

Vaccines to Prevent Cholera

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Introduction

Cholera, the acute diarrheal disease caused by *Vibrio cholerae* serogroups O1 and occasionally O139, is of cardinal public health importance because of the severity of the clinical illness it can cause (“cholera gravis”, leading to death if untreated), its explosive epidemic behavior and its propensity to occur in extensive pandemics involving many countries over many years. The oral cholera vaccines that have become available in recent years are employed to dampen the intensity of seasonal disease in endemic areas, protect high risk populations such as refugees interned in camps in cholera-endemic or cholera-proximal areas, and protect travelers from cholera-free countries/regions who must travel to countries/regions where cholera is epidemic or endemic. The remaining potential use of cholera vaccines, arguably the most important, aims to control large explosive epidemics in immunologically-naïve populations (so called “virgin soil” epidemics) such as when cholera returned to South America in 1991 after a century of absence and the 2010 outbreak in Haiti that followed a devastating earthquake.^{1,2} Virgin soil epidemics severely strain the resources of national and local public health authorities and disrupt civil society. The control of such epidemics demands a vaccine that can confer a high level of protection upon immunologically-naïve persons within just a few days of administration of a single dose. One of the new vaccines has characteristics (single dose, protection as early as 8–10 days following vaccination) that may allow it to be amenable to the control of virgin soil epidemics,³ particularly when such epidemics accompany complex emergencies (earthquakes, floods, wars).

Etiologic Agents

Circa 206 O serogroups of *V. cholerae* are recognized but only two, O1 and O139, routinely express cholera enterotoxin and attachment pili and cause epidemic cholera.⁴ Within the O1 serogroup there are two main serotypes, Inaba and Ogawa; a third serotype, Hikojima, is rare. Serogroup O1 strains are also classified into two biotypes, classical or El Tor. Almost all cholera disease currently occurring in the world is due to variants of the El Tor biotype. Emerging El Tor variants have been identified that express classical biotype cholera enterotoxin and sometimes classical toxin co-regulated pili (TCP), the organelles by which *V. cholerae* attaches to enterocytes as a key step in the pathogenesis of cholera.⁵⁻⁸ These “El Tor hybrids” expressing classical enterotoxin may cause more severe clinical disease than bona fide El Tor strains.⁹

Epidemiology

The Ganges River delta in South Asia is the ancestral home of cholera where between pandemics it persists as “Asiatic cholera.” The seventh pandemic of cholera, due to *V. cholerae* O1 El Tor, originated in the early 1960s on the island of Sulawesi, Indonesia, and progressively spread in waves over the ensuing six decades to involve at one time or another almost all of the world’s developing and transitional countries;⁴ in many it has remained endemic in sub-populations and niches.⁴ Thus, cholera is now endemic in many countries of South and Southeast Asia, sub-Saharan Africa and a few countries in the Americas. During the early 1990s it was endemic for several years in Peru, Ecuador, and some other Latin American countries.^{10,11}

When cholera invades new territory with immunologically-naive populations, the highest incidence of disease is observed in young adult males. If the disease becomes endemic, the incidence increases in women and children and eventually peak incidence is observed in young children. Cholera exhibits a seasonal pattern almost everywhere that it is endemic.¹² When the new season begins, cholera cases emerge simultaneously in multiple geographically separate foci. This pattern has also been observed when cholera invades new territory. In 1991, when cholera re-invaded South America with an explosive and extensive epidemic in Peru, large outbreaks appeared almost simultaneously in three distinct cities spanning a 900-kilometer stretch of the Pacific Coast.¹² The explosive increase of cases observed at the onset of many epidemics may be the consequence of hyperinfective vibrios released into water sources lacking vibriophages. Conversely, curtailment of the epidemic may be the consequence of an increased prevalence of lytic phages in the water.^{13,14}

Reservoirs of Infection. Humans are the sole known natural host of *V. cholerae* O1 cholera disease and chronic carriers are rare.^{15,16} Thus, it was previously assumed that in endemic areas mild and asymptomatic infections served as the reservoir to maintain the disease until the next cholera season when conditions would once again favor enhanced transmission. However, epidemiologic observations in the 1970s refuted this assumption and ushered in a new understanding of cholera epidemiology that clarified much of the epidemiologic behavior that previously had been puzzling. Confirmation of a single case of cholera in Texas in 1973 in a fisherman caused by an unusual highly hemolytic El Tor Inaba strain,¹⁷ followed 5 years later by an outbreak of approximately two dozen cases of the identical strain in which poorly cooked seafood was incriminated as the vehicle,¹⁸ led to identification of an environmental focus of infection along the Gulf of Mexico coast of the U.S.A.¹⁹ This El Tor Inaba strain was found to constitute autochthonous flora of the brackish waters of Gulf estuaries, where it was associated with crustacea (shrimp, etc.) eaten as local seafood. Identification of a similar environmental focus of free-living enterotoxigenic *V. cholerae* O1 El Tor in Queensland, Australia, supports the hypothesis that brackish water environmental niches can serve as the reservoir of *V. cholerae* O1.²⁰

V. cholerae can enter a “viable but nonculturable” state that allows them to survive harsh environmental conditions through a form of bacterial hibernation.^{16,21} When the toxigenic *V. cholerae* eventually encounter favorable conditions of temperature, salinity and pH, they can rejuvenate, regaining the potential to actively metabolize and grow.²¹ These may also be the conditions under which zooplankton blooms occur.

