffa and 1,255 in Forécariah (Figure 3). The median age of participants was 15 years (inter-quartile-range (IQR): 5–30). There were fewer males than females in the survey sample (47.6% and 44.1% males in Boffa and Forécariah respectively).

Oral Cholera Vaccine Coverage

Vaccination card retention was higher for children (81.7%) than adults (74.8%), and higher for females (82.4%) than males (73.2%).

Overall, 89.4% [95%CI: 86.4–91.8%] and 87.7% [95%CI: 84.2–90.6%] were vaccinated during the first round and 79.8% [95%CI: 75.6–83.4%] and 82.9% [95%CI: 76.6–87.7%] during the second round in Boffa and Forécariah respectively. The two dose (fully vaccinated) vaccine coverage (including card and oral reporting) was 75.8% [95%CI: 71.2–79.9%, deff = 10.1] in Boffa and 75.9% [95%CI: 69.8–80.9%, deff = 5.0] in Forécariah. Considering incomplete vaccination, 93.3% [95%CI: 91.1–95.0%, deff = 5.9] received at least one dose in Boffa and 94.9% [95%CI: 91.8–96.9%, deff = 3.7] in Forécariah. The dropout rate between the first and second dose was 15.2% [95%CI: 12.2–18.7%] and 13.6% [95%CI: 9.7–18.7%] in each site respectively. Vaccine coverage was lowest among adults in both prefectures (Figure 4).

Vaccine coverage with two doses was similar among females and males in Boffa (76.6% [95%CI: 71.9–80.7%] vs. 75.0% [95%CI: 69.8–79.4%]), but higher among females in Forécariah (79.4% [95%CI: 74.4–83.6%] vs. 71.4% [95%CI: 63.3–78.3%]). Vaccine coverage among women in childbearing age (15–49 years old) was statistically higher than among men of same age in Forécariah (72.6% [95%CI: 65.4–78.8%] vs. 53.4% [95%CI: 41.6–64.8%], $p<0.001$), but not in Boffa (70.1% [95%CI: 63.8–75.7%] vs. 64.3% [95%CI: 56.1–71.7%], $p = 0.1$). No major differences were observed in vaccination coverage by sub-prefecture (Table 1).

Regarding the awareness campaign, 95.7% of survey participants [95%CI: 94.2–96.8%] reported being aware of the campaign. Among individuals not vaccinated, the main reason was "absence during the campaign" for both the first and second rounds. The second most reported reason was "not having time to go for the vaccination" and the third, "sick during the campaign" (Table 2).AEFI was reported as the reason for non-vaccination by 0.9% of non-vaccinated individuals during the second round. A small percentage of participants considered that the vaccine made them feel sick (3.9% [95%CI 2.4–4.7%]). A large proportion of participants reported that the taste of the vaccine was bad (77.6% [95%CI 69.5–84.1%]). Among those vaccinated 1.4% [95%CI: 0.8–2.2%] reported spitting out or vomiting the vaccine. However, 98.9% [95%CI 97.8–99.5%] reported that they would be vaccinated again in a future cholera campaign.

Surveillance of Adverse Events following Immunization

Overall, 48 patients (15 per 100,000 vaccinated) spontaneously reported symptoms that were linked with the vaccine by the health personnel and considered as AEFI with 35 (20 per 100,000 vaccinated) after the first round and 13 (9 per 100,000 vaccinated) after the second round. In total, 29 were women (60%) and the median age was 2**7** years (IQR: 16–36 years); 8 (17%) were children 1 to 4 years. Seven patients reported having a history of allergies (15%). The cause of the allergy was specified for two patients (quinine and chloroquine). The average delay between vaccination and symptom onset was 24 hours with a median delay of 7 hours (IQR: 1–24 hours). One quarter reported the symptoms in the following hour after vaccination. Symptoms reported (n = 139) were mainly gastro-intestinal: 28 (20%) diarrhea, 22 (16%) vomiting, 14 (10%) stomachache and 12 (9%) nausea. In addition, 15 patients (11%) reported fever and general weakness. No patient was transferred to a hospital and no deaths were reported.

Discussion

The high coverage and good acceptability of the campaigns, conducted in a rural mobile population in Guinea, is encouraging. The percentage of people reporting AEFIs was low and almost all participants reported that they would be vaccinated in a future campaign. However, more evidence is needed about the feasibility of reactive campaigns from densely populated urban scenarios where cholera burden is high and cholera outbreaks evolve faster [16–20]. Also the acceptability of target campaigns in such a context should be assessed from a political, public health and community point of view. Determining the short-term protection given by the first dose is a clear priority as an effective one-dose regimen would facilitate the ease and timeliness of reactive campaigns in all contexts.

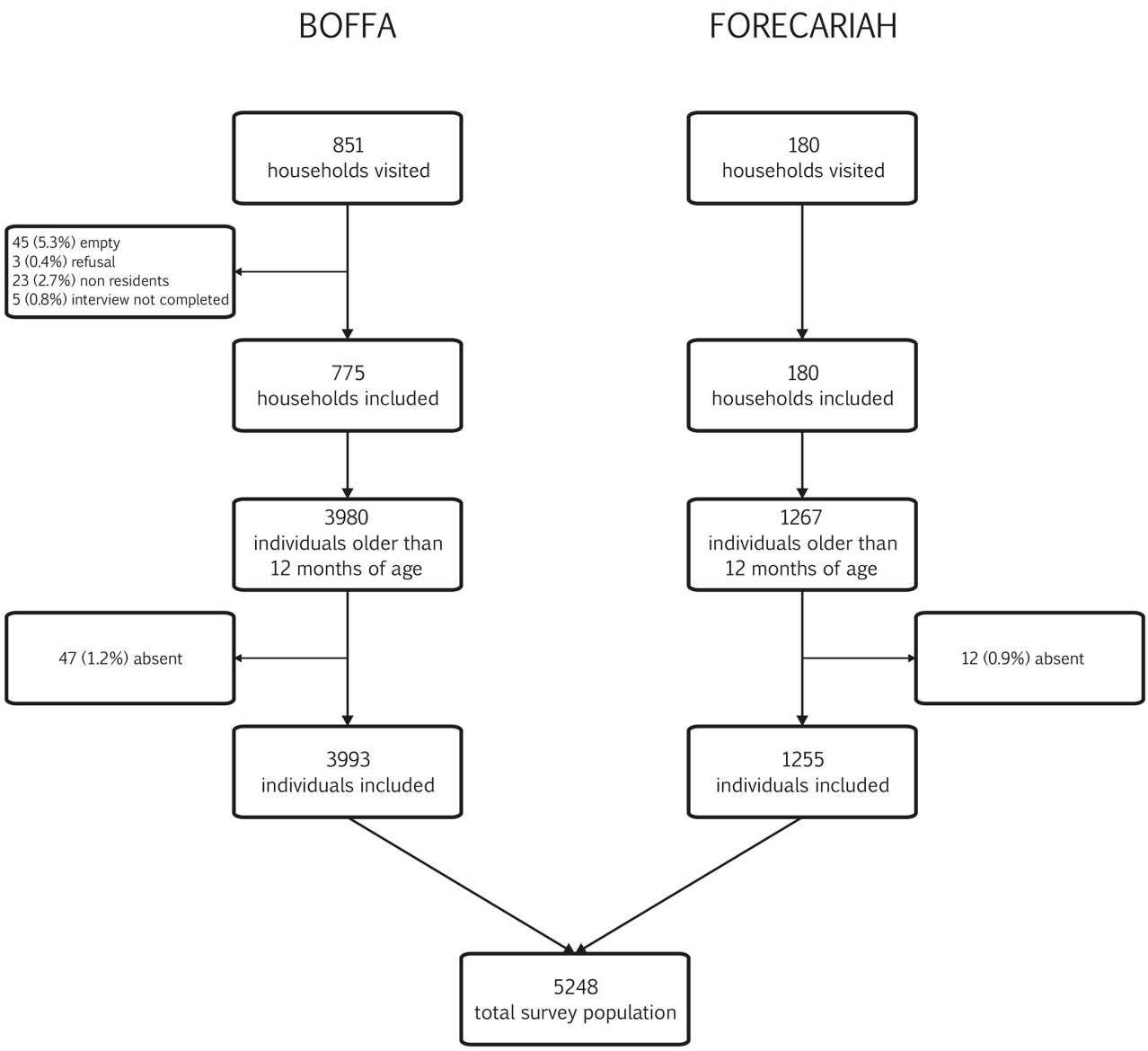

Figure 3. Study flow chart: Number of households visited, number of households included, number of individuals in the targeted age group (older than 12 months of age) residing in the households included in the survey and final number of individuals included in the study.
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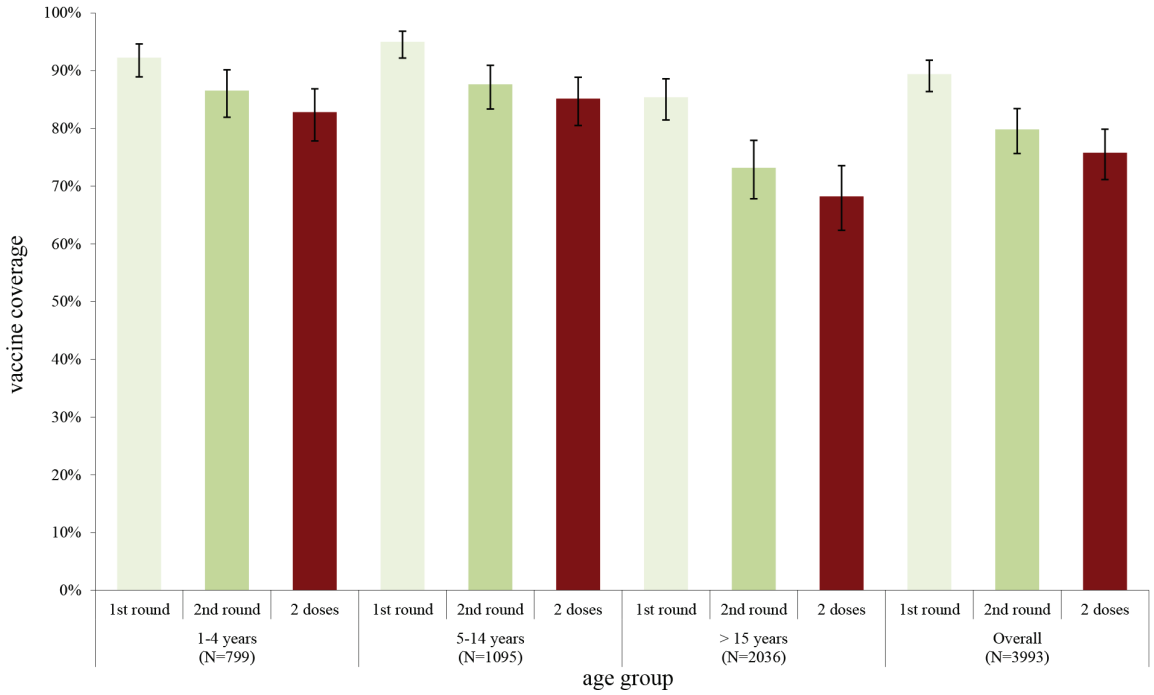
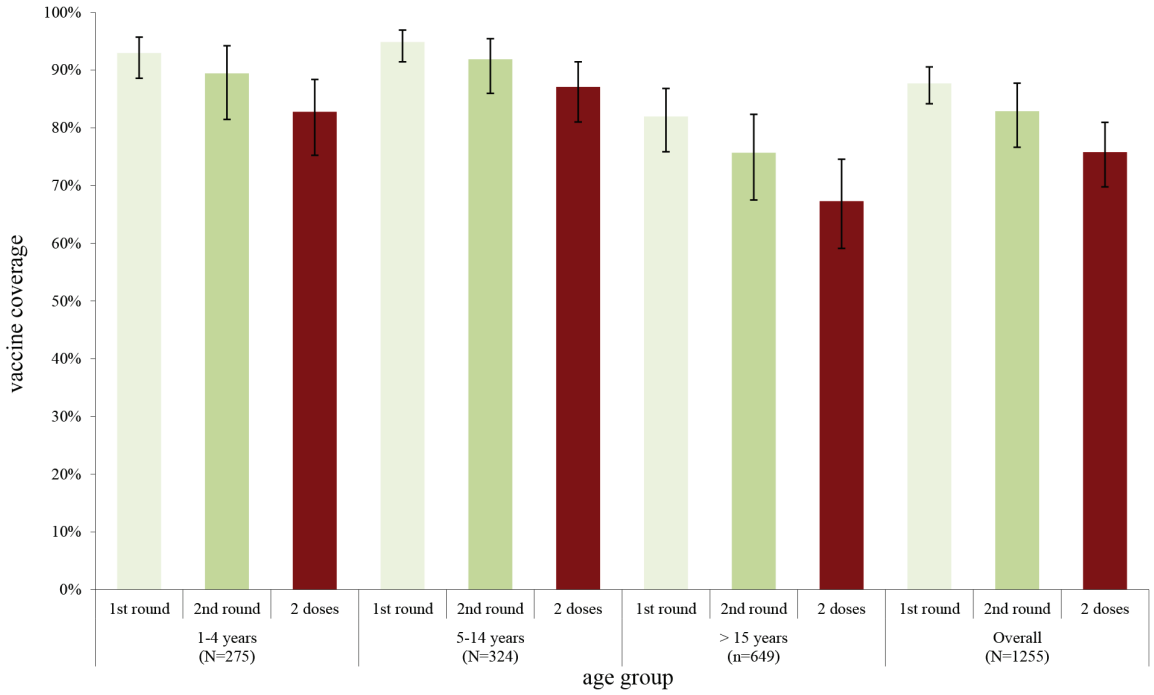
There are several key limitations of note. Despite the short time span between the vaccination campaign and the data collection for the surveys, we were not able to card-confirm vaccination status for 25% of participants and as a result some information bias may be present. Considering those individuals as not-vaccinated (worst-case scenario), two-dose coverage would decrease to 61% in Boffa and 64% in Forécariah. Second, the precision of estimates was better than expected because the number of participants recruited was higher (linked with the household size composition) than originally planned. However, population estimates in the surveyed areas are likely to be inaccurate. In most areas, no major differences were observed between administrative and survey coverage, but in Kaback an important deviation was observed. Inaccuracies in the population data could have caused some imbalances in the allocations of clusters; as described, we tried to avoid this problem using spatial sampling in Kaback.

An additional limitation concerns the use of a quantitative approach to explore campaign acceptability. Although reasons for non-vaccination were specifically collected using an open question, we cannot exclude the possibility that the population may not have understood certain awareness and education messages. A qualitative assessment would aid in understanding better reasons for non-vaccination, elucidate possible solutions and provide a better understanding of the perception of the vaccination campaigns by the population.

There are few examples where OCVs have been used as public health tools. Dukoral was used pre-emptively in refugee camps in Uganda and Darfur [21,22] and in endemic areas (Zanzibar and



A)



B)

Figure 4. Vaccine coverage by age group of the cholera mass vaccination campaign in Boffa (panel A) and Forécariah (panel B) prefectures, first round, second round and two doses (fully vaccinated), April–June 2012.
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Table 1. Vaccine coverage by sub-prefecture of the cholera mass vaccination campaign in Boffa and Forécariah prefectures, first round, second round and two doses (fully vaccinated), April–June 2012.

	First round		Second round		Full coverage (two doses)	
	n/N (%)*	[95% CI]	n/N (%)*	[95% CI]	n/N (%)*	[95% CI]
Boffa prefecture						
Boffa (n = 850)	773/847 (91)	[82–96]	692/847 (82)	[74–89]	655/847 (78)	[68–86]
Douprou (n = 535)	477/534 (88)	[81–93]	428/534 (79)	[70–86]	411/534 (76)	[67–83]
Koba (n = 957)	835/949 (88)	[83–92]	672/947 (71)	[62–80]	645/946 (69)	[59–77]
Mankountan (n = 577)	535/577 (93)	[88–96]	506/577 (89)	[82–93]	484/577 (84)	[76–90]
Tamita (n = 203)	190/203 (93)	[85–97]	165/202 (80)	[71–87]	160/202 (78)	[66–86]
Tougnifili (n = 811)	725/811 (88)	[77–94]	676/811 (83)	[73–89]	636/811 (77)	[64–86]
Forécariah prefecture						
Kaback (n = 754)	657/744 (87)	[84–90]	605/744 (80)	[72–86]	565/744 (74)	[67–81]
Kakossa (n = 501)	447/501 (88)	[80–93]	451/501 (88)	[76–93]	88/501 (78)	[68–86]

*The vaccine coverage estimates were weighted considering the study design and the confidences intervals were adjusted by the design effect.
doi:10.1371/journal.pntd.0002465.t001

Mozambique) [23,24]. Shanchol has been recently used in Haiti in a pilot campaign [25]. To our knowledge there are only two published examples of reactive campaigns using OCV, and both were conducted in Asia [26,27] using vaccines not prequalified by the WHO. The coverage and acceptability of these campaigns varied depending on the setting and the approach (pre-emptive vs. reactive). High coverage was obtained in Uganda, Darfur and Micronesia [21,22,26] and lower coverage was obtained in

Table 2. Reason for non-vaccination among individuals not vaccinated, Boffa and Forécariah prefectures, April–June 2012.

