

Promising Phase I/II Trial for Cuban Vaccine Candidate amidst Growing Cholera Threat

Text & Photo by Gail Reed

In August, the World Health Organization (WHO) published alarming data on the re-emergence of cholera in many African and Asian countries, noting that the outbreaks run “parallel with the ever-increasing proportion of vulnerable populations living in unsanitary conditions.” A total of 236,896 cases were notified to the WHO in 2006 from 52 countries, a case level not seen since the ,90s and representing an increase of 79% over 2005. Deaths reached more than 6,000, a three-fold increase from 2005. However, the WHO has long warned that cholera is widely under-reported, and in August renewed its description of the disease as “a global threat to public health and one of the key indicators of social development.” [1]

Until social determinants drastically improve for millions, oral cholera vaccines (OCV’s) may provide a life-saving opportunity to stem outbreaks – especially in high-risk areas where the case-fatality rate (CFR) reaches up to 30% – when they are used in conjunction with effective public health and clinical strategies in epidemic-prone settings. This is the current position of the WHO, a reversal of earlier thinking that doubted the value of cholera vaccines. However, due to several catastrophic epidemics in the 90s and new research, the WHO reassessed the vaccines’ potential in 2002.[2] Since then, others have concurred that OCV’s may become effective partners in rolling back the ever-more-aggressive *Vibrio cholerae* bacterium, causal agent of the disease.

In this context, results just in from a Cuban vaccine candidate’s Phase I/II trial show promise.

Vaccine Candidate:	<i>V. cholerae</i> 638
Characteristics:	Live oral attenuated El Tor Ogawa vaccine, constructed by deleting the entire CT generic element CTXΦ from <i>V. cholerae</i> 01 El Tor Ogawa strain C7258; inserting <i>Clostridium thermocellum</i> endoglucanase A gene (<i>celA</i>) into the hemagglutinin/protease <i>hapA</i> gene (the latter permitting rapid identification of the strain).
Presentation:	Flask containing lyophilized pharmaceutical active ingredient with lyoprotectors and one sachet containing the anti-acid.
Dose:	One
Developer:	Finlay Institute, Havana, Cuba
Clinical Trial:	Phase I/II (safety, reactogenicity, immunogenicity)
Conducted by:	National Institute of Health, Mozambique
Monitoring:	Finlay Institute, Havana, Cuba
Participants:	120 presumed-healthy adult male and female volunteers (≥18 years)
Trial Period:	One month
Results:	The vaccine candidate is safe (no severe adverse events, and percent of subjects reporting any degree of adverse effects was not statistically significant); 97% immunogenicity (subjects who received the vaccine

developed significant titers of vibriocidal antibodies).

Source: *Finlay Institute, Havana, Cuba*

According to an announcement by the Mozambican Health Ministry in August, the vaccine proved to be safe, subjects reporting no severe adverse events, and produced a seroconversion rate of 97.4% of vibriocidal antibody response.[3]

At Havana's Finlay Institute, where the vaccine was developed, *Cuba Health Reports (CHR)* spoke with Dr Hilda María García who also monitored the Mozambique trial. "We still have a long way to go," she said, "but these results indicate that we're on the right track." Dr García has specialized in cholera since 1984, but it wasn't until the 1991 cholera epidemic in Peru that Cuban health authorities went to work on a vaccine. "We were faced with the prospect of further epidemics in Latin America," recalled Dr García, "with cholera right at our doorstep."

V. cholerae O1 El Tor Ogawa C7258 cholera strain isolated during the Peruvian epidemic became the basis for the attenuated, genetically-modified 638 strain, the active ingredient for the new vaccine candidate. The 638 strain was created by Havana's National Center for Scientific Research (CNIC) in 1996, after which the Finlay Institute began working on further technological and pharmaceutical development of the vaccine candidate.



Dr Hilda María García, cholera specialist, Finlay Institute, Havana

Since no animal model reproduces the pathology of cholera, to demonstrate the safety, non-reactogenicity, and immunogenicity of the 638 strain, fresh cultures of the strain underwent 14 clinical trials in healthy adults in Cuba from 1997 to 2001, carried out at the Pedro Kourí Tropical Medicine Institute, Havana. "Safety and reactogenicity were particularly important for us, since live attenuated vaccines like ours tend to cause more adverse reactions."

An article in *Expert Review of Vaccines* sums up results: "No significant adverse reactions were observed in volunteers immunized with strain 638. Four out of the 42 volunteers who ingested strain 638 and one out of 14 who received placebo had loose stools. The vaccine strain was recoverable from the stools of 37 out of 42 volunteers. Among the vaccinees receiving $4 \times 10^7 - 2 \times 10^9$ cfu of *V. cholerae* 638, serum vibriocidal antibody responses occurred in 71%-82% and anti-Ogawa LPS IgA ASC responses occurred in 85%-100% of patients."

In addition Dr García said, the strain was also tested in Ecuador, at the Instituto de Seguro Social, Guayaquil in 1999, with similar conditions and results as the Cuban studies.