Modes of Transmission. Our practical knowledge of the vehicles of transmission of cholera stems from case-control investigations that have documented waterborne transmission and an array of food vehicles.^{22,23} When El Tor cholera struck the Pacific coast of several Andean countries of South America in 1991, improperly functioning municipal water supplies and sewage systems, contaminated surface waters, and unsafe domestic water storage methods fostered facile waterborne cholera transmission.^{1,24} Beverages prepared with contaminated water and sold by street vendors, ice, and even commercial bottled water have been incriminated.²⁵

V. cholerae O1 may be associated with seafood vehicles by means of their natural adherence to the chitinous exoskeletons of shrimp, crabs, and oysters in certain estuarine environments,^{18,21,26} or food may be secondarily contaminated during preparation or handling.²⁷ The most commonly implicated food vehicle worldwide has been raw or undercooked seafood, including mussels, shrimps, oysters, clams, cockles, fish, salt fish, and ceviche (uncooked fish or shellfish marinated in lemon or lime juice).

Cooked grains, rice and beans with sauces have also been incriminated in cholera transmission, particularly in Africa. A small inoculum of enterotoxigenic *V. cholerae* O1 introduced by an infected food handler into one of these types of food and stored without refrigeration can increase by several logs within 8 to 12 hours. Cholera has also been transmitted by vegetables and fruit irrigated with raw sewage or “freshened” by dousing with sewage-contaminated water.²⁸

During outbreaks or seasonal epidemics, cholera may spread via multiple modes of transmission. Depending on local customs, climate, and other factors, particular modes and vehicles of transmission predominate.²⁹ Finally, if pathogenic *V. cholerae* O1 and O139 persist in environmental reservoirs, then transmission across long distances can occur via the ballast water of large ships, as they intake ballast water in one port and discharge it prior to entering another port thousands of miles away.³⁰

Epidemiologists recognize that person-to-person contact spread of cholera virtually never occurs. Transmission is essentially always via food or water vehicles.

It has been hypothesized that for a few hours after being shed in enormous numbers by cholera patients purging rice water stools, toxigenic *V. cholerae* remain in a hyper-transmissible state.^{13,14,31} Thus, if a case of severe cholera occurs in a crowded setting where other susceptible human hosts and facile modes of transmission exist, the infectious dose may be unusually low and spread of disease may be explosive.¹³

Host Risk Factors. Certain host factors markedly increase the risk of developing cholera gravis, including O blood group,^{32,33} hypochlorhydria,^{34,35} and a lack of background immunity.³⁶ Persons of blood group O are at increased risk of developing cholera gravis than persons of other blood groups. When cholera invades a new territory with an immunologically naive population, persons with hypochlorhydria from partial gastrectomy, *Helicobacter pylori* chronic gastritis, etc., have frequently been the index case.³⁷ The highest incidence of cholera in endemic areas is often children 1 to 4 years of age. The age-specific incidence falls thereafter and the prevalence and geometric mean titer of serum vibriocidal antibody rise, as increasing immunity is acquired.³⁸ One interesting exception to this pattern is women of childbearing age who exhibit an inordinately high incidence.¹²

International Surveillance and Disease Notification. Since cholera was the disease for which modern public health surveillance and reporting was first organized, it bears the code 001 in the international classification of diseases. By international convention, cholera is a notifiable disease along with plague and yellow fever. In 2014, 190,549 cases of cholera were reported to the World Health Organization (WHO) from 42 countries; 55% were from Africa and 15% from the Americas. The true number of cholera cases globally is much higher and the annual burden is estimated to be 1.4–4.0 million cases and 21,000–143,000 deaths. For reasons involving trade, fear of food embargoes and effects on tourism, many countries delay reporting cholera cases to the WHO or do not report at all. For example, international health statistics in the late 1980s and 1990s indicated that Bangladesh had little or no cholera. Yet at the same time large-scale field trials to evaluate cholera vaccines were carried out in which hundreds of confirmed cases were documented.³⁹