	1st round		2nd round	
	N = 521		N = 952	
Reason	**n**	**%**	**n**	**%**
Impossibility to go to the vaccination site				
Absent during the campaign	411	78.89	672	70.59
The person did not have the time to be vaccinated	30	5.76	81	8.51
Sick during the campaign	24	4.61	42	4.41
The person was hospitalized at the time of vaccination	3	0.58	3	0.32
Lack of information				
Not informed about the campaign	17	3.26	28	2.94
The person did not know the date of the campaign	3	0.58	26	2.73
The person did not know the place of vaccination	1	0.19	2	0.21
The caregiver thought that the child was too young	8	1.54	8	0.84
The person thought that he/she was too old	4	0.77	4	0.42
The person thought that one dose was enough	0	0.00	2	0.21
Logistic constraints				
Vaccination site considered too far	3	0.58	5	0.53
No vaccines available at the vaccination site	0	0.00	8	0.84
Waiting time too long	0	0.00	8	0.84
Refusals				
Cultural beliefs	1	0.19	1	0.11
Bad experience with previous vaccinations	1	0.19	8	0.84
Adverse events during the first round	0	0.00	8	0.85
The vaccine was considered dangerous	0	0.00	1	0.11
Other	11	2.11	34	3.57
No explanation	4	0.77	11	1.16

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Mozambique, Zanzibar and Vietnam [23,24,27]. In Guinea we obtained 76% coverage for two doses and 93% of the population received at least one dose, which represents, to our knowledge, one of the highest coverage reached [21–24,26,27]. The high coverage obtained is a promising outcome considering that this was one of the largest campaigns conducted in terms of number of doses administered, the specificities of the population (rural and mobile), and the short time available for preparation of the campaign, which has been one of the major arguments against outbreak response with OCV. There are several factors that likely influenced the population to participate in the campaign: first, the campaign was conducted in response to an outbreak and the possibility of even partial protection against a frightening disease was motivating. Second, the population may have been reassured by the involvement of the MoH, public health authorities and MSF; as an example, the vaccination campaign was inaugurated in Boffa with the presence of the Minister of Health. This involvement was also crucial to mobilize human resources and to organize the campaign considering the local specificities. Finally, both the awareness campaign and the vaccination strategy itself (decentralized with sites organized in each village or settlement) involved the communities. This aimed to ensure awareness and provide vaccination opportunities to remote places and difficult to reach population which likely contributed to this high coverage. Vaccination activities started early in the morning and finished late in the afternoon to maximize the opportunities for workers in the main fishing ports. Despite these efforts, the lowest coverage was obtained in adult males.

Significant differences where observed by sex in Forécariah, especially in individuals between 15–49 years old. The vaccination campaign in Forécariah coincided with an intense period in agriculture activities, which was a barrier for the participation in the campaign, especially for the male adults. In addition, the Red Cross Society of Guinea distributed soap and a bottle of chlorine solution to women of childbearing age in Forécariah during the second round of vaccination, which likely increased the coverage in this group. Distribution of soap and chlorine was one of the control measures implemented by the MoH in response to the outbreak in the affected places, but this activity was successfully integrated in Forécariah within the vaccination sites. This suggests that synergies among different preventive approaches is an element to consider in future campaigns both to provide a more comprehensive message on cholera prevention and to improve the vaccine coverage itself.

The number of AEFI reported through the surveillance system was low, without severe AEFI reported. Only a small proportion of non-vaccinated individuals during the second round of vaccination reported AEFI as a cause of non-vaccination. This result is coherent with previous publications on vaccine safety where mild symptoms (mostly not requiring medical attention) have been reported [28,29]. The proportion of vaccinated individuals reporting AEFIs was lower in our study than in the cluster randomized clinical trial conducted in Kolkata (15 vs. 76 per 100,000) [28]. This difference is probably explained by: first, our surveillance system was passive compared with the active case finding implemented in Kolkata; and second, access to health care was likely more difficult in the vaccinated area in Guinea (remote rural area) than in the urban context of Kolkata.

With respect to the proportion of vaccinees vomiting or spitting out the vaccine after intake, we found a higher percentage than previously documented with Dukoral (no data available for Shanchol) [23]. For administration of Dukoral, the vaccine has to be diluted in water containing a buffer solution. Although administration with water is not necessary for Shanchol, we offered water after vaccine intake. Most vaccinated individuals did not like the taste of the vaccine and offering water may have contributed to fewer incomplete vaccine courses. Additional information should be collected in future campaigns using Shanchol, considering that providing water considerably increased the logistic complexity of the campaign.

In order to facilitate the use of OCV as an additional tool, WHO and partners are in the process of creating a vaccine stockpile dedicated to outbreak response [30]. Here, we showed that high coverage can be reached within a few weeks, even in rural areas, and that the campaigns were well accepted by the population. Good documentation of these interventions is essential to elucidate the strategies leading to successful outcomes as well as key implementation barriers. Synergies between different axes in cholera control interventions should be pursued and other examples of integrated cholera response than the one presented here should serve also to determine the best use of vaccines for cholera prevention and control.

Supporting Information

Checklist S1 STROBE checklist.
(PDF)

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Author Contributions

Conceived and designed the experiments: FJL LG IC KS BT MH AAD CI MS DL RFG. Performed the experiments: FJL LG IC KS. Analyzed the data: FJL LG RFG. Contributed reagents/materials/analysis tools: FJL LG IC. Wrote the paper: FJL LG IC KS BT MH AAD CI MS DL RFG.

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PLOS | NEGLECTED TROPICAL DISEASES

Use of a Cholera Rapid Diagnostic Test during a Mass Vaccination Campaign in Response to an Epidemic in Guinea, 2012

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Abstract

Background: During the 2012 cholera outbreak in the Republic of Guinea, the Ministry of Health, supported by Médecins Sans Frontières - Operational Center Geneva, used the oral cholera vaccine Shanchol as a part of the emergency response. The rapid diagnostic test (RDT) Crystal VC, widely used during outbreaks, detects lipopolysaccharide antigens of *Vibrio cholerae* O1 and O139, both included in Shanchol. In the context of reactive use of a whole-cell cholera vaccine in a region where cholera cases have been reported, it is essential to know what proportion of vaccinated individuals would be reactive to the RDT and for how long after vaccination.

Methodology/Principal Findings: A total of 108 vaccinated individuals, selected systematically among all persons older than one year, were included at vaccination sites and 106 were included in the analysis. Stools samples of this cohort of vaccinated participants were collected and tested with the RDT every day until the test was negative for two consecutive visits or for a maximum of 7 days. A total of 94.3% of cholera vaccine recipients had a positive test after vaccination; all except one of these positive results were reactive only with the O139 antigen. The mean time to become negative in those with an initial positive result after vaccination was 3.8 days, standard deviation 1.1 days.

Conclusions/Significance: The RDT Crystal VC becomes positive in persons recently vaccinated against cholera, although almost exclusively to the O139 antigen. This reactivity largely disappeared within five days after vaccination. These results suggest that the test can be used normally as soon as 24 hours after vaccination in a context of O1 epidemics, which represent the vast majority of cases, and after a period of five days in areas where *V. cholerae* O139 is present. The reason why only O139 test line became positive remains to be investigated.

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Introduction

Cholera is an acute diarrhoeal infection caused by ingestion of the bacterium *Vibrio cholerae*. Two serogroups– O1 and O139 – are responsible for cholera epidemics. While *V. cholerae* O1 causes the majority of outbreaks over the world, O139 – first identified in Bangladesh in 1992 – is confined to South-East Asia [1], where its incidence has declined over the years [2]. Globally, O139 accounts for a small minority of cholera cases [3], and local transmission has never been reported in Africa or America. Rapid identification of initial cases of cholera in the early phase of an epidemic is critical for implementation of a timely public health response [4] to control the spread and duration of the outbreak. Currently, cholera diagnosis relies on the microbiological identification of the pathogen by stool culture, which remains the gold standard to confirm the diagnosis [5]. However, this procedure requires laboratory infrastructure, adequate transport procedures and trained staff [5]. As rapid diagnostic tests (RDT) require less time, a minimum laboratory infrastructure and basic technical skills, they are used to confirm cholera outbreaks in places where high laboratory standards are difficult to obtain [6].

In 2003, the Institut Pasteur developed a cholera RDT based on the qualitative detection of lipopolysaccharide (LPS) antigen of both *Vibrio cholerae* O1 and O139 serogroups from stool specimens. This test uses one-step, vertical-flow immunochromatography principle and monoclonal antibodies against the core and O-specific polysaccharides of each serogroup for capture and detection of antigens [7,8]. The O1 specific antigenic determinant is common to Ogawa and Inaba serotypes [8,9] and the one for O139 is common to both O139 capsular polysaccharide and LPS.

Author Summary

The rapid diagnostic test (RDT) Crystal VC detects lipopolysaccharide antigens from V. *cholerae* O1 and O139 in stool samples, which are also present in the oral cholera vaccine Shanchol. It is important to take into consideration the possibility of a positive result to the RDT due to vaccination and not to cholera in recently vaccinated individuals. During a large mass cholera vaccination campaign in Kabak (Guinea) in 2012, we conducted a study to estimate the proportion of positive results to the RDT in recipients of the oral cholera vaccine at different time points after vaccination. The results of this study show that ingestion of the cholera vaccine led to a positive RDT, although almost exclusively to the O139 antigen, in the majority of vaccinated people. From the fifth day after vaccination, only a small minority of vaccinated individuals remained positive for the RDT and none of the specimens tested the seventh day of follow-up were positive. Our findings provide the first data on the use of the RDT Crystal VC in vaccinated people. This test should be used carefully during the first week after reactive mass oral cholera vaccination campaigns in areas where *V. cholerae* O139 is present.

This cross-reactivity between O139 LPS and capsular polysaccharide explains that antibodies react with both encapsulated and non-encapsulated *V. cholerae* O139 strains [10]. The RDT is produced by Span Diagnostics (Surat, India) under the trade name Crystal VC [5]. Several evaluations have shown good sensitivity, ranging from 92% to 100% [7,11–12]. In contrast, the specificity was lower and most evaluations in field conditions have shown specificities from 71% to 77% when compared with culture as the gold standard [4,11–13]. Nevertheless, the use of culture as gold standard may underestimate specificity, and re-analysis of the data using statistical methods for evaluation with an imperfect gold standard showed that the specificity could be around 85% [14]. After these evaluations, the manufacturer SPAN changed the test presentation (order of the lines and addition of a dilution buffer), but the test in this new version has not been formally evaluated. This test is widely used for epidemiological purposes during outbreaks.

In 2012, the Republic of Guinea faced an O1 cholera epidemic, with the first cases notified in the prefecture of Forécariah in February. In light of the ongoing cholera epidemic and the 2009 World Health Organization (WHO) recommendations calling for the consideration of oral cholera vaccines as a part of the epidemic response [15], the Ministry of Health and Public Hygiene (MHPH) of Guinea supported by Médecins Sans Frontières – Operational Center Geneva (MSF-OCG), implemented a vaccination campaign in the prefectures of Boffa and Forécariah. The vaccine Shanchol (Shantha Biotechnics, India), prequalified by the WHO, contains killed bacteria *V. cholerae* O1 and O139 and, given in two doses 14 days apart, provides nearly 70% protection for at least 2 years after vaccination [16]. A total of 7,531 cases including 138 deaths (case fatality ratio of 1.8%) were reported to the MHPH of Guinea between the beginning of the epidemic and its end, which was declared on 6 February 2013, after six consecutive weeks without any new case notification [17].

Given that the RDT Crystal VC detects the LPS antigens of *V. cholerae* O1 and O139 in feces, which are also contained in the oral vaccine Shanchol, we hypothesized that the stools of vaccinated individuals could become positive by the rapid test due to the vaccine only, in the absence of viable bacteria. In a reactive campaign during an outbreak, positive test results due to the vaccine could interfere with the use of the tests in suspected cholera cases. The aim of this study was to estimate the proportion of positive results of the test Crystal VC in recipients of the cholera vaccine Shanchol at different time points after vaccination and the mean time to become negative (in those with an initial positive result for O1 or O139) after vaccination.

Methods

Ethics statement

The study protocol was approved by the Ethical Review Board (ERB) of Guinea and the MSF ERB. Written informed consent was obtained from adults or from the guardians of participants less than 18 years of age. Privacy and confidentiality in the data collected from the participants were ensured both during and after the conduct of the study.

Setting, population and study design

The study took place in Kabak (Forécariah Prefecture, Guinea) during the second round of the mass vaccination campaign carried out by the MHPH/MSF in June 2012. The study population corresponded to the population targeted by the vaccination campaign (all residents of Kabak aged one year and above). Individuals were included if they were vaccinated and accepted to participate. They were excluded if they had watery diarrhea on inclusion (to exclude potential cholera cases) and/or a high probability of not being present for all the follow-up visits. The cohort of vaccinated participants meeting study criteria was followed-up prospectively.

We estimated that 96 individuals were needed to achieve a minimum precision of 10% around a proportion of 50% of positive RDT, as there were no data on the prevalence of positive tests in the vaccinated population. We increased the sample size to 106 to account for an expected 10% of loss to follow-up. A systematic sampling method (one every 10 individual) was used in every vaccination site.

Recruitment and follow-up procedures

Participants were recruited in 4 of the 31 vaccination sites, selected arbitrarily, as vaccination sites were not thought to have any influence on the study outcomes. Demographic information was collected at inclusion through a face-to-face interview (mainly in Soussou, the local language) and information on stool production and basic clinical symptoms during follow-up visits using an individual standardized case report form (CRF). Participants were asked to collect stool in a pot provided by the study team. Participants' homes were visited daily to collect stool specimens, complete a follow-up form and to provide them with a new pot for the next stool. We transported the stools to the laboratory and tested them with the RDT. Laboratory technicians completed the information with the RDT results. Follow-up was considered finalized when 2 consecutive negative RDT results were obtained or after 7 days.

Field use of the rapid diagnostic test

The stool samples were tested with the RDT at Kabak Health Center following the manufacturer's instructions by a laboratory technician trained to the use of the test. Crystal VC tests used were manufactured in 2011 and 2012 by Span Diagnostics Ltd., India (catalogue reference number 161C101-10). A small portion of stool was mixed with a buffer and 200 µL (4 drops) of the mix was placed in a test tube. The dipstick test was left in the tube for 20 minutes before reading. If only the control line appeared, the

test was negative. If 2 or 3 lines appeared, the test was positive for either *V. cholerae* O139, O1, or both. If the control line was absent, the test was considered invalid and repeated once.

Laboratory control of the rapid diagnostic test

Ten by ten dilutions of the Shanchol vaccine were prepared using the dilution buffer provided in the RDT kit. Undiluted and diluted vaccine solutions up to a 10^9-fold dilution were tested with the RDT following the manufacturer's recommendations.

A bacterial suspension adjusted to an optical density at 600 nm ($OD_{600\ nm}$) of 0.8 was prepared in the dilution buffer provided in the RDT kit from an overnight culture of *V. cholerae* O1 and O139 strains. Such an OD value was previously estimated to correspond to 2×10^8 *V. cholerae*/mL by colony counting of 10-fold serial dilutions spread on agar plates and incubated over night at 37°C. This initial solution was used to prepare solutions at 2×10^7 and 2×10^6 bacteria/mL using the dilution buffer provided in the kit, undiluted and diluted solutions were tested with the RDT following the manufacturer's recommendations.

Data analysis

Qualitative variables were described through their frequency and percentages. Continuous variables were described through their mean, median, standard deviation (SD) and percentiles (P_{25} and P_{75}). We calculated the proportion of positive results for O1 or O139 for each day of follow-up including in the numerator the number of positive results and in the denominator the sum of the total number of tests performed and the number of cases for whom follow-up was stopped after obtaining two consecutive negative results. Missing data (absent or no stool sample) were excluded from this calculation. The 95% exact confidence intervals (95%CI) of the proportion estimate were calculated. To estimate the mean time to obtain a negative RDT result after vaccination (time to become negative) we counted the number of days needed to obtain a first negative result in the group of people who obtained previously a positive result for O1 or O139 after vaccination. Statistically significant differences by gender and age were assessed with a linear regression model. A p value$<$0.05 was considered significant.