The article in *Expert Review of Vaccines* adds that "*V. cholerae* 638 was then evaluated for protective efficacy in a randomized, double-blind, placebo-controlled trial in volunteers in Cuba. Among 24 of the vaccinees, 96% developed vibriocidal responses, and 50% developed an anti-LPS IgA response in serum. At 1 month after vaccination, 12 vaccinees and 9 placebo recipients were challenged with 7×10^5 cfu of virulent strain *V. cholerae* 3008. None of the 12 vaccinees, but 7 of the volunteers from the placebo group, had diarrhea; 2 of the latter developed severe cholera." [4,5,6]

Dr García told *CHR*: "The promising results of this phase carried out with fresh cultures of the strain prompted the Finlay Institute to move ahead, installing technology according to Good Manufacturing Practices (GMP) capable of safely producing a live attenuated cholera vaccine (requiring biosafety level-2

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laboratories).” Once the Institute received the requisite licensing from Cuba’s Biological Safety Center, scientists then evolved plans for scaling up production of the final formulation of the vaccine candidate, presented in flasks of the lyophilized 638 strain with lyoprotectors and other additives. This vaccine candidate was used in Phase I/II trials in Cuba and Mozambique.

In 2005, researchers proceeded to submit a vaccine candidate to a double-blind, placebo-controlled study in healthy adult volunteers, conducted by Cuba’s Pedro Kourí Tropical Medicine Institute, testing mainly for safety and immunogenicity.

“However,” explained Dr García, “testing a cholera vaccine in Cuban volunteers is very different from trials in a country such as Mozambique, where cholera is present along with very different nutritional, epidemiological, and environmental conditions.”

“Our agreement with Mozambique’s National Institute of Health allowed the vaccine candidate to take another step forward,” emphasized Dr García, “in the context of South-South cooperation.” The two countries are now preparing a second set of Phase I/II trials in children aged 2-11 years. This is particularly important because one of the main hurdles of cholera vaccine candidates to date has been their reduced protection for children, who are most vulnerable to the effects of cholera worldwide.

Cholera Background

In 2006, 99% of the over 200,000 cases of cholera reported came from African countries, which reported an 87% case increase over 2005. Mozambique reported 6,306 cases, the sixth highest number after Angola, Ethiopia, Sudan, Democratic Republic of the Congo and Tanzania, in that order.[1]

Cholera is a severe diarrheal disease resulting from ingesting food or water contaminated with *V. cholerae* bacteria. Fluid loss causes dehydration in untreated persons, death occurring when 10%-15% of total body weight is lost, which can happen within hours of onset.

Fecal excretion of the bacteria into water supplies accounts for ongoing contamination and rapid dissemination in areas where populations do not have adequate sanitary conditions. Thus, cholera is essentially a disease of the poor, endemic in “cholera belts” in parts of Asia and sub-Saharan Africa besieged by cyclical epidemics, and among refugee populations.

Since the end of the 20th Century, the world has been threatened by the seventh great pandemic, in which the main cholera biotype is El Tor, serogroup O-1 (serotypes Inaba and Ogawa).

Available Vaccines

There are two internationally-licensed vaccines against cholera, used primarily for travellers:[7]

- WC/rBS (Dukoral®) an oral multiple-dose killed whole-cell *V. cholerae* O1 vaccine, although oral live vaccines are generally considered more effective
- CVD 103-HgR (Orochol®, Mutacol®) a single-dose live, orally-administered attenuated vaccine (originally developed against classical *V. cholerae*, although it has showed some level of cross-protection for El Tor). However, as of 2006, this vaccine was not being manufactured commercially[4]

Additional vaccines in development include:

- Peru-15, a non motile derivative of *V cholerae* O1 El Tor Inaba C6709 strain
http://www.medicc.org/publications/cuba_health_reports/020.php

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- A number of vaccines are in development for *V.cholerae* O 139, appearing at present primarily in Asia
- Several vaccines have been produced and tested in Vietnam, including a variant of WC/rBS (the latter licensed only in Vietnam)

However, to date, no cholera vaccine has been able to reproduce the approximate three-year natural immunity conferred by the cholera infection itself.[4]

“Despite more than 100 years of research, an effective vaccine providing long-lasting immunity against cholera has not been obtained.”

Shahjahan Kabir, Academic Research and Information Management,
Uppsala, Sweden in *The Lancet*, March 2007

Challenges Ahead

In addition to protection over time, several challenges remain for all cholera vaccines, including the Cuban candidate:

- Proven protection in specific populations (children, HIV+ persons, persons suffering from parasitic diseases or other conditions, etc.).
- Cold chain issues: until now, all vaccines and vaccine candidates require a cold chain, but in cholera endemic and epidemic-prone regions – some of the poorest in the world – this can present serious problems for vaccine distribution.
- All also require water be added to the vaccine ingredients, raising the issue of water accessibility and quality.
- And finally price: cholera vaccines have tended to be sold at prices that will keep them in the category of “tourist vaccines,” with little relevance to the populations where cholera is endemic.

While the Cuban vaccine candidate itself faces many hurdles – not the least of which is raising the hefty financing needed for Phase III trials – Dr García is confident on one point: if the vaccine proves effective, the Finlay Institute’s aim is to transfer the technology “fairly and as swiftly as good manufacturing and licensing processes permit” to production facilities in countries in need.

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