The Disease

Cholera infection exhibits a spectrum of clinical illness ranging from asymptomatic shedding of vibrios in the stool to life-threatening watery diarrhea accompanied by overt severe dehydration (cholera gravis). Up to three-quarters of cholera infections may be sub-clinical, and among symptomatic patients only a minority may manifest severe purging. The propensity to develop cholera gravis is strongly associated with two host risk factors: blood group O and hypochlorhydria. If the prodigious losses of body water and electrolytes are not promptly replaced in cholera patients who are actively purging "rice water stools" (e.g., at the rate of one liter per hour in an adult), the patient may rapidly dehydrate, suffer renal shutdown, shock and acidosis, and die within hours of the onset of illness. Patients with cholera gravis exhibit the classic signs and symptoms of severe dehydration including weak or absent peripheral pulses, hypotension, sunken eyes, loss of skin turgor, and decreased urine output. Table 1 compares the concentrations of serum electrolytes in normal adult serum and in rice water stools of adults with cholera gravis. The purging of large volumes of rice water stools as evident in cholera gravis is physiologically equivalent to loss of plasma leading to hemoconcentration, hypovolemia, hypotension, decreased renal blood flow, and overt hypovolemic shock.

Table 1. Concentrations of Electrolytes in Normal Adult Sera and in the Rice Water Stools of Adults with Cholera Gravis

	Normal Adult Serum	Rice Water Stools From Cholera Gravis Patients
Na+	135-145 mEq/ml	135 mEq/ml
K+	135-145 mEq/ml	15 mEq/ml
Cl-	95-105 mEq/ml	100 mEq/ml
HCO ₃	24-30 mEq/ml	40 mEq/ml

Pathogenesis and Immunity

V. cholerae O1 comprises a sophisticated, multi-step, delivery system for cholera toxin, the virulence attribute responsible for the severe purging of voluminous watery stools characteristic of cholera gravis. In volunteers the ingestion of as little 5 mcg of purified cholera enterotoxin can induce diarrheal illness and 5 mcg has led to a clinical syndrome that closely resembles the severe purging of cholera gravis.⁴⁰ Subsequent volunteer studies with *V. cholerae* O1 vaccine strains that harbored deletions in genes encoding the enzymatically active (A) subunit, both A and B (binding) subunits of cholera toxin or the entire cholera toxin virulence cassette (which encodes two other toxins and a minor colonization factor) showed that some strains retained the ability to cause mild diarrhea and other gastrointestinal symptoms,⁴¹ possibly by invoking intestinal inflammation.^{42,43} Whereas ingestion of purified cholera toxin alone can induce a syndrome of severe purging, the fully pathogenic vibrios that cause natural cholera encode multiple virulence factors that direct a stepwise progression to severe diarrhea.

Following ingestion, pathogenic *V. cholerae* O1 or O139 must survive the formidable gastric acid barrier and transit the pylorus to reach the proximal small intestine, the critical site of host-parasite interaction. Ingestion without buffer of 10⁶ viable pathogenic *V. cholerae* by fasting North American volunteers resulted in neither infection nor diarrhea because the vibrios were destroyed by gastric acid.⁴⁴ In contrast, when 10⁶ vibrios are administered with sodium bicarbonate buffer or food that protects the vibrios during gastric transit, cholera

develops in approximately 90% of the volunteers.⁴⁴ Indeed, when administered with buffer, as few as 10^3 *V. cholerae* O1 El Tor cause diarrhea in ~67% of volunteers,⁴⁴ although the stool volume is less than in subjects who ingest higher doses of vibrios.

Once in the small intestine, the vibrios sense their environment by means of ToxR, a protein that is the product of a master regulatory gene, *toxR*.⁴⁵ Activation of *toxR* leads to expression of cholera toxin and toxin coregulated pili (TCP), the key intestinal colonization factor,^{46,47} and to the indirect activation (via *toxT*) of approximately 17 other genes involved with bacterial adaptation to survival in the human intestine. As neuraminidase and other vibrio enzymes break down the mucus barrier on the surface of the intestine, motility plays a critical role as the unipolar flagellum propels the organisms toward the enterocyte surface, attracted by chemotactins.

TCP constitutes the major intestinal colonization factor for *V. cholerae* O1 and O139.^{46,47} TCP of El Tor and O139 are genetically and antigenically identical but differ somewhat from TCP of classical biotype. Genes for TCP biogenesis are found within a 40-Kb *Vibrio* Pathogenicity Island (VPI). A mutant strain of *V. cholerae* O1, unable to express TCP, was unable to colonize the intestine of volunteers or to stimulate good vibriocidal antibody responses.⁴⁸

Experimental challenge studies in volunteers showed that a single episode of clinical cholera due to either serotype (Inaba or Ogawa) within a biotype stimulated 90-100% protection against clinical illness upon subsequent experimental challenge with either the homologous or heterologous serotype of *V. cholerae* O1 and the protection elicited by classical biotype infection endured for at least three years.^{44,49,50} These observations of potent infection-derived immunity were corroborated in the field with natural cholera illness,^{12,51} refuting early suggestions that an initial episode of cholera elicited little or only short-lived protection.⁵²