Data were entered in an EpiData version 3.1 database (EpiData, Odense, Denmark) and analyzed using Stata version 11 (StataCorp, College Station, Texas, USA).

Results

Recruitment and follow-up

A total of 108 individuals were recruited during 2 days in 4 vaccination sites. Two individuals were excluded from the analysis (one was absent during all follow-up visits and for the other, follow-up was stopped accidentally by the study team).

Follow-up of the remaining 106 participants is described in Figure 1. Participants were followed for a median time of 5 days (minimum of 2 and 7 as maximum). Almost half of them (49.1%) were followed for 4 (23.6%) or 5 days (26.4%).

Participant's characteristics, symptoms and delay in stool collection and testing

Among the 106 participants, 79.2% (84) were females and the median age was 25 years (P_{25}-P_{75} = 2–80). The majority of participants were older than 15 (84.8%) and the proportion of children under five was 5.7%.

In total, 18 participants declared having diarrhea during follow-up, and two reported vomiting. Other symptoms such as constipation, stomachache or headache were declared by 37 participants.

The average delay was 3.9 hours (SD = 4.4) between stool production and collection and 6.6 hours (SD = 5.9) between stool collection and performance of the RDT (including collection and transport of samples to the laboratory) by the laboratory technicians. As a result, there was an average delay of 10.5 hours (SD = 6.6) between stool production and performance of the RDT.

Proportion of positive tests after vaccination

Of the 106 participants, 100 (94.3%) became positive with the O139 line after vaccination and 6 never had a positive result. On the first day of follow-up (day 1) 71.1% were positive. On day 3, almost half of the tests remained positive (49.5%) and on day 5 and 6 this percentage decreased below 3% (Table 1).

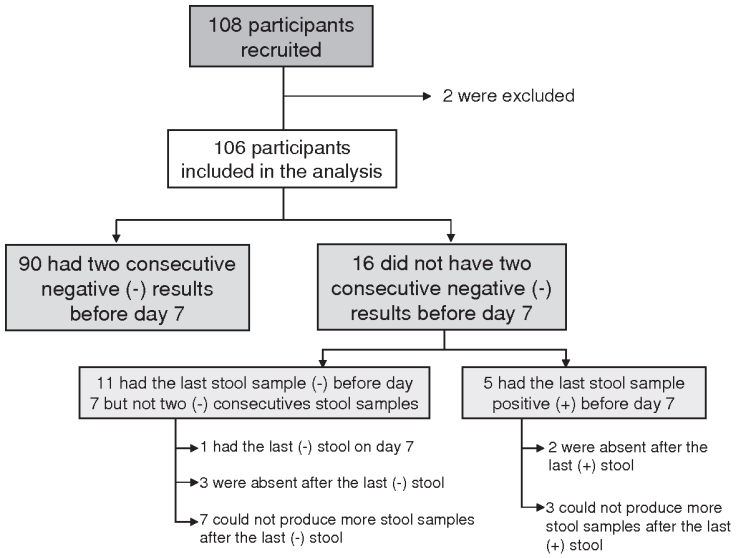

Figure 1. Study participants, exclusions and follow-up results, Kabak, 2012.
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Table 1. Rapid diagnostic test results in vaccinated participants by day of follow-up, Kabak, 2012.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day7
A. Tests performed	97	97	90	76	46	23	6
a.1. Positive result (+)	69	80	47	20	2	1	0
a.2. Negative result (−)	28	17	43	56	44	22	6
B. Follow-up stopped after 2(−)	0	0	5	17	42	67	85
C. Absent	1	0	0	1	1	4	5
D. No sample available	8	9	11	12	17	12	10
Total[1]	106	106	106	106	106	106	106
Proportion[2] of positives (%)	71.1	82.5	49.5	21.5	2.3	1.1	0.0
95%CI of the proportion	61.5–79.9	73.4–89.4	39.1–59.9	13.7–31.2	0.3–8.1	0.0–6.0	0.0–4.0[3]

[1]The total is the sum of A+B+C+D.
[2]The proportion is the result of the formula (a.1/(A+B))*100.
[3]97.5% Confidence Interval, one-sided.
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Only one participant became positive with the O1 line (together with the O139 line) on the first day of monitoring, and both lines became negative subsequently.

Time to become negative

Of the 100 participants with at least one positive result, five could not be tested on day 7 as they were absent or did not produce stools, although they had a positive result with their last specimen collected (Figure 1). Among these 5 participants, 3 had their last positive stool on day 3, 1 on day 4 and 1 on day 5. For the remaining 95 cases with O139 positive tests, we calculated the time to become negative after vaccination.

For all participants, the mean time to become negative after vaccination was 3.8 days (SD = 1.1) and the median time was 4 days (P_{25}-P_{75} = 3–5). For males, the mean time to become negative after vaccination was 4.3 days (SD = 1.4) and 3.6 (SD = 1) for females (p = 0.03), with a median of 4 days for both males and females. A linear regression model showed that a longer time to become negative was associated to an older age (p = 0.002) and to male sex (p = 0.012) (Table 2).

Laboratory testing of the rapid diagnostic test

The Crystal VC RDT gave positive results for both O1 and O139 when the strip was inserted directly into the vaccine solution prior to ingestion, and remained positive up to 10^4-fold dilutions of the vaccine. At a 10^5-fold dilution, only the O139 line remained positive and none of them were positive at higher dilutions (Table 3).

Table 2. Linear regression model of time to become negative by age and sex, Kabak, 2012.

	Coefficient	95% Confidence Interval	p
Age[1]	0.020	(0.008–0.032)	0.002
Sex[2]	0.669	(0.153–1.186)	0.012

[1]The coefficient shows the increase in days in the time to become negative per year of age.
[2]The coefficient shows the increase in days in the time to become negative for males compared to females.
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The RDT gave a positive signal with the O1 test line at bacterial concentration of 2×10^8 and 2×10^7, but was negative at 2×10^6 bacteria/mL, while all dilutions of *V. cholerae* O139 culture tested down to 2×10^6 bacteria/mL were positive for the O139 line (Table 3).

Discussion

To our knowledge, this is the first study showing that healthy individuals vaccinated with the oral vaccine Shanchol become positive with the cholera rapid test Crystal VC in the first days following vaccination. The proportion of vaccinated individuals

Table 3. Rapid diagnostic test results performed in vaccine and bacterial suspension dilutions, Pasteur Institute, 2012.

	Control line	Line T1 O139	Line T2 O1
Vaccine dilutions			
Tube 1 (10-fold dilution)	+++	+++	+++
Tube 2 (10^2-fold dilution)	+++	+++	+++
Tube 3 (10^3-fold dilution)	+++	+++	++
Tube 4 (10^4-fold dilution)	+++	++	+
Tube 5 (10^5-fold dilution)	+++	+	−
Tube 6 (10^6-fold dilution)	+++	−	−
Tube 7 (10^7-fold dilution)	+++	−	−
Tube 8 (10^8-fold dilution)	+++	−	−
Tube 9 (10^9-fold dilution)	+++	−	−
O1 and O139 strains dilutions	+++		
O1 - Tube 1 (2×10^8bacteria/mL)	+++	−	+++
O1 - Tube 2 (2×10^7 bacteria/mL)	+++	−	++
O1 - Tube 3 (2×10^6 bacteria/mL)	+++	−	−
O139 - Tube 1 (2×10^8 bacteria/mL)	+++	+++	−
O139 - Tube 2 (2×10^7 bacteria/mL)	+++	+++	−
O139 - Tube 3 (2×10^6 bacteria/mL)	+++	++	−

Intensity of the positive line: (+) very weak positive; (++) weak positive; (+++) positive.
Negative result: (−).
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positive for the Crystal VC test after vaccination was high (94.3%) for the O139 component of the test, but low with the O1 component. This proportion of O139-positive tests decreased rapidly to half on the third day after vaccination and to one-fifth on the fourth day of follow-up. The median duration required to have a negative result for those cases presenting a previous positive test was 4 days.

Almost all positive tests (except for one) were positive only for O139 line, despite the fact that the Shanchol vaccines contains the two strains *V. cholerae* O1 and O139, with a higher amount of O1 (1500 Elisa units of *V. cholerae* O1 LPS and 600 Elisa units of *V. cholerae* O139 LPS for a dose of 1.5 mL) [18]. This could be due to a higher sensitivity of the RDT for the O139, as suggested by the results of sensitivity against bacterial cultures showing that the O139 line was reactive with higher bacterial dilutions than the O1 line. Such results were already reported by Nato et al. [7] when evaluating the initial version of the RDT, but are in contradiction with those observed by Mukherjee et al. [13] with the first version of the Crystal VC test, which was reactive at 10^6 bacteria/mL for *V. cholerae* O1 and 10^7 bacterial/mL for *V. cholerae* O139. These differences of analytical sensitivity between the different versions of the RDT emphasize the need for a proper diagnostic performance evaluation of each new version of the test.

Including pre-vaccination stool status of our study population as well as unvaccinated participants could have provided useful information on the magnitude of potential false positive reactions due to factors unrelated to vaccination, i.e. non-specific reactions, which could have been expected considering the reported moderate specificity of the test [4,11–13], or positivity due to asymptomatic carriers. The sharp increase and subsequent decrease in the proportion of O139 positive tests after vaccination are not in favour of such assumptions and suggest that the positive results were due to the vaccine alone. Of the 75 tests done after day 5, only three (4%) were positive for O139, and overall only one test was positive for O1 which is lower than the number of false positives that could be expected based on the test specificity. However, it should be noted that this study was conducted in people without cholera symptoms while the previous evaluations were conducted in suspected cholera cases.

There are several limitations worth noting. First, women and adults were overrepresented in our study sample. Although women were more vaccinated than men were during the vaccination campaign carried out in Kabak, the proportion of women in our study (79.3%) was clearly higher than the vaccinated population (59.5%) [19]. This is likely due to the fact that the majority of men presented early at the vaccination site and were more likely to be excluded given their potential absence for work during the follow-up period. However, although there was a small difference in the mean time to become negative between men and women (4.3 days vs. 3.6), the median was the same for both sexes (4 days) thereby not likely affecting the results presented here. The median age in the study was 25 years compared to 15 for the vaccinated population [19]. Considering that the time to become negative was longer for the older participants, it is likely that we slightly overestimated the time to become negative. Nonetheless, the differences by age were small in magnitude (0.2 days per 10 years of age) and they do not change the interpretation of the results neither our recommendations regarding the use of the cholera RDT in vaccinated areas. Second, we could not conclude on five cases who had a positive result with their last specimen collected, and for whom further samples could not be collected because they were absent or unable to produce stool samples. When designing the study, we decided to limit the follow-up period to 7 days, based on the expected time for gastrointestinal transit of the killed bacteria. Although extending the follow-up of participants until they became negative for the rapid test would have been useful for concluding on these 5 individuals, we consider that this limit was reasonable in the absence of any other data. In addition, even if we consider that these five people were still positive at day 7, the percentage of positive tests would be still low (5.2%), lower than the expected for non-cholera cases considering the specificity of the test. Third, we did not perform culture to exclude participants with possible cholera or asymptomatic carriage of *V. cholerae*. Although initially planned in the protocol for participants with diarrhea or with a positive RDT at the end of follow-up (day 7), no culture was performed since symptoms were found unreliable and none of the specimens tested on the seventh day of follow-up were positive. Finally, specimens were tested on average ten hours after stool production and without the possibility of storage at 4°C due to the lack of electricity in Kabak. This delay seems reasonable given the difficulties to collect the samples immediately after production, although it is unclear the degree to which antigens degrade during this period, which could potentially affect the RDT results.

The results of the study confirm our hypothesis that the rapid test Crystal VC can become positive in persons recently vaccinated against cholera, although only with the O139 line, probably linked to its higher analytical sensitivity. However, tests become negative rapidly and five days after vaccination the proportion of positive tests among vaccinated is less than 3%. As the current global pandemic is almost exclusively caused by *Vibrio cholerae* O1, our results suggest that the current Crystal VC kit can be used normally as soon as 24 h after receiving Shanchol in a context of *V. cholerae* O1 epidemic, and after a period of five days in areas where *V. cholerae* O139 is present. Other cholera rapid diagnostic tests based on the LPS detection are available in the market [20] and could also become positive in recently vaccinated individuals. Thus, an evaluation of other tests or future versions of the Crystal VC test is recommended if they are to be used in the context of oral cholera vaccination campaigns. Finally, we strongly recommend that the diagnostic performances of the current modified version of the Crystal VC test be evaluated with respect to the different sensitivities of the O1 and O139 lines.

Supporting Information

Checklist S1 STROBE checklist.
(PDF)

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Author Contributions

Conceived and designed the experiments: IMP ALP FJL RFG IC KS SS MH MLQ. Performed the experiments: IMP ALP MLQ. Analyzed the data: IMP ALP FJL. Contributed reagents/materials/analysis tools: MH. Wrote the paper: IMP ALP . Contributed to critical revision of the protocol and the manuscript for important intellectual content: FJL MLQ IC RFG KS SS MH. Final approval of the version to be published: IMP FJL KS SS MH RFG IC MLQ ALP.

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Epidemic use of killed whole cell V. cholerae vaccine, Guinea, 2012

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Abstract:	Background: The use of cholera vaccines to prevent and control cholera is currently under debate. Shanchol (Shantha-Biotech, Hyderabad, India) is one of the two WHO prequalified oral cholera vaccines; however, vaccine effectiveness under field conditions and protection conferred in the first months after administration remains unknown. The main objective of this study was to estimate the short-term vaccine effectiveness of two doses of Shanchol used as a part of the integrated response to a cholera outbreak in Africa. Methods: We conducted a matched case-control study in the Republic of Guinea between May 20 and October 19, 2012. Suspected cholera cases were confirmed by rapid test, control subjects were selected among neighbors of the same age and sex as the case-patients. The odds of vaccination were compared between case-patients and control-subjects in bivariate and adjusted conditional logistic regression models. Vaccine effectiveness was calculated as 1-odds ratio per 100. Results: Overall, 40 case-patients and 160 control-subjects were included in the study for the primary analysis between June 8 and October 19,

	2012. Vaccination with two complete doses was associated with significant protection against cholera after adjustment for potentially confounding variables (86.6%; 95%confidence interval: 56.7%-95.8%; P value=0.001). Conclusion: This study shows the effectiveness of Shanchol when used in response to an outbreak in an African country, which supports the addition of vaccination as an outbreak response tool. This study supports also the on-going efforts to establish a cholera vaccine stockpile for emergency use, which would enhance current outbreak prevention and control strategies.

Epidemic use of killed whole cell *V. cholerae* vaccine, Guinea, 2012

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Abstract

Background: The use of cholera vaccines to prevent and control cholera is currently under debate. Shanchol (Shantha-Biotech, Hyderabad, India) is one of the two WHO prequalified oral cholera vaccines; however, vaccine effectiveness under field conditions and protection conferred in the first months after administration remains unknown. The main objective of this study was to estimate the short-term vaccine effectiveness of two doses of Shanchol used as a part of the integrated response to a cholera outbreak in Africa.

Methods: We conducted a matched case-control study in the Republic of Guinea between May 20 and October 19, 2012. Suspected cholera cases were confirmed by rapid test, control subjects were selected among neighbors of the same age and sex as the case-patients. The odds of vaccination were compared between case-patients and control-subjects in bivariate and adjusted conditional logistic regression models. Vaccine effectiveness was calculated as 1-odds ratio per 100.

Results: Overall, 40 case-patients and 160 control-subjects were included in the study for the primary analysis between June 8 and October 19, 2012. Vaccination with two complete doses was associated with significant protection against cholera after adjustment for potentially confounding variables (86.6%; 95%confidence interval: 56.7%-95.8%; P value=0.001).

Conclusion: This study shows the effectiveness of Shanchol when used in response to an outbreak in an African country, which supports the addition of vaccination as an outbreak response tool. This study supports also the on-going efforts to establish a cholera vaccine stockpile for emergency use, which would enhance current outbreak prevention and control strategies.

Recent large scale cholera outbreaks have shown the limits of traditional response strategies[1]. The devastating consequences of cholera epidemics in vulnerable populations have opened a debate about ways to improve preparedness and response plans. Two documents issued by the World Health Organization (WHO) in 2010 and 2011 stimulated debate: a revised position paper regarding oral cholera vaccines (OCV)[2] and the prequalification of Shanchol (Shantha Biotech, Hyderabad, India)[3].