Immune Response

Following *V. cholerae* O1 infection, robust serum vibriocidal antibody responses and rises in immunoglobulin G (IgG) cholera antitoxin are observed.^{53,54} Approximately 90% of complement-dependent vibriocidal antibodies are directed toward the O antigen with the remaining 10% of antibodies directed against protein antigens. In immunologically primed individuals, strong secretory IgA (SIgA) intestinal antibody responses are recorded following cholera infection. However, significant rises in SIgA anti-LPS and antitoxin are surprisingly sparse in nonprimed individuals. The detection of gut-derived, trafficking IgA antibody secreting cells that make specific antibody to LPS and CT antigens is a good measure of priming of the intestinal immune system.⁵⁵

Whereas infection-derived immunity to cholera is believed to be mediated by intestinal mucosal SIgA antibodies, curiously, serum vibriocidal antibodies are the best correlate of protection.^{36,56,57} These serum antibodies may be a proxy for the stimulation of intestinal antibodies. Serum anti-B subunit responses are more prominent in pediatric cholera patients, while serum antibody responses to LPS and TCP are more prominent in adults.⁵⁸ Whereas high titers of specific vibriocidal antibodies appear after *V. cholerae* O1 infection, vibriocidal responses following O139 infection are weak and rather nonspecific.⁵⁹ A correlate of protection for O139 cholera has not yet been identified.

Diagnosis

The diagnosis of cholera is confirmed by isolating *Vibrio cholerae* from stool cultures on selective media such as thiosulfate-citrate-bilesalt-sucrose (TCBS) both directly and after enrichment in alkaline peptone water;⁶⁰ suspicious colonies are agglutinated with typing sera (directly or after sub-culture). Rapid non-culture tests that detect *V. cholerae* O1 and/or O139 lipopolysaccharide antigens are useful in field situations.⁶¹⁻⁶³

Treatment

Appropriate antimicrobials are an important adjunct to fluid therapy, as they diminish the volume and duration of purging and rapidly curtail the excretion of vibrios, thereby diminishing the chance of secondary transmission. Patients surviving from hypovolemic shock and severe dehydration manifest certain complications, such as hypoglycemia, that must be recognized and promptly treated. If these fundamental guidelines are followed properly, case fatality, even during explosive epidemics in developing countries, can be kept below 1%.^{64,65} Failure to comply with these basic proven clinical rules can result in unacceptably high case fatality.^{66,67}

Fluid Therapy. Patients suffering from severe dehydration of cholera with or without overt shock usually lose ~10% of their body weight and must be rapidly rehydrated with intravenous fluids. Fluid therapy is divided into two phases: (1) rehydration phase — the rapid replacement of water and electrolyte deficits, and (2) maintenance phase — the infusion of fluids to replace ongoing losses. Fluid and electrolyte deficits should be replenished as rapidly as possible (within 2–4 hours of initiation). The time recommended for rehydration in adult and pediatric patients is 3 and 6 hours, respectively. In adults, 30% of the total required fluid is administered in the first 30 minutes, while in children this volume is administered over one hour. Patients with cholera gravis generally require multiple liters of intravenous fluids to stabilize them to the point where oral rehydration can begin; at the earliest opportunity, they are carefully weaned from intravenous fluids. Adults with cholera gravis typically require 8–12 liters of intravenous fluids before oral hydration alone can keep up with losses. The most extensively used intravenous rehydration fluid worldwide for treatment of cholera is Ringer's lactate, because it is so widely available. Ringer's lactate contains Na⁺ 130 mEq/L, K⁺ 4 mEq/L, Ca⁺⁺ 3 mEq/L, Cl⁻ 111 mEq/L, and lactate (precursor of HCO₃⁻) 29 mEq/L. Because the concentration of K⁺ in Ringer's lactate is too low, supplemental K⁺ must be administered either by adding a sterile KCl (or similar potassium salt) solution to the Ringer's solution to increase the concentration of K⁺ to 15–20 mEq/L, or by initiating oral rehydration.

The volume of all diarrheal losses and vomitus must be measured in the patient with cholera. Once the patient has had replacement of his or her deficit and is in the stage of maintenance therapy, fluid management is generally based on 6-hour periods. The total fluid loss during the previous 6-hour period constitutes the volume of fluids that will be administered to the patient during the next 4–6 hours. As diarrheal losses begin to diminish, the 6-hourly replacement requirements decrease accordingly.

Aggressive rehydration therapy with fluid and electrolytes leads to rapid clinical improvement in the patient (e.g., elevation of blood pressure, stronger pulse, improved skin turgor, and enhanced consciousness) reflected in simple laboratory assays (e.g., fall in hematocrit and plasma specific gravity). Once renal perfusion is re-established normal homeostatic mechanisms begin to combat acidosis and regulate serum electrolyte concentrations.

Patients with mild or moderate dehydration and moderate purge rates (< 500 mL per hour) can generally be managed with oral rehydration alone. Oral rehydration therapy is based on the physiological fact that glucose-mediated cotransport of sodium and water across the mucosal surface of the small intestine epithelium remains intact during cholera infection despite the effect of cholera toxin.⁶⁸ If the diarrhea is copious, large volumes of oral rehydration fluids must be ingested to keep up with ongoing losses.

The oral rehydration solution (ORS) recommended by WHO for treatment of cholera is composed of Na⁺ 90 mEq/L, Cl⁻ 80 mEq/L, K⁺ 20 mEq/L, citrate⁻ 30 mEq/L, and glucose 111 mmol/L. Packets containing sufficient salts and glucose to prepare 1 liter of rehydration solution are widely available in developing countries. Each packet contains 3.5 g of NaCl, 2.9 g of sodium citrate, 1.5 g of KCl, and 20 g of glucose. In some Asian countries cereal-based oral rehydration solutions that provide multiple actively transported substrates are used to treat cholera;⁶⁹ some controlled trials showed no advantage over glucose-based ORS.⁷⁰ Reduced osmolarity rehydration solutions (Na⁺ 75 mEq/L, Cl⁻ 65 mEq/L, K⁺ 20 mEq/L, citrate⁻ 30 mEq/L and glucose 75 mmol/L) are controversial for treatment of cholera.⁷¹ Although the rate and volume of purging are reduced versus standard ORS, some patients develop hyponatremia (albeit usually asymptomatic).

The regimen for calculating the amount of oral rehydration solution to be administered to replace ongoing losses differs by age. Since the Na⁺ concentration in cholera stools is approximately 135 mEq/L in adults, one-and-a-half volumes of oral rehydration solution containing 90 mEq/L should be given for every volume of watery diarrheal stool passed in order to adequately replace Na⁺ losses. In contrast, in young children in whom the Na⁺ concentration of cholera stools is only approximately 100 mEq/L, ongoing losses can be replaced on the basis of a 1:1 ratio of oral rehydration solution to volume of diarrheal stool. There is a practical limit to the volume of oral rehydration solution that can be consumed on an hourly basis; in adults and teenagers the upper limit is approximately 750 mL/hour.

Antimicrobial Therapy. Appropriate antibiotics significantly decrease the duration of diarrhea, total diarrheal stool volume, and duration of excretion of *V. cholerae*, and therefore constitute an important adjunct to rehydration therapy. Resistance of *V. cholerae* O1 to commonly used antibiotics is increasing. Tetracycline and its long acting derivative, doxycycline, were used extensively in the past to treat cholera but resistance to these drugs in endemic areas in Asia and Africa has decreased their utility. Nevertheless, they remain useful where monitoring of vibrio strains documents their sensitivity. The regimen for teenagers and adults is 500 mg four times daily for 3 to 5 days and the pediatric dosage for tetracycline is 50 mg/kg/day in four divided doses for 3 to 5 days. Doxycycline requires only once daily administration (300 mg for adults and teenagers and 4 to 6 mg/kg for children, for 3 to 5 days). The very short course of tetracycline therapy used for the treatment of cholera precludes staining of teeth and other adverse reactions otherwise encountered with long courses of this antibiotic.

In areas where tetracycline-resistant *V. cholerae* are prevalent ciprofloxacin 250 mg once daily for 3 days is the preferred regimen;⁷² some, but not all, trials with single-dose ciprofloxacin have also given good results.⁷³⁻⁷⁵ Single-dose azithromycin (1 g in adults) has been shown to be effective in treating cholera in both adults and children. In one randomized, controlled clinical trial, a single dose of azithromycin (20 mg/kg, maximum dose 1 g) was as effective as three days of erythromycin therapy (12.5 mg/kg every 6 hours).⁷⁶ Trimethoprim-sulfamethoxazole use should be avoided in areas where O139 is known to be prevalent, since *V. cholerae* O139 is typically resistant to this antimicrobial.⁷⁷ During epidemics in developing countries, single-day or single-dose antibiotic therapy (such as 1 g of ciprofloxacin or 300 mg of doxycycline for adults or 1 gm of azithromycin) may be necessary in resource constrained settings,^{75,78} particularly if antibiotics are in short supply. However, the concern with single-dose therapy is that this may accelerate the emergence of resistance.