The two OCV currently prequalified by the WHO are killed whole cell *V. cholerae* O1 vaccines; Shanchol contains in addition *V. cholerae* O139, and Dukoral (SBL Vaccine/Crucell, Sweden) contains a recombinant cholera toxin B-subunit. Dukoral showed 84% short-term protection (six months) under field conditions, and has been successfully used both in Asia and Africa[4,5]. Conversely, the effectiveness of Shanchol under field conditions needs to be determined as the efficacy of the vaccine has been only measured under experimental conditions in Kolkata, India[6]. Furthermore, as the trial was not designed to evaluate the short-term, but rather long-term protection (at two, three and five years), the protection offered by Shanchol within the first months after vaccination remains unknown[6–8].

Shanchol has important difference compared with Dukoral; its price is considerably lower (1.85 vs. 5.25 US$ per dose[9]), it does not require buffer and occupies lower storage volume[10], which reduces the logistic burden to implement mass vaccination campaigns. Evidence about the protection conferred by Shanchol in the first months after administration under field conditions is essential when considering its use for outbreak response. This is especially true at a time when WHO and its partners are in the process of creating a cholera vaccine stockpile for emergency use[11].

The results presented here estimates the effectiveness of Shanchol in an African country when used in response to an outbreak.

Methods

Study oversight

This study was funded by Médecins Sans Frontières (MSF). MSF had no role in study design, data collection or analysis. The authors assume full responsibility for the analyses and interpretation of the data. The study protocol was approved by the Ethical Review Boards of the Republic of Guinea (Guinea) and MSF. Vaccine and treatment were provided free of charge and participation in the study was voluntary. Written consent was obtained from participants or their parents/guardians. Adverse events following immunization (AEFI) were documented through passive surveillance during 14 days as described elsewhere[12].

Study setting

The 7th cholera pandemic spread to Guinea in 1970 with the largest outbreak to date observed in 1994 with more than 30,000 cases and 670 deaths reported nationwide. From 2003 to 2007, cholera outbreaks were reported in the capital Conakry and in the coastal zones during the rainy season (July to September). A new cholera outbreak was declared in Guinea in February 2012 (see the Supplementary Appendix). The National Microbiology Laboratory (Conakry, Guinea) confirmed the circulation of *V. cholerae* O1 El Tor. Outbreak response interventions were implemented, including free-of-charge medical treatment, as well as water, sanitation and hygiene promotion activities[13–15]. In addition, non-selective mass vaccination campaigns were implemented in the prefectures of Boffa and Forécariah following WHO recommendations[2]. In Boffa, the coastal part of the six sub-prefectures bordering the ocean (Koba, Boffa-centre, Douprou, Tougnifily, and part of Mankountan and Tamita) were vaccinated from April 18 to May 14, and in Forécariah, the sub-prefectures of Kaback and Kakossa were vaccinated from May 27 to June 15 (Supplementary Appendix, Figure S3). Details of the vaccination campaigns have been published elsewhere[12,16].

Surveillance for cholera

The surveillance system was reinforced in one prefectural hospital, eight health centers and 23 health posts between April 16 and October 31, 2012 in the vaccinated areas. The suspected cholera case definition (acute non-bloody watery diarrhea with more than three liquid stools in 24 hours) was standardized and a case-based prompt notification system with cholera specific registers was implemented in collaboration with the African Cholera Surveillance Network (Africhol). Medical staff was also trained in the use of a rapid diagnostic test (RDT) for cholera (Crystal-VC tests, SPAN Diagnostics, India, Lot numbers: 4000007832 and 4000008589).

Definition and Selection of Case-Patients

All suspected cholera cases seeking care in a health center of the study area between one week after the end of the vaccination campaigns and October 31, 2012, were eligible to be included as case-patients if they provided written informed consent and fulfilled the following criteria: resident in the study area since April 16, 2012; older than 12 months; a positive cholera RDT; and their residence could be located after discharge. Only the first episode of acute watery diarrhea was included. A flow chart explaining the inclusion of participants in the study is shown in Figure 1.

To assess whether effectiveness results could be attributed to bias, case-patients with non-choleric diarrhea (negative RDT result) were also compared with control-subjects that did not have diarrhea (indicator bias analysis)[4].

Definition and Selection of Control-subjects

A neighbor of the same sex and within the same age group (1- 4, 5- 9, 10-19, 20-29, 30-39, over 40 years of age) as the case-patient was eligible to be a control if: he or she had not sought treatment for diarrhea between January 1, 2012 and the date of onset of the matched case-patient's diarrheal illness; and if he or she would have sought treatment in a health center if severe, watery diarrhea had developed. Eligibility

for selection also required the same informed consent, residency and age criteria as those applied to the case-patients.

Four neighbor control-subjects were selected for each case-patient and included in the primary analysis and the indicator bias analysis (Figure 1). Beginning with the first household to the left of the case-patient and a sampling interval of three households, one control-subject was recruited per household until four control-subjects were recruited.

Ascertainment of Vaccination and Potentially Confounding Variables

Receipt of the cholera vaccine during the mass immunization program was ascertained in face-to-face home interviews. Participants were asked whether they had been vaccinated and, if so, to show the vaccination cards. For those who reported that they had been vaccinated but were not in possession of a card, vaccination status and the completeness of dose ingestion were recorded as orally reported by the individual. Clinical, demographic, socioeconomic, and environmental variables were ascertained through questionnaires.

Laboratory Procedures

For each patient included in the study, a stool sample was collected and used to perform the RDT. The doctor/nurse in charge of the health center performed the test and interpreted the result following manufacturer's instructions.

In addition, for those patients with a positive result to the RDT, a filter paper disc was dipped into fresh stool and placed into a microtube with 2-3 drops of normal saline solution (NaCl 0.9%). Tubes were kept at room temperature and sent to Institut Pasteur, Paris for isolation of *V. cholerae* according to standard methods[17]. PCR was systematically performed on all specimens. Detection of the *rfb* was done as described by Hoshino *et al.*[18]. Presence of PCR inhibitors and bacterial DNA were respectively controlled

by PCR amplification of an exogenous internal positive control (Applied Biosystems® TaqMan®) incorporated to each sample and amplification of the 16S rRNA gene.

Statistical Analysis

The primary analysis assessed the protection conferred by the receipt of two completely ingested doses of vaccine against confirmed cholera by RDT. We calculated that 90 cases and 360 controls (ratio 1:4) would be needed assuming 50% vaccine effectiveness, alpha error 5% and 80% power. The secondary analysis assessed the protection conferred by an incomplete course of vaccine (one complete dose or incomplete dose(s) due to spitting or vomiting part of a dose) against confirmed cholera by RDT. We also conducted a sub-analysis considering as case-patients: (i) those with presence of *V. cholerae* confirmed by culture and/or PCR and (ii) those with diarrhea but with a negative result to the RDT (indicator bias analysis).

We compared the odds of vaccination between case-patients and control-subjects through conditional logistic regression to account for the matching design; we fit a model with indicator variables for non-vaccinated, incomplete and complete dosing. We calculated the level of vaccine protection as (1- odds ratio) x 100.

We compared demographic, environmental, and socioeconomic factors between case-patients and their matched control-subjects in order to assess their potential as confounders of vaccine protection. We considered as possible confounders variables with a P values <0.2 in the bivariate models. We obtained an adjusted estimate of vaccine protection by co-variables that significantly contributed to improve the likelihood of the model. All P values and 95% confidence intervals were two-sided. Statistical significance was determined as a P value less than 0.05. Stata/SE 10 software was used for analyses.

Results

Baseline Information

From May 21 to October 31, 2012, 239 patients with acute, non-bloody diarrhea were treated at health centers in the study area (Figure 2); 5 died, yielding a case fatality ratio of 2%. Overall, 40 case-patients and 160 control-subjects were included in the primary analysis (Figures 1 and 2). None of the case-patients enrolled in the study died. The median age of participants was 28.0 years (inter-quartile-range: 16.5-39.0). There were fewer females (35.0%) than males (Table 1). Half of the cases sought care on the same day of symptom onset. Dehydration was present in 70% of cases at admission.

Of 36 case-patients included in the primary analysis for whom a specimen was sent for culture and PCR analysis, 18 (50%) were positive for *V. cholerae O1*, El Tor-Ogawa; 13 were positive for culture and PCR and 5 PCR positive but culture negative. All the 36 samples showed a weak amplification signal of the 16S rRNA gene. Among the 18 negative specimens, 5 had an almost undetectable amplification signal.

In addition, 43 watery diarrhea case-patients with a negative RDT result and 172 control-subjects were recruited for the indicator bias analysis (Figures 1 and 2).

Analysis of confounders and effect modifiers

Case-patients with cholera confirmed by RDT were more likely than the control-subjects to eat in public places and to share the latrine with individuals who had had cholera (Tables 1 and S1). Baseline characteristics of the non-cholera case-patients and the matched control-subjects included in the indicator bias analysis are shown in the Supplementary Appendix (Table S2).

Vaccine Effectiveness Analysis

Vaccination with two complete doses was associated with significant protection against cholera, in the crude analysis and after adjustment for potential confounders (86.6%; 95% confidence interval: 56.7 to 95.8%; P value=0.001) (Table 2). The precision of the vaccine effectiveness estimate for an incomplete course of vaccine was inconclusive (42.8%; 95% confidence interval: -83.6 to 82.2%; P value=0.35).

In the sub-analysis including only cases that were culture and/or PCR confirmed, vaccination with two complete doses was also associated with significant protection against cholera (91.6%; 95% confidence interval: 58.6 to 98.3%; P value=0.002) (Table 3).

The odds of vaccination between non-cholera watery diarrhea cases and control-subjects did not vary between these two groups (Table 3).

Discussion

The results presented here show high effectiveness of two complete doses of Shanchol when administered as part of the response to a cholera epidemic in Africa. Our results represent an estimate of the short-term protection of Shanchol and are in line with previous results with Dukoral[4]. This is highly relevant considering the fact that oral vaccines have shown low levels of protection in low-income African settings in the past[19–21].

This study was carried out under real field conditions during a cholera outbreak with several limitations to note. The outbreak response immunization was part of the control strategy implemented by the Ministry of Health with the support of MSF in response to the epidemic; thus, the exposure in the population was not controlled. Overall, 316,250 vaccines were delivered and 48 non-severe adverse events following immunization were notified (see Supplementary Appendix);the vaccine coverage was high[12], ranging from 69% in Koba to 84% in Makountan sub-prefectures. High vaccination coverage reduces transmission in vaccinated communities (herd protection)[22–24], thereby directly and indirectly reducing the risk of cholera. In the past, this has limited opportunities to measure effectiveness [25] due to difficulties in recruiting case-patients. In our study, most of the cases were recruited from Koba (Boffa prefecture) where a small local outbreak was reported (August-October 2012). This area showed the lowest vaccination coverage[12] and borders Dubreka where high transmission (overall attack rate 17 per 10,000) was reported (Supplementary Appendix). It is important to mention that the small sample size did not translate into low power (99%) as the observed effectiveness was higher than the assumption considered for the sample size calculation. However, the small sample size limited the possibility of conducting stratified analyses by age and to estimate single dose effectiveness with sufficient precision. This latter figure although not significant, was substantially lower than the protection conferred by two doses, and the point estimate was in line with previous findings with other OCV[4,6,24].

Despite the wide and systematic distribution of vaccination cards and the short time span between the vaccination campaign and the data collection, 25% of the vaccinated people interviewed were not able to provide their vaccination card at the time of the study. As we were not able to confirm vaccination status for all participants, some information bias may be present. Nevertheless, even if we consider all the individuals who were unable to find their vaccination card as not-vaccinated (worst-case scenario), the vaccine effectiveness would still reach 82% (Supplementary Appendix).

Further, case-control studies of vaccine effectiveness may also be prone to bias related to differences in health seeking behavior. In order to measure this potential bias we conducted a supplementary analysis, measuring the odds of vaccination among non-cholera watery diarrhea cases and a sample of matched control-subjects (indicator bias analysis). As the odds of vaccination did not vary significantly between these two groups, we interpret this finding as absence of large health seeking behavior bias.

Despite difficulties inherent in assessing effectiveness under field conditions, we were able to provide estimates of the short-term protection (first six months) of Shanchol in Africa where the impact of OCV is expected to be the highest in reducing mortality[1,26] and where problems with the protection provided by oral vaccines have been documented in the past[21]. The crude and the adjusted effectiveness estimates were similar after exploring the effect of a large number of well-described possible confounders, as well as when using only PCR or culture confirmed cases. The low proportion of PCR positive samples could be related to the small amount or the poor quality of biological material, as assessed by the weak amplification signal of the 16S rRNA gene. False positive RDT results cannot be excluded[27,28]; this non-differential misclassification would have underestimated the vaccine effectiveness.

The results presented here suggest that the short-term protection of Shanchol can be higher in the first six months than the protection remaining two years post-vaccination (67% estimate reported by Sur et al.)[6], probably as a result of waning immunity. It is possible that this difference is also partially explained by the fact that some non-vaccinated participants may have naturally acquired immunity, as cholera is

endemic in Kolkata. In addition, our estimate might include some indirect protection, although indirect effects were minimized through the matched design[22,23].

An aspect that does not seem to have decreased the short-term protection provided by Shanchol is the cold chain strategy used in Guinea, where vaccines were stored under cold chain, but were transported and used at ambient temperature on the vaccination day. The vial temperature monitor was checked for stability before administration (all remained valid). These results are not surprising considering the good heat stability of Dukoral[29], but this requires more robust documentation which will allow for more flexible delivery strategies in the future. Another aspect that can substantially simplify the use of OCV in outbreak settings is a single dose regimen. Our study was underpowered to provide precise estimates of the one-dose protection. Determining the short-term protection given by one dose is a clear priority towards the implementation of efficient and timely reactive campaigns.

In conclusion, our estimates on the short-term effectiveness of Shanchol provide essential information for the rapid use of OCV to improve the current strategies for outbreak prevention and control. This evidence supports the current WHO recommendation of exploring the role of OCV in response to cholera outbreaks and should serve to recommend strongly the addition of OCV among the tools to be used in response to epidemics.

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Competing Interests

All authors declare that they have no competing interests.

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Table 1. Characteristics of the case-patients and control-subjects included in the vaccine effectiveness study, Boffa and Forécariah, Guinea, 2012.

	Control-subjects		Case-patient		
	n	(%)	n	(%)	P value
Total included	160		40		
Males	104	(65.0)	26	(65.0)	
Female	56	(35.0)	14	(35.0)	
Age in years (median and IQR*)	28	16-39	28	18-36	
Profession					0.18
Trader	29	(18.1)	8	(20.0)	
Farmer	37	(23.1)	16	(40.0)	
Pupil / student	29	(18.1)	3	(7.5)	
Fisherman	10	(6.3)	3	(7.5)	
Housewife	10	(6.3)	1	(2.5)	
Unemployed	22	(13.8)	6	(15.0)	
Other	23	(14.4)	3	(7.5)	
Head of household's educational attainment					0.13
None	43	(27.2)	13	(32.5)	
Primary	5	(3.2)	4	(10.0)	
Secondary	21	(13.3)	2	(5.0)	
University	5	(3.2)	0	(0.0)	
Literate	84	(53.2)	21	(52.5)	
Telephone					0.10
No	32	(20.0)	13	(32.5)	
Yes	128	(80.0)	27	(67.5)	
Household size					0.063
0-4 members	34	(21.3)	17	(42.5)	
5-7 members	40	(25.0)	7	(17.5)	
8-12 members	49	(30.6)	9	(22.5)	
>12 members	37	(23.1)	7	(17.5)	
Proportion of children attending school in the household					0.13
None of them	33	(22.9)	14	(37.8)	
Less than half	42	(29.2)	11	(29.7)	
More than half	51	(35.4)	8	(21.6)	
All of them	18	(12.5)	4	(10.8)	
Distance to the closest health center					0.10
Need of transport	107	(66.9)	31	(77.5)	
Walking distance	53	(33.1)	9	(22.5)	
Other cholera cases in the household					0.15
No	155	(97.5)	37	(92.5)	
Yes	4	(2.5)	3	(7.5)	

Treatment of drinking water†					0.15
No	26	(16.3)	11	(28.2)	
Yes	34	(21.3)	5	(12.8)	
Eating food in a public space					0.02
Never	72	(45.0)	11	(28.2)	
Sometimes	49	(30.6)	20	(51.3)	
Everyday	39	(24.4)	8	(20.5)	
Usual place of defecation					0.12
Latrine	81	(50.6)	17	(42.5)	
Pit in the yard	56	(35.0)	14	(35.0)	
In the ground	23	(14.4)	9	(22.5)	
Sharing the latrine with someone suffering from cholera					0.001
No	131	(96.3)	24	(80.0)	
Yes	5	(3.7)	6	(20.0)	

* IQR: inter-quartile-range: upper and lower quartile

† Defined as water treated with chlorine or boiling before consumption

Table 2. Vaccine effectiveness estimates and 95 percent confidence interval (95%CI) of a complete (two doses) and an incomplete vaccine course. Boffa and Forécariah, Guinea, 2012.