Cholera Vaccines

There are currently four licensed cholera vaccines, all administered orally, including:

1. Dukoral® (Crucell) consists of a mix of killed whole cell *V. cholerae* O1 bacteria of both biotypes and serotypes plus 1 mg of cholera toxin B subunit.^{79,80}
2. Shanchol™ (Shanta, Hyderabad, India) contains a mix of killed vibrios of both O1 (both biotypes and serotypes) and O139 *V. cholerae*.^{81,82}
3. Euvichol® Plus (Eubiologics, Seoul, Korea) contains the identical formulation of vibrios as Shanchol and Euvichol but in a simple, highly practical presentation.⁸³
4. Vaxchora® (PaxVax Bermuda, Ltd., Hamilton, Bermuda [part of PaxVax, Redwood City, CA) live single-dose oral cholera vaccine consists of genetically-engineered *V. cholerae* O1 strain CVD 103-HgR.^{3,84,85}

A detailed comparison of the salient features of these vaccines is summarized in Table 2.

Table 2. Salient Characteristics of Four Licensed Oral Cholera Vaccines

Parameter of Comparison	Dukoral	Shanchol	Euvichol Plus	Vaxchora (CVD 103-HgR)	PxVx0200 (CVD 103-HgR) high dose (~10 ⁹ cfu)
Components	Heat inactivated <i>V. cholerae</i> O1 classical Inaba (2.5x10 ¹⁰), classical Ogawa (2.5x10 ¹⁰), formalin-inactivated classical Ogawa (2.5x10 ¹⁰), formalin-inactivated El Tor Inaba (2.5x10 ¹⁰) and 1 mg of recombinant cholera toxin B subunit suspended in 3 ml of buffer	Heat inactivated <i>V. cholerae</i> O1 classical Inaba (2.5x10 ¹⁰), classical Ogawa (2.5x10 ¹⁰), formalin-inactivated classical Ogawa (2.5x10 ¹⁰), formalin-inactivated El Tor Inaba (2.5x10 ¹⁰) and 1 mg of recombinant cholera toxin B subunit suspended in 1.5 ml of buffer	Heat inactivated <i>V. cholerae</i> O1 classical Inaba (300 Elisa units [EU]), classical Ogawa (300 EU), formalin-inactivated classical Ogawa (300 EU), formalin-inactivated El Tor Inaba (300 EU) and formalin-inactivated O139 (300 EU) suspended in 1.5 ml of buffer	Recombinant <i>V. cholerae</i> O1 classical Inaba strain CVD 103-HgR with deletion of <i>ctxA</i> and insertion of a Hg ⁺⁺ resistance marker in <i>hlyA</i> (inactivating Hemolysin A) (~10 ⁸ colony forming units [cfu])	Recombinant <i>V. cholerae</i> O1 classical Inaba strain CVD 103-HgR with deletion of <i>ctxA</i> and insertion of a Hg ⁺⁺ resistance marker in <i>hlyA</i> (inactivating Hemolysin A) (~10 ⁹ colony forming units [cfu])
No. of doses	2	2	2	1	1
Interval between doses	2 weeks	2 weeks	2 weeks	-	-
Well tolerated	Yes	Yes	Yes	Yes	Yes
Efficacy or effectiveness in endemic populations	~ 50%	~ 65%	~ 65% (by extrapolation from Shanchol)	The high-dose (10 ⁹ cfu) formulation will be used in endemic populations	Yes. (79% efficacy by extrapolation from Orochol E) ⁹⁷
Efficacy in industrialized country adults	Yes	No	No	Yes ^{3,85}	Yes (by extrapolation from Vaxchora ^{3,85})