Vaccination status	Control-subjects		Case-patient		VE*	95%CI	P value	aVE†	95%CI	P value
	N	(%)	N	(%)	%			%		
Unvaccinated	23	(14.4)	15	(37.5)	Ref			Ref		
Incomplete course‡	36	(22.5)	14	(35.0)	38.9%	(-55.2% - 76.0%)	0.30	42.8%	(-83.6% - 82.2%)	0.35
Full course (two doses)	101	(63.1)	11	(27.5)	84.0%	(59.7% - 93.6%)	<0.001	86.6%	(56.7% - 95.8%)	0.001
Total	160	(100.0)	40	(100.0)						

* VE: crude vaccine effectiveness estimates, calculated as 1-odds ratio.

† AVE: adjusted vaccine effectiveness. Adjusted by: number of individuals living in the household, treatment of water before consumption and sharing the latrine with a cholera case

‡ Incomplete course: individuals who took only one dose or who spitted out or vomited one of the two doses of vaccine

Table 3. Vaccine effectiveness estimates and 95 percent confidence intervals (95%CI) in the sub-analysis containing only culture and/or PCR confirmed cases and in the sub-analysis using watery diarrhea cases with negative RDT result. Boffa and Forécariah, Guinea, 2012.

Vaccination status	control		Case		VE*	95%CI	P value
	N	(%)	N	(%)	%		
Culture-PCR sub-analysis†							
Unvaccinated	10	(13.9)	8	(44.4)	Ref		
Incomplete course‡	17	(23.6)	6	(33.3)	66.2%	(-53.0% - 92.6%)	0.16
Full course (two doses)	45	(62.5)	4	(22.2)	91.6%	(58.6% - 98.3%)	0.002
Total	72	(100.0)	18	(100.0)			
Indicator bias analysis§							
Unvaccinated	9	(5.2)	4	(9.3)	Ref		
Incomplete course‡	35	(20.4)	7	(16.3)	48.1%	(-177.1% - 90.3%)	0.44
Full course (two doses)	128	(74.4)	32	(74.4)	25.2%	(-225.2% - 82.8%)	0.70
Total	172	(100.0)	43	(100.0)			

* VE: crude vaccine effectiveness estimates, calculated as 1-odds ratio.

† In this sub-analysis only cholera cases confirmed by culture and/or PCR were included in the analysis.

‡Incomplete course: individuals who took only one dose or who spit out or vomited one of the two doses of vaccine.

§ In this sub-analysis case-patients with non-choleric diarrhea (negative RDT result) were also compared with control-subjects that did not have diarrhea in an attempt to assess whether the results with respect to effectiveness could be attributed to bias.

Figures

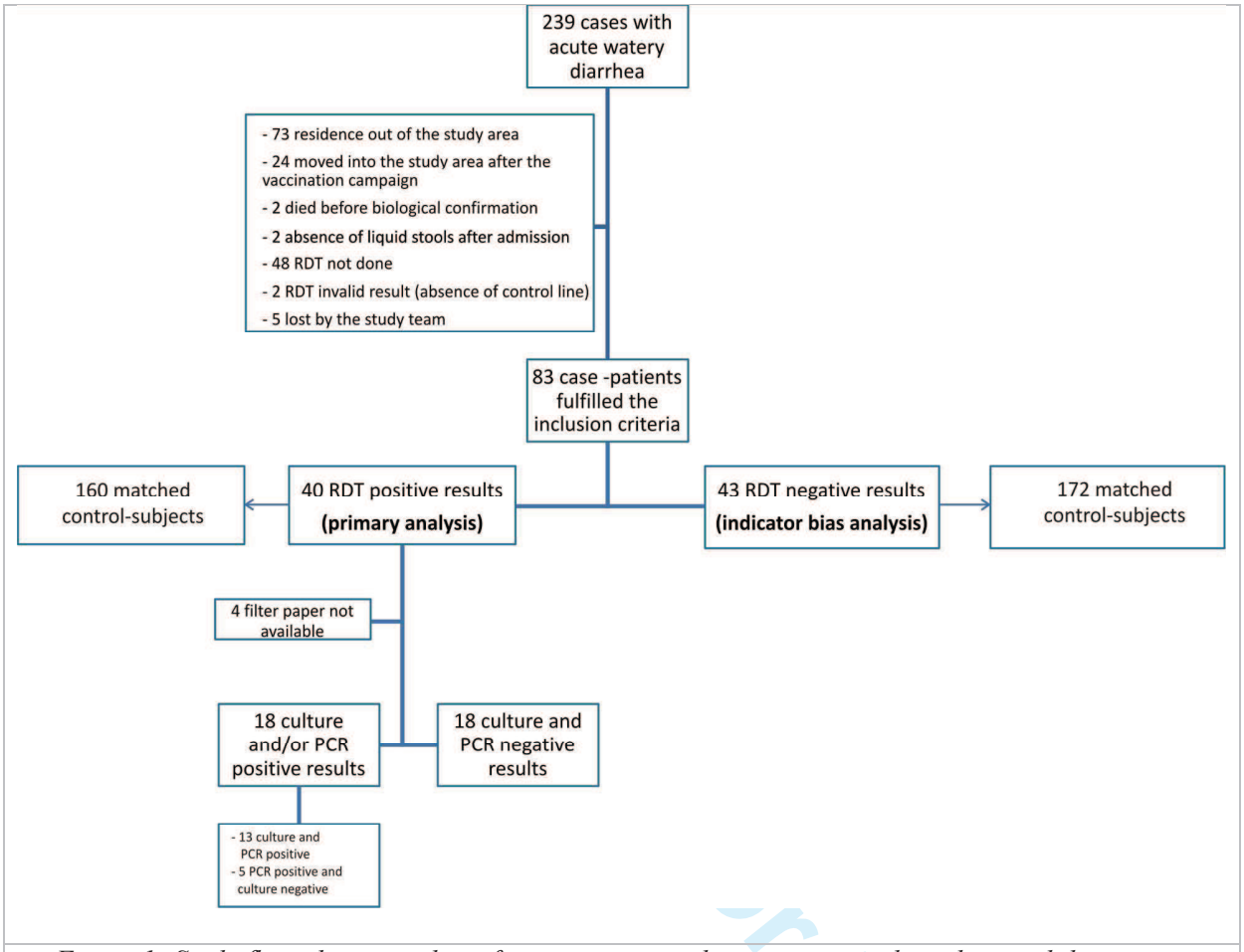


Figure 1: Study flow chart: number of case-patients with a positive result to the rapid diagnostic test (RDT) included in the primary analysis, number of case-patients with a negative result to the RDT included in the indicator bias analysis, and number of case-patients included in the sub-analysis with culture and/or PCR positive cases. Four matched control-subjects were selected for each case-patient.

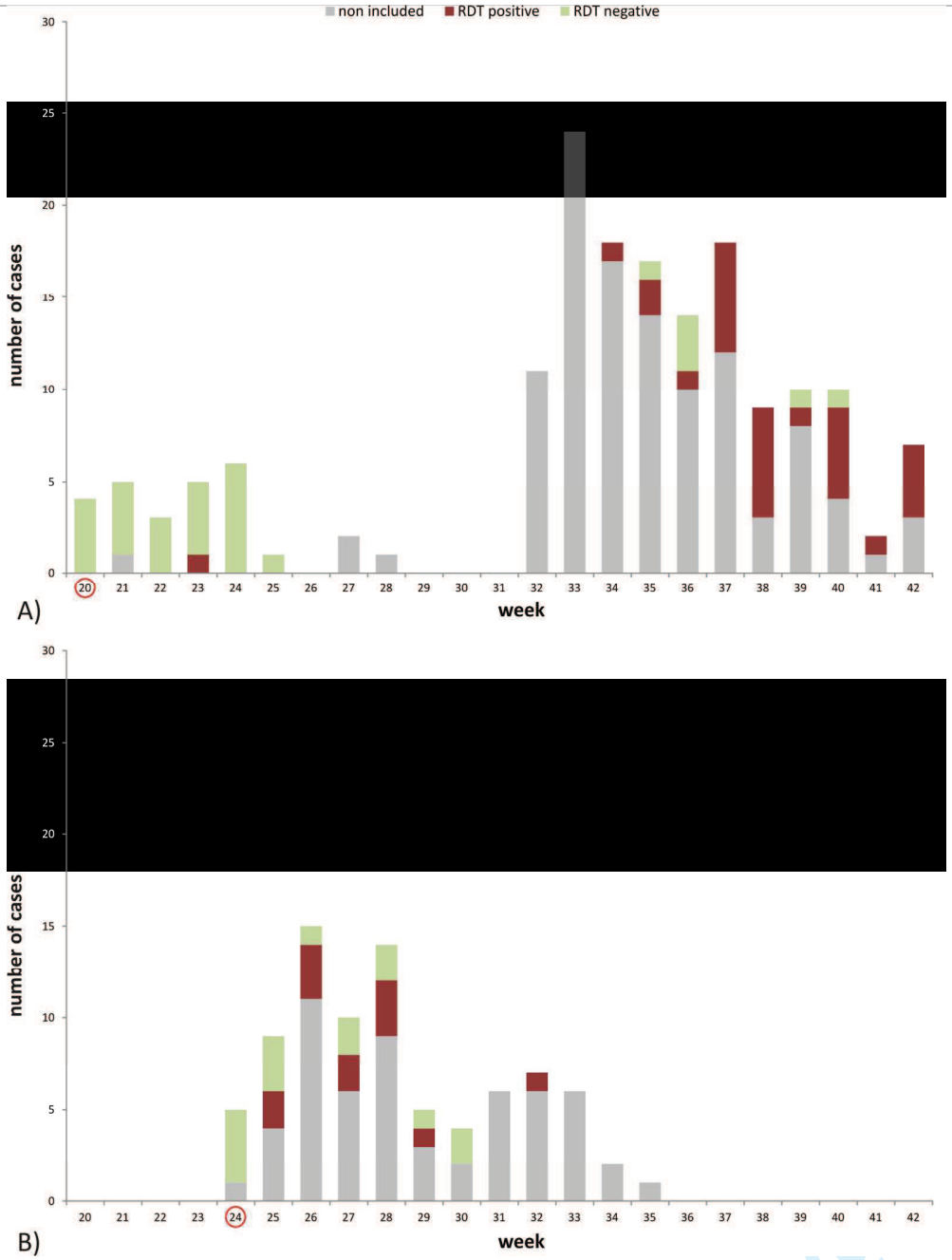
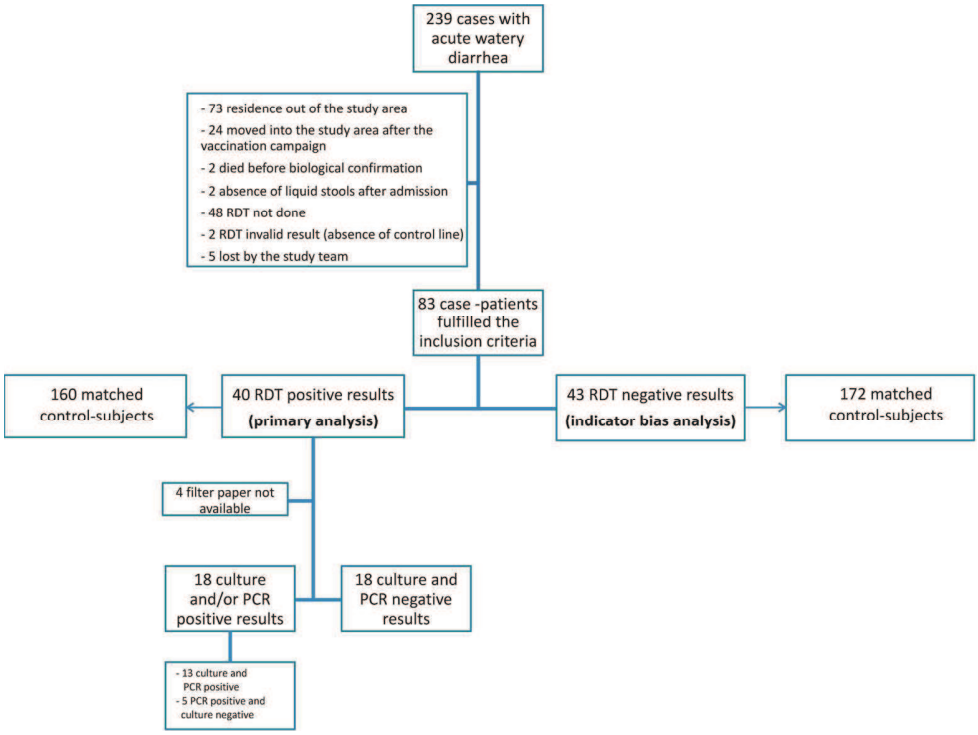
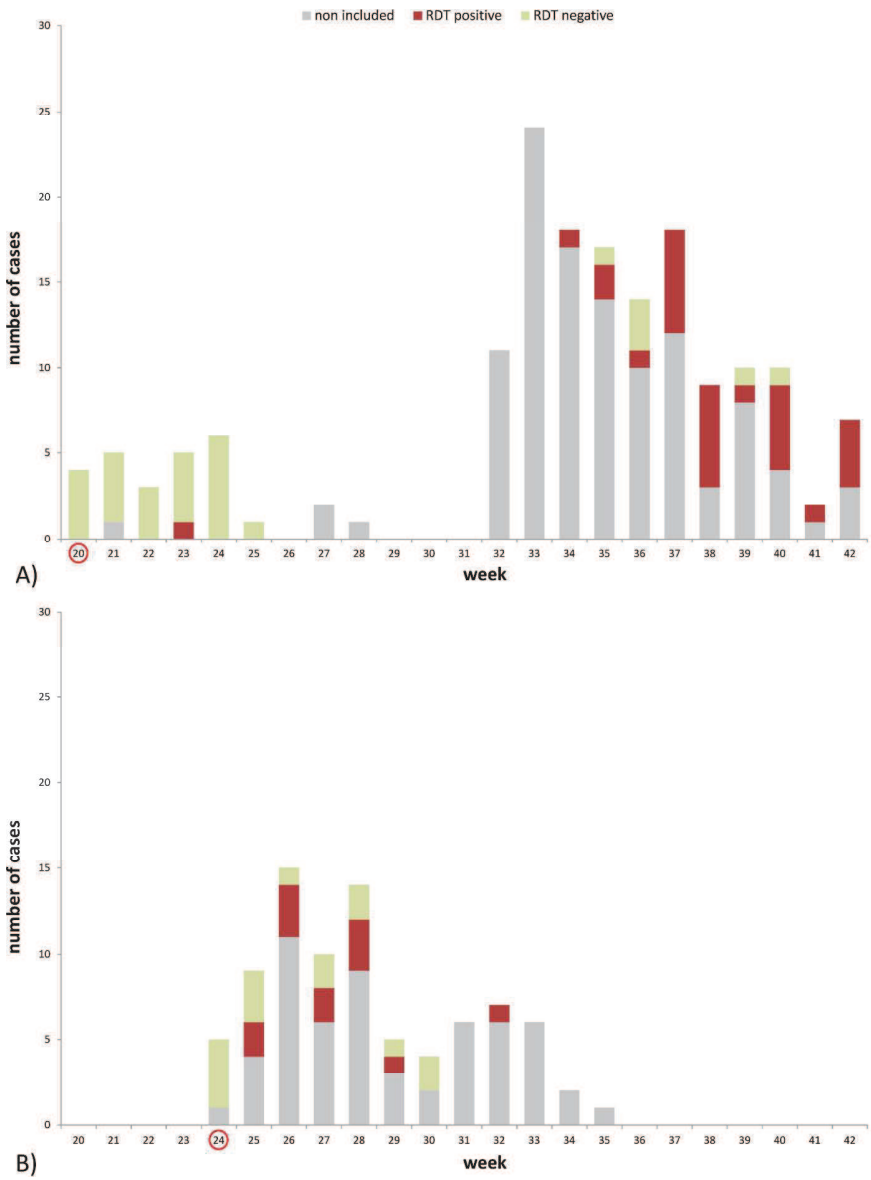


Figure 2: Acute diarrhea cases reported in the study areas after the starting date of the study (week 20 in Boffa prefecture, panel A; and week 24 in Forécariah prefecture, panel B). The cholera cases confirmed by rapid test (RDT) included in the study are represented in red and the non-cholera cases (RDT negatives) included in the indicator bias analysis are represented in green.