Parameter of Comparison	Dukoral	Shanchol	Euvichol Plus	Vaxchora (CVD 103-HgR)	PxVx0200 (CVD 103-HgR) high dose (~ 10 ⁹ cfu)
Duration of efficacy	3-4 yrs ¹⁰⁶	5 years ⁹²	Extrapolation from Shanchol data	At least 6 months (by extrapolation from Mutacol) ⁹⁶	At least 6 months (by extrapolation from Mutacol)
Onset of efficacy following first dose	Not known. Likely ≥ 21 days	Not known. Likely ≥ 21 days	Not known. Likely ≥ 21 days	8–10 days ^{3,96}	8–10 days ^{3,96}
Herd immunity	Yes	Yes	Likely	Likely	Likely
Boostable immune responses	Yes	Yes	Extrapolation from Shanchol data	Yes, but only after at least 4 months following primary immunization	Yes, but only after at least 4 months following primary immunization
Immunogenicity in toddlers and pre-school children	Yes	Yes	Extrapolation from Shanchol data	Yes (extrapolation from Orochol E data) ¹⁰⁷⁻¹⁰⁹	Yes (extrapolation from Orochol E data) ¹⁰⁷⁻¹⁰⁹
Efficacy in toddlers and pre-school children	Yes	Yes (lower than in older children and adults)	Extrapolation from Shanchol	?	Age ≥2 years (extrapolation from Orochol E data ⁹⁷)
Safety & immunogenicity in pregnant women	Yes ¹¹⁰	Yes ¹¹¹	Extrapolation from Shanchol	?	?
Safety & immunogenicity in HIV-positive persons	Yes	Yes ¹¹²	Extrapolation from Shanchol	Yes (extrapolation from Orochol E data)	Yes
Presentation	Liquid suspension of vaccine in a glass vial containing a single dose and accompanied by an aluminum foil sachet with buffer. The buffer sachet is emptied into a cup with 150 of cool water, stirred and the 3 ml of vaccine suspension is added and further mixed. For children (age 2 years and above, one-half of the 150 ml buffer solution should be discarded (leaving 75 ml) before adding the 3 ml of vaccine.	Liquid suspension of vaccine in glass vials containing a single dose. The cap of the vial is removed by hand or with a forceps and the 1.5 ml contents of the vial are transferred to the mouth of the vaccinee.	Liquid suspension of vaccine in plastic tubes with easily removal tips for direct transfer of the 1.5 ml of liquid vaccine directly into to the mouth of the vaccinee.	Double sachets, one sachet containing lyophilized vaccine and the other sachet containing buffer powder. The contents of the buffer sachet is put into a cup and 100 ml of water is added and the suspension stirred. The contents of the vaccine sachet are then added to reconstitute the lyophilized vaccine. The resultant 100 ml vaccine cocktail is then ingested.	Double sachets, one sachet containing lyophilized vaccine and the other sachet containing buffer powder. The contents of the buffer sachet is put into a cup and 100 ml of water is added and the suspension stirred. The contents of the vaccine sachet are then added to reconstitute the lyophilized vaccine. The resultant 100 ml vaccine cocktail is then ingested.
Strategy for delivering the vaccine	Mostly via campaigns	Mostly via campaigns	Mostly via campaigns	Travel clinics	Mostly via campaigns

Non-Living Oral Vaccines. Dukoral is the commercial product of a non-living oral cholera vaccine prototype that was tested in U.S. volunteers and then in a randomized controlled field trial in Bangladesh in the 1980s.^{39,86} The prototype vaccine contained purified B subunit prepared from holotoxin by biochemical separation of the B subunit from the toxic A subunit. The current commercial formulation, Dukoral, contains recombinant B subunit.⁸⁷ Dukoral has been shown to be well tolerated and protective against cholera in post-licensure evaluations.^{88,89} The B subunit enhances Dukoral's anti-bacterial immunity by adding antitoxic immunity that is also effective against enterotoxigenic *Escherichia coli* producing heat-labile enterotoxin; however, the additive protection of antitoxic immunity is short-lived, lasting only 4-6 months.^{90,91} Dukoral, administered as two doses 2 weeks apart, is used by European and Canadian travelers for protection against travelers' diarrhea caused by LT-producing *E.coli*. Although Dukoral has been pre-qualified by the World Health Organization for procurement by U.N. agencies, heretofore it has been little used for control of endemic or epidemic cholera other than in demonstration projects.

Shanchol demonstrated its ability to diminish the incidence of cholera in highly endemic neighborhoods of Kolkata, India.⁹² Two doses of Shanchol administered two weeks apart conferred 65% efficacy (95% CI, 52–74%) against cholera overall (all ages combined).⁹² However, there was a clear hierarchy of protection with young children 1–4 years of age (who suffer the highest incidence of cholera) having the lowest level of efficacy. Over the 5 years of surveillance, the efficacy was 75% in persons ≥ 15 years of age, 68% in children age 5–14 years, and 42% in children 1–4 years of age at the time of enrollment and vaccination.⁹² The impact of prior immunologic priming was evident during the first year of follow-up when the point estimate of efficacy was only 17% in children age 1–4 years but was 81% in older children age 5–14 years and 66% in individuals age 15 years and above.⁸² Shanchol was also efficacious in a nested case/control study following a mass vaccination to control seasonal cholera in Guinea;⁹³ this trial also highlighted the complexities of organizing reactive immunization campaigns and the desirability of a single-dose regimen.⁹⁴ A single-dose of Shanchol was systematically evaluated in a massive randomized placebo-controlled field trial in urban slums in Dhaka, Bangladesh. A single dose gave 63% (95% CI, -39–90%) protection among children 5–14 years of age, 56% (16–77) protection among persons \geq age 15 years but only 16% (-49–53%) efficacy among children <5 years of age.⁹⁵ The incidence of cholera in children <5 years (1.75/10⁵ person days) was 8.3-fold higher than among children 5–14 years (0.21/10⁵ person days) and 5.8-fold higher than among persons age ≥ 15 years, presumably indicating the ability of single-dose Shanchol to work well in persons with considerable prior background immunity to cholera but not performing well in immunologically less-primed hosts.⁹⁵ Heretofore, Shanchol has been the oral cholera vaccine most extensively utilized from the WHO cholera vaccine stockpile.