Study flow chart: number of case-patients with a positive result to the rapid diagnostic test (RDT) included in the primary analysis, number of case-patients with a negative result to the RDT included in the indicator bias analysis, and number of case-patients included in the sub-analysis with culture and/or PCR positive cases. Four matched control-subjects were selected for each case-patient.
160x120mm (300 x 300 DPI)



Acute diarrhea cases reported in the study areas after the starting date of the study (week 20 in Boffa prefecture, panel A; and week 24 in Forécariah prefecture, panel B). The cholera cases confirmed by rapid test (RDT) included in the study are represented in red and the non-cholera cases (RDT negatives) included in the indicator bias analysis are represented in green.
252x336mm (300 x 300 DPI)

Annex 2. Data collection form: feasability assessment

Vacciantion Site	
Vaccination Session*	
Time spent for preparation of the session	
Session duration	
Time spent for closing of the vaccination point	
Daily number of doses being administered	
Per gender	
Per age-group (2-4, 5-14, >=15)	
Per hour	
Number of personal in charge of the vaccination point per category	
Screening	
Animators	
Vaccination cards	
Preparators	
Vaccinators	
Cleaners	
Registers	
Community memebers	
Med responsable	
Log responsable	
Coordinator	
Logistics for consumable	
Cool boxes	
Vaccine vials	
Litres of water	
Quantity of non-consumable items	
Chairs	
Tables	
Benches	

* Vaccination session = one day session on each vaccination point

Annex 3. Vaccine coverage and acceptability questionnaire

A	**DONNEES ENQUETEURS**		
A1	*DATE DE L'ENQUETE : __/__/2012*	*A2*	*NUMERO DE L'EQUIPE : _____*
A3	*SOUS-PREFECTURE :* *BOFFA-CENTRE 1 [_] DOUPROU 2 [_] KOBA 3 [_] TAMITA 4 [_] TOUGNIFILI 5 [_] MANKOUNTAN 6 [_]*		
A4	*VILLAGE : _____*	*A5*	*N° DE GRAPPE : _____*
A6	*N° DE LA MAISON : C _ _ _*		
A7	NOMBRE DE PERSONNE RESIDANT DANS LA MAISON : [ ]		

B. VACCINATION CONTRE LA POLIOMYELITE

Pendant le pèlerinage et juste après, une campagne de vaccination contre la polio a été organisée à Boffa. Elle concernait tous les enfants âgés de moins de 5 ans. Des vaccinateurs passaient de maison à maison (de porte à porte) et donnaient un médicament dans la bouche des enfants. (Présenter la boîte à image).

B1. Des vaccinateurs sont-ils passés dans cette maison ? Non [ ] Oui [ ] Ne sait pas [ ]

B2. Combien d'enfants de moins de 1 an résident dans cette maison ? [ ]

	Id	**Age** (en mois)	**Sexe** Homme=0 / Femme=1	**Vacciné contre la polio?** Non = 0 / Oui = 1 / Ne sait pas =9
Enfants de moins de 1 an	1.1			
	1.2			
	1.3			
	1.4			

B3. A part lui/elle/eux, combien d'enfants de moins de 5 ans résident dans cette maison ? [ ]

	Id	**Age** (en années)	**Sexe** Homme=0 / Femme=1	**Vacciné contre la polio?** Non = 0 / Oui = 1 / Ne sait pas =9
Enfants âgés de 1 à 4 ans	2.1			
	2.2			
	2.3			
	2.4			
	2.5			
	2.6			
	2.7			

C. VACCINATION CONTRE LE CHOLERA

La semaine passée, une campagne de vaccination contre le choléra a été organisée à Boffa par le Ministère de la Santé avec l'appui de Médecins sans Frontières. Elle concernait toutes les personnes à partir de 1 an. Il fallait se rendre dans un site de vaccination où on remplissait une carte de vaccination avant de recevoir un médicament dans la bouche. (Présenter la boîte à image).

Une campagne similaire avait été organisée avant le pèlerinage.

C.1. Des personnes résidant dans cette maison sont-elles allées se faire vacciner ? Aucune 1 [ ] Certaines 2 [ ] Toutes 3 [ ] Ne sait pas 9 [ ]

Si certaines ou toutes sont allées se faire vacciner, pourriez-vous aller chercher les cartes de vaccination ?

Si la personne ne sait pas répondre à cette question, demandez si un autre adulte de la maison peut répondre à cette question. Si aucun autre adulte ne sait répondre à cette question, demandez si vous pouvez repasser plus tard.

C.2. Pour les enfants âgés de 1 à 4 ans (*reporter l'âge et le sexe depuis le tableau B3, prenez les autres informations en discutant avec les parents des enfants puis vérifiez sur la carte si possible*).

Id	Age	Sexe	Vacciné contre le choléra ?	Nombre de doses reçues	1er tour *du 18/04/2012 au 23/04/2012 (avant pèlerinage)*					2ème tour *du 09/05/2012 au 14/05/2012 (après le pèlerinage)*				
					dose reçue ?	Vérifié sur carte ?	Date de vaccination	Avez-vous recraché ou vomi ?	Raisons non vaccination	2eme dose reçue ?	Vérifié sur carte ?	Date de vaccination	Avez-vous recraché ou vomi ?	Raisons de non vaccination
	(en années)	Homme=0 Femme=1	Non = 0 Oui = 1 NSP =9	0 = 0 1 =1 2 = 2 >2 = 3 NSP = 9	Non=0 Oui=1 NSP=9	Non=0 Oui=1	jj / mm / aa	Non = 0 Oui = 1 NSP =9	Voir codes (NA si vacciné)	Non=0 Oui=1 NSP=9	Non=0 Oui=1	jj / mm / aa	Non = 0 Oui = 1 NSP =9	Voir codes (NA si vacciné)
2.1							__ / __ /12					__ / __ /12		
2.2							__ / __ /12					__ / __ /12		
2.3							__ / __ /12					__ / __ /12		
2.4							__ / __ /12					__ / __ /12		
2.5							__ / __ /12					__ / __ /12		
2.6							__ / __ /12					__ / __ /12		
2.7							__ / __ /12					__ / __ /12		
2.8							__ / __ /12					__ / __ /12		

C.3. Pour les enfants âgés de 5 à 14 ans (*prenez les informations en discutant avec les parents des enfants, puis vérifiez sur la carte si possible*).

Id	Age	Sexe	Vacciné contre le choléra ?	Nombre de doses reçues	1er tour *du 18/04/2012 au 23/04/2012 (avant pèlerinage)*					2ème tour *du 09/05/2012 au 14/05/2012 (après le pèlerinage)*				
					Dose reçue ?	Vérifié sur carte ?	Date de vaccination	Avez-vous recraché ou vomi ?	Raisons de non vaccination	Dose reçue ?	Vérifié sur carte ?	Date de vaccination	Avez-vous recraché ou vomi ?	Raisons de non vaccination
	(en années)	Homme=0 Femme=1	Non = 0 Oui = 1 NSP =9	0 = 0 1 =1 2 = 2 >2 = 3 NSP = 9	Non=0 Oui=1 NSP=9	Non=0 Oui=1	jj / mm / aa	Non = 0 Oui = 1 NSP =9	Voir codes (NA si vacciné)	Non=0 Oui=1 NSP=9	Non=0 Oui=1	jj / mm / aa	Non = 0 Oui = 1 NSP =9	Voir codes (NA si vacciné)
3.1							__ / __ /12					__ / __ /12		
3.2							__ / __ /12					__ / __ /12		
3.3							__ / __ /12					__ / __ /12		
3.4							__ / __ /12					__ / __ /12		
3.5							__ / __ /12					__ / __ /12		
3.6							__ / __ /12					__ / __ /12		
3.7							__ / __ /12					__ / __ /12		
3.8							__ / __ /12					__ / __ /12		

C.4. Pour les adultes (15 ans et plus) (*prenez les informations de la carte si possible, sinon en discutant avec les personnes concernées ou le responsable du ménage s'ils sont absents*).

Id	Age	Sexe	Vacciné contre le choléra ?	Nombre de doses reçues	1er tour *du 18/04/2012 au 23/04/2012 (avant pèlerinage)*					2ème tour *du 09/05/2012 au 14/05/2012 (après le pèlerinage)*				
					Dose reçue ?	Vérifié sur carte ?	Date de vaccination	Avez-vous recraché ou vomi ?	Raisons de non vaccination	Dose reçue ?	Vérifié sur carte ?	Date de vaccination	Avez-vous recraché ou vomi ?	Raisons de non vaccination
	(en années)	Homme=0 Femme=1	Non = 0 Oui = 1 NSP =9	0 = 0 1 =1 2 = 2 >2 = 3 NSP = 9	Non=0 Oui=1 NSP=9	Non=0 Oui=1	jj / mm / aa	Non = 0 Oui = 1 NSP =9	Voir codes	Non=0 Oui=1 NSP=9	Non=0 Oui=1	jj / mm / aa	Non = 0 Oui = 1 NSP =9	Voir codes
4.1							__ / __ /12					__ / __ /12		
4.2							__ / __ /12					__ / __ /12		
4.3							__ / __ /12					__ / __ /12		
4.4							__ / __ /12					__ / __ /12		
4.5							__ / __ /12					__ / __ /12		
4.6							__ / __ /12					__ / __ /12		
4.7							__ / __ /12					__ / __ /12		
4.8							__ / __ /12					__ / __ /12		
4.9							__ / __ /12					__ / __ /12		
4.10							__ / __ /12					__ / __ /12		
4.11							__ / __ /12					__ / __ /12		
4.12							__ / __ /12					__ / __ /12		

D. ACCEPTABILITE DU VACCIN CONTRE LE CHOLERA

D.1. Pour les enfants âgés de 1 à 14 ans (*reporter l'âge et le sexe depuis les tableaux B3 et C3, prenez les autres informations en discutant avec les parents des enfants ou leur responsable*).

	Id	Age	Sexe	Effets indésirables					
				1er tour			*2ème tour*		
				Malade?	***Symptômes ?***	***Consultation ?***	***Malade?***	***Symptômes ?***	***Consultation ?***
		(en années)	Homme =0 Femme= 1	*Non = 0 Oui = 1 NA=8 NSP =9*	*Codes, séparés par une virgule*	*Non = 0 Oui = 1 NA=8 NSP =9*	*Non = 0 Oui = 1 NA=8 NSP =9*	*Codes, séparés par une virgule*	*Non = 0 Oui = 1 NA=8 NSP =9*
Enfants âgés de 1 à 4 ans	2.1								
	2.2								
	2.3								
	2.4								
	2.5								
	2.6								
	2.7								
	2.8								
Enfants âgés de 5 à 14 ans	3.1								
	3.2								
	3.3								
	3.4								
	3.5								
	3.6								
	3.7								
	3.8								

D.3. Pour les adultes (15 ans et plus) présents, poser leur les questions suivantes :

Id	Age	Sexe	Présent?	Effets indésirables						Acceptabilité					Complè-tement protégé avec 1 dose ?	Complè-tement protégé avec 2 doses ?
				1er tour			*2ème tour*			**Entendu parler campagne ?**	**Effet sur la santé ?**	**Rendre malade ?**	***Goût ?***	**Vacciné à nouveau ?**		
				Malade?	***Symptômes ?***	***Consulta-tion ?***	***Malade?***	***Symptômes ?***	***Consulta-tion ?***							
	(en années)	Homme =0 Femme= 1	Non = 0 Oui = 1	*Non = 0 Oui = 1 NA=8 NSP =9*	*Codes, séparés par une virgule*	*Non = 0 Oui = 1 NA=8 NSP =9*	*Non = 0 Oui = 1 NA=8 NSP =9*	*Codes, séparés par une virgule*	*Non = 0 Oui = 1 NA=8 NSP =9*	*Non = 0 Oui = 1 NSP =9*	*Pas d'effet=0 Mauvais effet=1 Bon effet = 2 NSP = 9*	*Non = 0 Oui = 1 NSP =9*	*Mauvais=0 Bon = 1 Pas d'opinion=8 Pas vacciné = 9*	*Non = 0 Oui = 1 NSP =9*	*Non = 0 Oui = 1 NSP =9*	*Non = 0 Oui = 1 NSP =9*
4.1																
4.2																
4.3																
4.4																
4.5																
4.6																
4.7																
4.8																
4.9																
4.10																
4.11																
4.12																

Annex 4. Notification form of adverse events following immunization

CENTRE/POSTE DE SANTE	
Nom du Centre santé/Poste de vaccination	
Village / Sous-prefecture	
Date de la consultation __ / __ / 2012	
Nom et prénom de la personne remplissant la feuille : _____	

IDENTIFICATION DE LA PERSONNE SOUFFRANT D'EFFETS INDESIRABLES	
Nom	
Prénom	
Sexe	Homme H [ ] Femme F [ ]
Si femme, enceinte ?	Oui 1 [ ] Non 0 [ ] Ne sait pas 9 [ ]
Date de naissance/Age	_ _ / _ _ / _ _ _ _ Age _____ ans
Carte de vaccination	Oui 1 [ ] Non 0 [ ]

Nom du Vaccin	Date de vaccination	Heure de vaccination	Dose (1er/ 2ème)	Numéro de lot	Date d'expiration

RENSEIGNEMENTS MEDICAUX								
La personne a-t-elle des antécédents allergiques ?	**Oui 1 [ ] Non 0 [ ] Ne sait pas 9 [ ]**							
Si oui, à quoi la personne est-elle allergique ?								
Date et heure où les symptômes ont débuté ?								
La personne présente-t-elle les symptômes suivants ? (Cochez les symptômes et indiquer sévérité)								
- réaction allergique			**- démangeaisons**			**- Douleur à la gorge**		
- Déshydratation			**- bouche sèche**			**- toux**		
- Douleur abdominale			**- fièvre > 38°C**			**- Fatigue**		
- diarrhée			**- ulcère oral**			**- Étourdissement**		
- vomissement			**- éruption cutanée**			**- Maux de tête**		
- nausées			**- Modification de la couleur des urines**			**- autres**		
Décrire les symptômes : **Pour le cas sévères (grade 3, 4 et 5) une feuille supplémentaire sera remplie par le Dr. Soumah**								
RESULTATS								
Résultat : Résolu 1 [ ] Traitement ambulatoire 2 [ ] Transféré 3 [ ] Décédé 4 [ ] Inconnu 5 [ ] **Si décédé, date de décès : _ _ / _ _ / _ _ _ _ Autopsie : oui [ ] Non [ ] Inconnu [ ]**								
Si la personne a été transférée, indiquez où ?								
RELATION AVEC LE VACCIN : **Non lié 1 [ ] Improbable 2 [ ] Possible 3 [ ] Probable 4 [ ] Très probable 5 [ ] Données insuffisantes 6 [ ]**								

Investigation nécessaire : Oui [ ] Non [ ] ; Si oui, date des investigations _ _ /_ _ /_ _ _ _ **Résultats des investigations :**

Annex 5. Informed consent : case-control study

Feuille d'information pour les cas se présentant dans une structure de prise en charge du choléra dans la zone d'étude.

Nous travaillons en collaboration avec le Ministère de la Santé et de l'Hygiène publique de République de Guinée. Nous menons une étude sur le choléra, qui est une maladie commune dans ce pays.

Le choléra cause de nombreux cas de diarrhées sévères chez les enfants et les adultes en Guinée. Un vaccin contre le choléra donné par voie orale (par la bouche) est maintenant qualifié par l'Organisation Mondiale de la Santé et disponible. Des études menées dans d'autres pays ont permis de démontrer que ce vaccin protégeait contre le choléra, sans effet secondaire. Le Ministère de la Santé et de l'Hygiène publique de République de Guinée, en collaboration avec des organisations internationales, a réalisé une campagne de vaccination de masse contre le choléra dans cinq sous-préfectures de la préfecture Boffa. Nous souhaiterons maintenant évaluer les résultats de cette campagne de masse.

Nous vous demandons votre permission pour vous inclure, ou inclure l'enfant sous votre responsabilité, dans cette étude. Vous (ou l'enfant sous votre responsabilité) avez été sélectionné car vous (ou l'enfant sous votre responsabilité) habitez dans une des sous-préfectures ayant bénéficié de la campagne de vaccination et êtes venus consulter ce centre de traitement à cause de diarrhées aqueuses survenues pendant ou après la campagne de vaccination.