There are no pre-licensure efficacy or post-licensure effectiveness data yet on Euvichol or Euvichol Plus. They were licensed based on their identity of formulation to Shanchol and clear demonstration of non-inferiority in eliciting seroconversion of serum vibriocidal antibody titers.⁸³ Euvichol and Euvichol Plus received rapid WHO prequalification and will now be able to expand the supply of oral cholera vaccine in the WHO stockpile. In Table 2 it is assumed that Euvichol Plus will provide similar effectiveness as Shanchol.

Vaxchora™ (strain CVD 103-HgR) was licensed by the U.S. FDA in June 2016 and in the U.S.A. and other industrialized country markets will provide a single-dose, rapidly acting (strong protection evident in 8-10 days) oral cholera vaccine for immunologically-naïve persons who must travel on short notice to places of high risk.⁸⁵ CVD 103-HgR has a deletion of the gene encoding the enzymatically-active A subunit of cholera toxin, while leaving intact the immunogenic B subunit. It also has a Hg⁺⁺ resistance marker inserted into *hlyA*, thereby inactivating Hemolysin A expression. In persons from industrialized countries, a single oral dose containing $\sim 10^8$ colony forming units (cfu) of CVD 103-HgR is well tolerated, elicits serum vibriocidal antibody seroconversion in >90% of vaccinees, has only modest excretion (18–25% have positive coprocultures from day 1–4 post-vaccination) and confers 90% efficacy

against challenge with wild type *V. cholerae* O1 10 days after vaccination. Upon challenge at 3 months following ingestion of a single dose, 80% vaccine efficacy was recorded.³ The volunteer challenge studies with Vaxchora identified serconversion of vibriocidal antibody as a strong correlate of protection.³

CVD 103-HgR was originally manufactured by the now defunct Swiss Serum and Vaccine Institute and commercialized under the trade name Orochol® in many countries and as Mutacol® in Canada. This earlier formulation protected volunteers against challenge with *V. cholerae* O1 of either El Tor or classical biotype and either Inaba or Ogawa serotype and conferred protection against challenge as soon as 8 days and as long as 6 months after vaccination.⁹⁶ A formulation containing one-log higher vaccine organisms, Orochol E (~10⁹ cfu), was commercialized for use in developing countries.⁹⁷ The reason for the one-log higher dosage for developing country populations is that environmental enteropathy, which is highly prevalent in the low socioeconomic levels of the population at highest risk of cholera and other enteric infections, dampens the immune response to the live oral vaccine. The higher number of cfu per dose overcomes this intestinal barrier.⁹⁸⁻¹⁰⁰ The biology of the need for the higher dose in impoverished developing country populations has been reviewed.¹⁰¹

Orochol E was evaluated in a large-scale, randomized, placebo-controlled, double-blinded field trial in North Jakarta neighborhoods where cholera was hyperendemic.¹⁰² Randomization was at the level of the individual in the Jakarta trial when the critical role of indirect protection was not yet appreciated.^{103,104} In this venue the vaccine did not show evidence of significant protection but shortly after the enrollment and vaccination, cholera incidence dropped by >80% in what was previously a hyperendemic ecology. One interpretation is that the live oral vaccine via indirect protection lowered the overall incidence in the community to a point where efficacy could not be demonstrated but the cholera burden was greatly diminished for four years.¹⁰² Orochol E's ability to protect populations in developing countries was later shown in a post-licensure reactive vaccination undertaken by the WHO during a cholera epidemic in Micronesia where 79% vaccine efficacy was calculated.⁹⁷ Clinical trials have begun with a high-dose formulation of CVD 103-HgR (PXVX0200) prepared by the manufacturer of Vaxchora to explore its utility for reactive vaccination.^{85,105} In one preliminary study in Mali, West Africa, a single dose of the high-dose formulation was significantly more immunogenic in stimulating serum vibriocidal antibodies than one or two doses of Shanchol used as the immunologic comparator.¹⁰⁵

Prevention and Control

Safe Water and Food. Since enteric fever pathogens are typically acquired via the ingestion of contaminated water or food, enteric precautions should be taken when living or traveling in endemic areas. Only treated (boiled or chemically treated) water should be consumed. Foods that may be fecally contaminated (e.g., uncooked salad vegetables) should be avoided. Travelers to cholera-endemic areas should be particularly careful of eating seafood dishes unless they are cooked to a high temperature.

Conclusion

Both for the prevention of disease in populations in cholera-endemic countries and for travelers from industrialized countries to cholera-endemic and epidemic regions of the world, several new and improved oral vaccine options now exist to prevent cholera disease. The global supply of cholera vaccines is also increasing. Judicious use of these vaccines can diminish the risk of cholera worldwide.

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