Si vous acceptez de participer, un échantillon de vos selles (ou des selles de l'enfant sous votre responsabilité) sera prélevé. Cet échantillon sera testé pour le choléra. Ceci sera complètement gratuit. Cette procédure est une procédure de routine pour le diagnostic du choléra. Vous (ou l'enfant sous votre responsabilité) serez traité selon les guides standards de prise en charge des cas de diarrhées. Nous vous poserons ensuite quelques questions vous concernant (ou concernant l'enfant sous votre responsabilité) comme par exemple le village où vous (ou l'enfant sous votre responsabilité) vivez, la source où vous prenez l'eau pour boire, le nombre de personnes vivant dans votre ménage, ou si vous avez reçu le vaccin contre le choléra lors de la campagne de masse. Vos réponses à ces questions nous aideront à évaluer les facteurs de risque et de protection contre le choléra. Les résultats de cette étude nous permettront d'améliorer les connaissances sur ce vaccin, et d'augmenter son utilisation en Guinée et dans d'autres pays.

Le questionnaire devrait durer environ 30 minutes. En participant à cette recherche, vous ne vous exposez à aucune sensation douloureuse.

Nous vous informerons du résultat du test diagnostic réalisé sur l'échantillon de selles que nous aurons prélevé pour savoir si vos diarrhées (ou celles de l'enfant sous votre responsabilité) sont dues ou non au choléra. Vous n'aurez aucune dépense financière liée à votre participation à l'étude, et vous ne recevrez pas d'argent. Toutes les informations que vous donnerez resteront confidentielles. Epicentre archivera les questionnaires dans un endroit sécurisé fermé à clef et les données ne pourront être utilisées que pour ce projet.

Vous (ou l'enfant sous votre responsabilité) n'êtes pas obligé de participer à ce projet de recherche, et votre refus n'aura pas de conséquence sur votre prise en charge et votre traitement. Vous (ou l'enfant sous votre responsabilité) bénéficierez du même traitement dont vous auriez bénéficié en participant à l'étude.

Vous (ou l'enfant sous votre responsabilité) pouvez choisir d'interrompre votre participation à ce projet de recherche à n'importe quel moment pour quelque raison que ce soit, sans perdre aucun de vos droits en tant que

patient. Votre traitement (ou celui de l'enfant sous votre responsabilité) n'en sera aucunement affecté.

Si vous avez la moindre question, vous pouvez nous la poser maintenant ou plus tard. Si vous souhaitez poser une question plus tard, vous devrez contacter *Dr. Melat Haile*, téléphone *X1*.

Ce projet de recherche a été revu et approuvé par le Comité national de bioéthique de République de Guinée, dont la tâche est de s'assurer que les personnes participant à cette recherche sont protégés. Si vous souhaitez en savoir plus sur ce comité d'éthique, contactez *Dr. Apha Amadou Diallo,* téléphone X2.

Certificat de consentement

J'ai été invité(e) (ou l'enfant dont je m'occupe a été invité) à participer à la recherche sur le choléra. J'ai lu les informations fournies ci-dessus ou quelqu'un me les a lues. J'ai eu la possibilité de poser des questions à ce sujet, et toutes les questions que j'ai posées ont trouvé une réponse satisfaisante. J'accepte volontairement de participer (ou que l'enfant dont je m'occupe participe) comme sujet d'étude et j'ai compris que j'ai le droit de me retirer de cette étude à n'importe quel moment sans que cela n'affecte d'aucune manière ma prise en charge médicale (ou celle de l'enfant dont je m'occupe).

Nom du sujet

_____

Date et Signature du sujet

_____

___/___/___(jj/mm/aa)

Si la personne n'est pas lettrée

Nom d'un témoin lettré
(si possible, cette personne devra être choisie par le participant et ne pas avoir de connexion avec l'équipe de recherche)

_____

Date et Signature du témoin

_____

___/___/___(jj/mm/aa)

Nom du chercheur

_____

Date et Signature du chercheur

_____

___/___/___(jj/mm/aa)

Annex 6. Vaccine effectiveness (case-control study) questionnaire

IDENTIFICATION DE LA STRUCTURE DE SOIN

X1 NOM DE LA STRUCTURE : _____

X2 VILLE / VILLAGE : _____

X3 DATE DE REMPLISSAGE DU QUESTIONNAIRE : ……/……./ 2012 (jj/mm/aaaa)

X4 Personne en charge du remplissage _____

PERSONNE **CAS** C [_]
SUSPECT DE CHOLERA

X5 **CODE CAS [ ]**

X6 COORDONNÉES GPS DONNÉES :

LAT: N .....

LONG : W …….....

PERSONNE QUI **N'A JAMAIS** T [_]
ATTRAPPE LE CHOLERA DEPUIS
FEVRIER 2012

X7 **Témoins communautaires :**
Pour le Cas *(code)* **[ ] → Témoin** A-D **[___]**

X8 COORDONNÉES GPS DONNÉES :

LAT: N .....

LONG : W …….....

IDENTIFICATION ***(à remplir pour les CAS et les TEMOINS)***

A1 NOM : ……..

A2 SEXE : HOMME H[_] FEMME F[_] A3 AGE [ ] ANS

A4 SOUS-PREFECTURE :
BOFFA-CENTRE 1[_] DOUPROU 2[_] KOBA 3[_] TAMITA 4[_] TOUGNIFILI 5[_] MANKOUNTAN 6[_]

A5 VILLAGE .....

A7 ADRSESE (N° ET RUE OU POINTS DE REPERE): _____
_____

Si la personne est malade de choléra, remplissez le tableau B à la page 2
Si la personne est non malade de choléra, remplissez le tableau C à la page 3

(CAS)

B	**PERSONNE CAS SUSPECT DE CHOLERA**
	INFORMATIONS RECUEILLIES A LA CONSULTATION DU CENTRE DE SANTE
B1	Numéro du cas dans le registre [ ]
B2	Date d'admission au centre de sante ……../……../…….
B3	Date du début de la diarrhée ……../……../…….
B4	Type de diarrhée: Aqueuse 1[_] Sanglante 2[_] Autre 3[_] ……
B5	Déshydratation : Pas de déshydratation (A) A[_] Un peu déshydraté (B) B[_] Déshydratation sévère (C) C[_] Ne sait pas N[_]
B6	Le malade est encore au centre de santé: Non 0[_] Oui 1[_]
B7	Si « Non », date de sortie : …...../…...../.....

VERIFICATION D'AUTRES INFORMATIONS D'ELIGIBILITE AUPRES DU MALADE :

		Oui	**Non**
B8	Viviez-vous dans un village de la préfecture de Boffa avant le 18 / 04 / 2012 ?	[_]	[_]
B9	Aviez-vous au moins 1 an au 22 / 04 / 2012 ?	[_]	[_]
B10	Avez-vous eu au **moins trois selles aqueuses** en 24 heures avant de vous rendre au centre de santé ?	[_]	[_]
B11	La diarrhée a-t-elle commencé **après le 25 / 04 / 2012 ?**	[_]	[_]
B12	Acceptez-vous de participer ?	[_]	[_]

ATTENTION :

Continuez le questionnaire UNIQUEMENT SI la personne choisie comme cas a répondu « OUI » à TOUTES les questions en haut.

(TEMOINS)

C	**PERSONNE QUI N'A JAMAIS ATTRAPE LE CHOLERA DEPUIS FEVRIER 2012**	
	Vérifier si la tranche d'âge de la personne non malade que vous allez interroger (témoin) est bien du même sexe et de la même tranche d'âge que la personne malade (cas) à laquelle elle est appariée :	
C1	Sexe du cas :	Homme H[_] Femme F[_]
C2	Sexe du témoin :	Homme H[_] Femme F[_]
C3	Tranche d'âge du cas:	1-4[_] 5-9[_] 10-19[_] 20-29[_] 30-39[_] 40-49[_] plus de 50 ans [_]
C4	Tranche d'âge du témoin	1-4[_] 5-9[_] 10-19[_] 20-29[_] 30-39[_] 40-49[_] plus de 50 ans [_]

Vérification d'autres informations d'éligibilité :

		Oui	**Non**
C5	Viviez-vous dans un village de la préfecture de Boffa avant le 18 / 04 / 2012 ?	**[_]**	**[_]**
C6	Aviez-vous au moins 1 an au 22 / 04 / 2012 ?	**[_]**	**[_]**
C7	Avez-vous souffert de diarrhées aqueuses aigües en 2012 ?	**[_]**	**[_]**
C8	Etes-vous allé dans un centre de santé en 2012 pour des diarrhées aqueuses aigües?	**[_]**	**[_]**
C9	Si non, si vous aviez eu des diarrhées aqueuses aigües, seriez-vous allé dans un centre de santé ?	**[_]**	**[_]**

ATTENTION :

Continuez le questionnaire UNIQUEMENT SI cette personne choisie comme témoin a répondu « OUI » aux questions C5, C6 et C9 en haut et « NON» aux questions C7 et C8.

(CAS ET TEMOINS)

D	**STATUT SOCIO-ÉCONOMIQUE**
D1	Quelle est votre profession ? Commerçant 1[_] Marchand 2[_] Cultivateur 3[_] Ecolier/Elève 4[_] Extracteur sel 5[_] Pêcheur 6[_] Ménagère 7[_] Pas de travail 8[_] Autre 9[_] …… Ne sait pas 99[_]
D2	Quel est le niveau d'études du chef du foyer ? Aucun 0[_] Primaire 1[_] Secondaire 2[_] Universitaire 3[_] Ne sait/rappelle pas 9[_]
D3	Votre famille possède-t-elle : Radios Non 0[_] Oui 1[_] Ne sait pas 9[_] Vélo Non 0[_] Oui 1[_] Ne sait pas 9[_] Téléphone portable Non 0[_] Oui 1[_] Ne sait pas 9[_] Générateur Non 0[_] Oui 1[_] Ne sait pas 9[_] Télévisions Non 0[_] Oui 1[_] Ne sait pas 9[_] Frigo Non 0[_] Oui 1[_] Ne sait pas 9[_] Cuisinière/Four (électrique ou gaz) Non 0[_] Oui 1[_] Ne sait pas 9[_] Bateau/Pirogue Non 0[_] Oui 1[_] Ne sait pas 9[_]
D4	Combien de personnes vivent dans le foyer ? _____ personnes
D5	Combien d'enfants de moins de 5 ans ?_____ Combien d'enfants entre 5 et 15 ans ? _____
D8	Combien d'enfants entre 5 et 15 ans vont à l'école ? _____
E	**CONDUITE VIS-A-VIS DU SOIN**
E1	Distance par rapport au centre de santé en km _____
E2	Combien de temps vous faut-il pour aller au centre de santé ? _____
E3	Par quel moyen de transport A pied 1[_] Moto 2[_] Voiture 3[_] Transport public 4[_] Autre 5[_], préciser _____
E4	Si vous aviez une diarrhée, où iriez-vous chercher des soins ? Lister dans l'ordre les endroits cités par la personne : _____ _____
F	**HISTORIQUE DU CHOLÉRA & DES CONTACTS A RISQUE**
F1	En ce moment, y a-t-il des cas de choléra (ou symptômes similaires) dans la maison ? Non 0[_] Oui 1[_] Ne sait pas 9[_]
F2	Dans les 7 derniers jours, y a-t-il eu des cas de choléra (ou symptômes similaires) dans votre entourage (voisins, amis, collègues…) ? Non 0[_] Oui 1[_] Ne sait/rappelle pas 9[_]
F3	Dans les 7 derniers jours, avez-vous voyagé ou reçu une visite de la part des personnes provenant d'une autre localité ? Non 0[_] Oui 1[_] Ne sait/rappelle pas 9[_]
F4	Dans les 7 derniers jours, avez-vous participé à un enterrement ou retrait de deuil de quelqu'un suspect de choléra ? Non 0[_] Oui 1[_] Ne sait/rappelle pas 9[_]

G	**APPROVISIONNEMENT EN EAU ET TRAITEMENT DE L'EAU**
G1	Dans quelle source d'eau vous approvisionnez-vous *le plus souvent* ? *(Ne suggérez pas la réponse, une seule réponse possible)* a. Pompe / Forage 1[_] b. Puits protégé (avec renforcement en ciment et un seul sceau) 2[_] c. Puits traditionnel (ouvert au ciel sans protection) 3[_] d. Eau d'une source naturelle (rivière, mare…) 4[_] e. Autre : _____ 5[_] f. Ne sait pas 9[_]
G2	Dans les 7 derniers jours, avez-vous pu traiter votre eau de boisson ? Non 0[_] Au moins une fois 1[_] Tous les jours 2[_] Ne sait pas 9[_]
G3	A la maison, le récipient utilisé pour stocker l'eau à boire est-t-il fermé par un couvercle ou par un bouchon ? Non 0[_] Oui 1[_] Ne sait pas 9[_]
H	**NOURRITURE CONSOMMEE DANS LES 7 DERNIERS JOURS**
H1	Dans les 7 derniers jours, avez-vous mangé de la nourriture vendue ou préparée dans un endroit publique (restaurant, sur la route) ? Jamais 0[_] Au moins une fois 1[_] Tous les jours 2[_] Ne sait pas 9[_]
I	**PRATIQUES D' HYGIENE**
I1	Avez-vous du savon/cendres (pour le lavage des mains) à la maison en ce moment ? Non 0[_] Oui 1[_] Ne sait/rappelle pas 9[_]
I2	D'habitude, quand vous lavez-vous les mains ? *(**Ne suggérez pas la réponse** et mettez une croix seulement aux réponses citées par le participant. Plusieurs réponses possibles.)* Je ne me lave pas les mains [_] Aucune réponse [_] Avant de manger [_] Après avoir mangé [_] Après avoir été aux toilettes [_] Après vous être occupé d'un enfant qui avait été aux toilettes [_] Avant de faire la cuisine [_] Autre [_] …….
J	**TOILETTES/LATRINES**
J1	Ou est-ce que vous faites le plus souvent vos besoins ? Latrine privée 1[_] Latrine partagée 2[_] Fosse dans la cour 3[_] Par terre 4[_] Dans la nature 5[_] Autre 6[_] ……. Ne sait pas 9[_]
J2	Qui d'autre se sert des Toilettes/Latrines/Fosse que vous utilisez ? Uniquement le foyer 1[_] Plusieurs foyers 2[_] N'importe qui 3[_] Ne sait pas 9[_]
J3	Quelqu'un qui partage vos latrines a-t-il eu le choléra ou toute autre diarrhée au cours du dernier mois ? Non 0[_] Oui 1[_] Ne sait pas 9[_]
J4	Les latrines sont-elles débordées ? Non 0[_] Oui 1[_] Pas possible de le voir 9[_]

K	STATUT VACCINAL
K1	Avez-vous été vacciné contre le choléra ? Non 0[_] Oui 1[_] Ne sait pas 9[_]
	Si oui, demandez à voir la carte de vaccination.
K2	Combien de doses avez-vous reçu ? 0 0[_] 1 1[_] 2 2[_] Plus de 2 3[_] NSP 9[_]
	1ère dose
K3	1ère dose reçue ? Non 0[_] Oui 1[_] Ne sait pas 9[_]
K4	Vérifié sur la carte de vaccination ? Non 0[_] Oui 1[_]
K5	Date de vaccination pour la 1ère dose : __ / __ / 2012 Ne sait pas [ ]
K6	Lieu de vaccination pour la 1ère dose : _____
K7	Avez-vous recraché ou vomi en prenant la 1ère dose ? Non 0[_] Oui 1[_] Ne sait pas 9[_]
	2ème dose
K8	2ème dose reçue ? Non 0[_] Oui 1[_] Ne sait pas 9[_]
K9	Vérifié sur la carte de vaccination ? Non 0[_] Oui 1[_]
K10	Date de vaccination pour la 2ème dose : __ / __ / 2012
K11	Lieu de vaccination pour la 2ème dose : _____
K12	Avez-vous recraché ou vomi en prenant la 2ème dose ? Non 0[_] Oui 1[_] Ne sait pas 9[_]
K13	D'après vous, une personne ayant reçu deux doses de vaccins contre le choléra peut-elle encore être malade du choléra ? Non 0[_] Oui 1[_] Ne sait pas 9[_]

INFORMATIONS SUR LES ENQUETEURS

Nom: …..... Signature: ……

INFORMATIONS SUR LES SUPERVISEURS

Nom: …..... Signature: ……

Annex 7. Characteristics of the case-patients and control-subjects: vaccine effectiveness study

Table 1. Characteristics of the case-patients and control-subjects included in the vaccine effectiveness study, Boffa and Forécariah, Guinea, 2012

	Controls		Cases		
	n	*(%)*	n	*(%)*	P value
Profession					0.18
Trader	29	*(18.1)*	8	*(20.0)*	
Farmer	37	*(23.1)*	16	*(40.0)*	
Pupil / student	29	*(18.1)*	3	*(7.5)*	
Fisherman	10	*(6.3)*	3	*(7.5)*	
Housewife	10	*(6.3)*	1	*(2.5)*	
Unemployment	22	*(13.8)*	6	*(15.0)*	
Other	23	*(14.4)*	3	*(7.5)*	
Head of the household's educational degree					0.13
Non	43	*(27.2)*	13	*(32.5)*	
Primary	5	*(3.2)*	4	*(10.0)*	
Secondary	21	*(13.3)*	2	*(5.0)*	
University	5	*(3.2)*	0	*(0.0)*	
Literate	84	*(53.2)*	21	*(52.5)*	
Radio	113	*(70.6)*	27	*(67.5)*	0.68
Bicycle	82	*(51.2)*	19	*(47.5)*	0.64
Telephone	128	*(80.0)*	27	*(67.5)*	0.10
Generator	36	*(22.5)*	6	*(15.0)*	0.28
Television	36	*(22.5)*	6	*(15.0)*	0.27
Fridge	1	*(0.6)*	0	*(0.0)*	0.50
Boat	26	*(16.3)*	9	*(22.5)*	0.29
Household size					0.06
0-4 members	34	*(21.3)*	17	*(42.5)*	
5-7 members	40	*(25.0)*	7	*(17.5)*	
8-12 members	49	*(30.6)*	9	*(22.5)*	
>12 members	37	*(23.1)*	7	*(17.5)*	

	Controls		Cases		
	n	*(%)*	n	*(%)*	P value
Proportion of children attending school in the household					0.60
None of them	29	*(17.6)*	4	*(9.8)*	
Less than half	63	*(38.2)*	17	*(41.5)*	
More than half	54	*(32.7)*	13	*(31.7)*	
All of them	19	*(11.5)*	7	*(17.1)*	
Distance to the closet health center					0.15
Need of transport	51	*(29.7)*	17	*(39.5)*	
Walking distance	121	*(70.3)*	26	*(60.5)*	
Other cholera cases in the household	6	*(3.5)*	2	*(4.7)*	0.69
Travelling or receiving a visit in the last week	35	*(20.3)*	11	*(25.6)*	0.34
Participation in a burial ceremony	2	*(1.2)*	1	*(2.3)*	0.23
Water source					0.11
Pump	84	*(48.8)*	20	*(46.5)*	
Protected well	39	*(22.7)*	14	*(32.6)*	
Unprotected well	6	*(3.5)*	2	*(4.7)*	
Water from natural source	42	*(24.4)*	7	*(16.3)*	
Other	1	*(0.6)*	0	*(0.0)*	
Treatment of the drinking water	43	*(25.4)*	11	*(25.6)*	0.66
Recipient to store drinking water with a lid	170	*(98.8)*	43	*(100.0)*	0.35
Eating food in a public space					0.21
Never	117	*(68.0)*	28	*(65.1)*	
Sometimes	27	*(15.7)*	10	*(23.3)*	
Soap available in the household	113	*(65.7)*	31	*(72.1)*	0.29
Washing hands before eating	144	*(83.7)*	37	*(86.0)*	0.83
Washing hands after eating	87	*(50.6)*	22	*(51.2)*	0.69
Washing hands after going to the toilet	93	*(54.1)*	25	*(58.1)*	0.47
Washing hands after cleaning a baby after defecation	16	*(9.3)*	3	*(7.0)*	0.59
Washing hands before cooking	23	*(13.4)*	6	*(14.0)*	0.91

	Controls		Cases		
	n	*(%)*	n	*(%)*	P value
Usual place of defecation					0.12
Latrine	81	*(50.6)*	17	*(42.5)*	
Pit in the yard	56	*(35.0)*	14	*(35.0)*	
In the ground	23	*(14.4)*	9	*(22.5)*	
Sharing the latrine					0.71
Just for the household	31	*(22.3)*	6	*(18.8)*	
Several households	59	*(42.4)*	13	*(40.6)*	
Anybody	49	*(35.3)*	13	*(40.6)*	
Sharing the latrine with someone suffering from cholera	5	*(3.7)*	6	*(20.0)*	0.001
Flooding latrine	13	*(9.5)*	4	*(12.9)*	0.54

Annex 7. Characteristics of the non-cholera watery diarrhea case-patients and control-subjects: inicator bias analysis.

Table 2. Characteristics of the non-cholera watery diarrhea case-patients and control-subjects included in the indicator bias analysis, Boffa and Forécariah, Guinea, 2012.

	Controls		Cases		
	n	*(%)*	n	*(%)*	P value
Profession					0.50
Trader	22	*(12.8)*	8	*(18.6)*	
Farmer	48	*(27.9)*	9	*(20.9)*	
Pupil / student	19	*(11.0)*	3	*(7.0)*	
Fisherman	5	*(2.9)*	2	*(4.7)*	
Housewife	26	*(15.1)*	8	*(18.6)*	
Unemployment	36	*(20.9)*	11	*(25.6)*	
Other	16	*(9.3)*	2	*(4.7)*	
Head of the household's educational degree					0.24
Non	34	*(19.9)*	13	*(31.0)*	
Primary	16	*(9.4)*	3	*(7.1)*	
Secondary	11	*(6.4)*	4	*(9.5)*	
University	5	*(2.9)*	3	*(7.1)*	
Literate	105	*(61.4)*	19	*(45.2)*	
Radio	123	*(71.5)*	28	*(65.1)*	0.30
Bicycle	91	*(52.9)*	21	*(48.8)*	0.55
Telephone	124	*(72.1)*	31	*(72.1)*	1.00
Generator	27	*(15.7)*	11	*(25.6)*	0.20
Television	23	*(13.4)*	13	*(30.2)*	0.03
Fridge	1	*(0.6)*	1	*(2.3)*	0.35
Boat	31	*(18.0)*	8	*(18.6)*	0.71
Household size					0.61
0-4 members	23	*(13.5)*	3	*(7.0)*	
5-7 members	41	*(24.1)*	9	*(20.9)*	
8-12 members	57	*(33.5)*	15	*(34.9)*	
>12 members	49	*(28.8)*	16	*(37.2)*	

	Controls		Cases		
	n	*(%)*	n	*(%)*	P value
Proportion of children attending school in the household					0.13
None of them	33	*(22.9)*	14	*(37.8)*	
Less than half	42	*(29.2)*	11	*(29.7)*	
More than half	51	*(35.4)*	8	*(21.6)*	
All of them	18	*(12.5)*	4	*(10.8)*	
Distance to the closet health center					0.10
Need of transport	107	*(66.9)*	31	*(77.5)*	
Walking distance	53	*(33.1)*	9	*(22.5)*	
Other cholera cases in the household	4	*(2.5)*	3	*(7.5)*	0.15
Travelling or receiving a visit in the last week	42	*(26.3)*	13	*(32.5)*	0.41
Participation in a burial ceremony	3	*(1.9)*	0	*(0.0)*	-
Water source					0.98
Pump	63	*(39.4)*	17	*(42.5)*	
Protected well	21	*(13.1)*	5	*(12.5)*	
Unprotected well	10	*(6.3)*	2	*(5.0)*	
Water from natural source	47	*(29.4)*	11	*(27.5)*	
Other	19	*(11.9)*	5	*(12.5)*	
Treatment of the drinking water	34	*(21.3)*	5	*(12.8)*	0.15
Recipient to store drinking water with a lid	158	*(98.8)*	40	*(100.0)*	0.35
Eating food in a public space					0.02
Never	72	*(45.0)*	11	*(28.2)*	
Sometimes	49	*(30.6)*	20	*(51.3)*	
Soap available in the household	78	*(49.1)*	16	*(40.0)*	0.30
Washing hands before eating	143	*(89.4)*	33	*(82.5)*	0.22
Washing hands after eating	24	*(15.0)*	4	*(10.0)*	0.37
Washing hands after going to the toilet	72	*(45.0)*	17	*(42.5)*	0.77
Washing hands after cleaning a baby after defecation	12	*(7.5)*	1	*(2.5)*	0.20
Washing hands before cooking	21	*(13.1)*	5	*(12.5)*	0.90

	Controls		Cases		
	n	*(%)*	n	*(%)*	P value
Usual place of defecation					0.28
Latrine	61	*(35.5)*	15	*(34.9)*	
Pit in the yard	81	*(47.1)*	17	*(39.5)*	
In the ground	30	*(17.4)*	11	*(25.6)*	
Sharing the latrine					0.17
Just for the household	73	*(48.7)*	13	*(38.2)*	
Several households	48	*(32.0)*	11	*(32.4)*	
Anybody	29	*(19.3)*	10	*(29.4)*	
Sharing the latrine with someone suffering from cholera	9	*(7.1)*	5	*(16.7)*	0.23
Flooding latrine	11	*(7.4)*	4	*(11.8)*	0.39

Annex 9. Vaccine coverage by age and sex of the cholera mass vaccination campaign in Boffa and Forécariah prefectures.

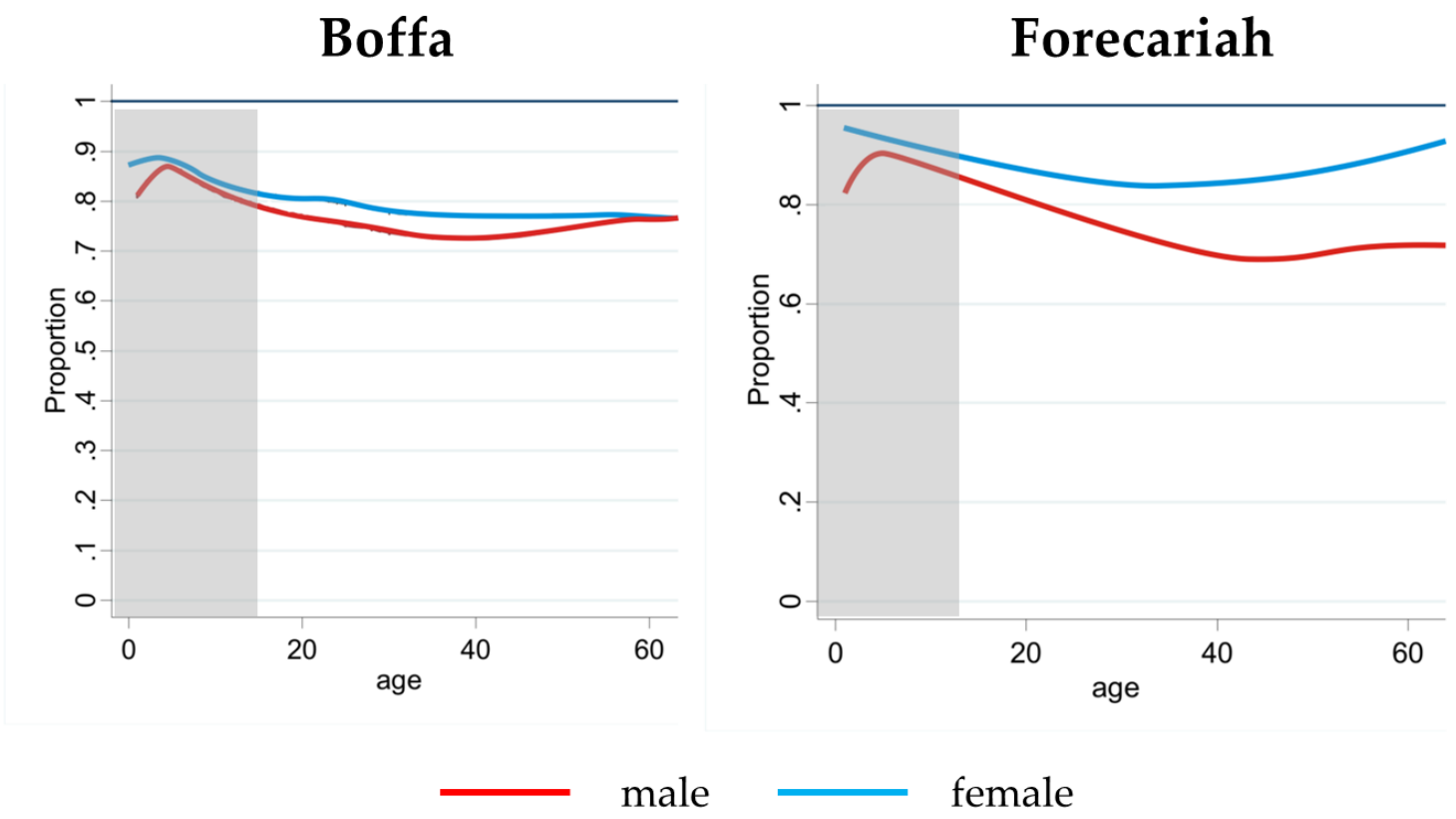


Figure 1: Vaccine coverage by age and sex of the cholera mass vaccination campaign in Boffa and Forécariah prefecture, first round, second round and two doses (fully vaccinated), April-June 2012.

Annex 10. Geographical distribution of the vaccine coverage in Boffa and Forécariah prefectures.

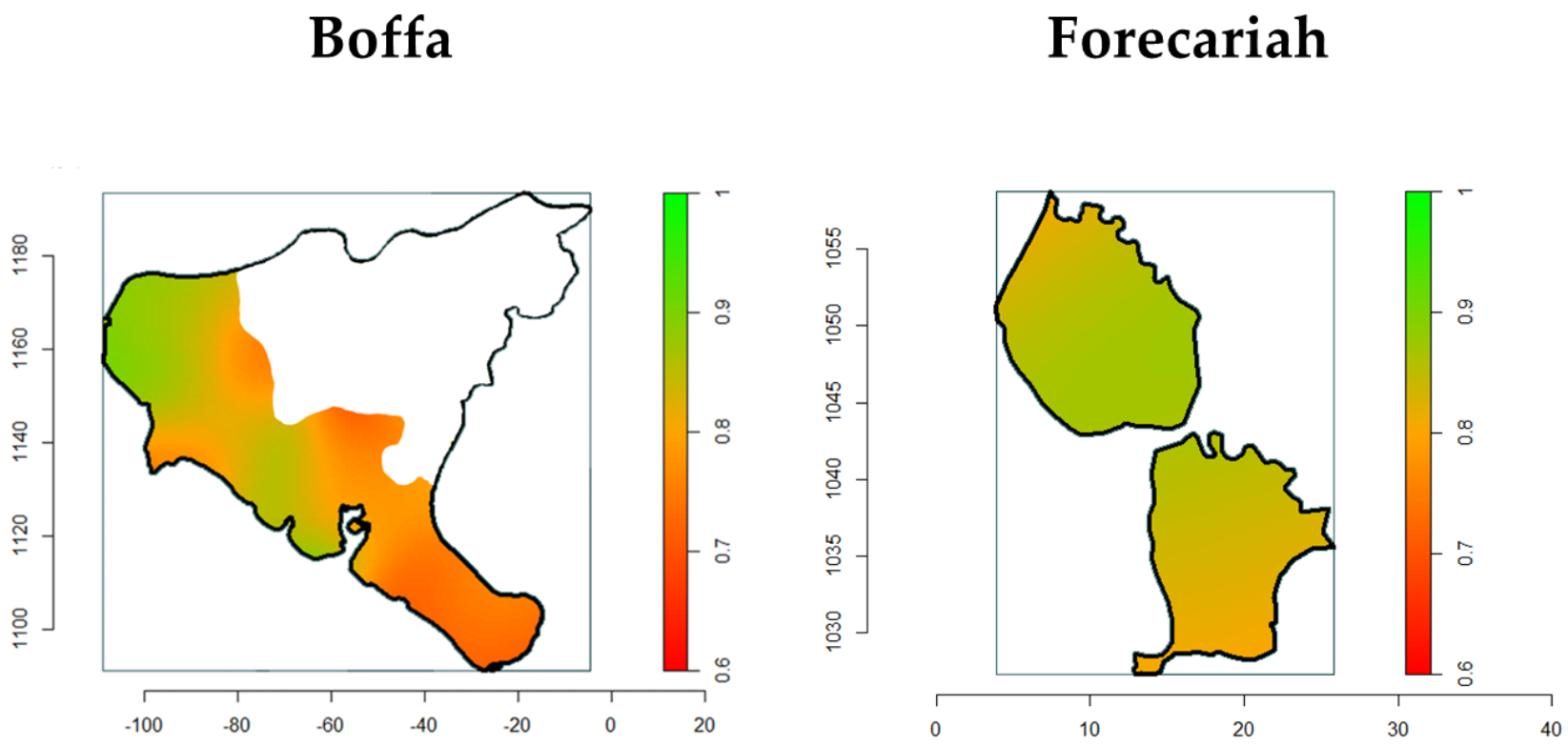


Figure 2: Vaccine coverage by age and sex of the cholera mass vaccination campaign in Boffa and Forécariah prefecture, first round, second round and two doses (fully vaccinated), April-June 2012.

* The estimates of the spatial distribution of the vaccine coverage were obtanied applying a Gaussian kernel function to the point estimate of each cluster. The analysis were conducting using the package "spatstat" of R software v.2.10.