CHOLERA AND OTHER BACTERIAL ENTERIC INFECTIONS PANELS

[Established in 1965 as Cholera Panels; renamed in 1987 and 2001]

Several years before the USJCMSP began, a cholera epidemic raged across Asia. So, in January 1965, when U.S. President Lyndon Johnson and Japanese Prime Minister Eisaku Sato agreed that medical experts from each country would collaborate in a new program to address health problems in Asia, cholera was on the agenda. Three months later, at their initial April 1965 meeting in Tokyo, Japanese and American experts in cholera, who had been named as members of the USJCMSP Joint Cholera Panel, described urgent goals:

> It is the strong feeling of the Joint Cholera Panel that the vigorous and integrated efforts of the U.S.–Japan Cooperative Medical Science Program will succeed in achieving its two major goals: the immediate goal of making effective therapy available to cholera patients throughout Asia, and the long range, but ultimately more important goal of developing an effective vaccine which consistently confers long-term immunity against cholera.[1]

Thus, before the USJCMSP held its first official meeting, from October 4–7 1965, in Honolulu, Hawaii, scientists from Japan and the United States had agreed on their approach to alleviating the severe health problems presented by cholera. In his October 1965 address to members of the USJCMSP in Honolulu, Dr. Charles Carpenter put the problem in perspective. "A greater number of cholera deaths were recorded in Asia in 1963 than at any time since World War II. At the present time, cholera is established over a wider area than at any time since 1945, and currently threatens Eastern Europe."[2] Dr. Carpenter was Chair of the U.S. Cholera Panel from 1965–1972, Member of the U.S. Delegation from 1972–1989, and Chair of the U.S. Delegation from 1990–2000.

Cholera is a potentially fatal enteric disease characterized by severe dehydrating diarrhea. The causative organism, *Vibrio cholerae*, is a gram-negative bacterium that persists in many environments in Southeast Asia, especially those with fecally contaminated water supplies. The primary symptom of cholera, severe watery diarrhea, is caused by an enterotoxin produced by the bacterium. Two serogroups of cholera are prevalent today, O1 and O139. The O1 serogroup contains two biotypes, "classical" cholera, which plagued Asian and European countries for many centuries, and El Tor, which emerged in the Celebes in 1961, and has spread to most continents. The second serogroup is O139, which Dr. Yoshifumi Takeda and his colleagues identified and characterized after its appearance in Madras, India, in 1992. Dr. Takeda became Chair of the USJCMSP Japanese Delegation in 2001, a position he holds today.

Cholera Ward

Throughout its history, the USJCMSP has made significant contributions to the treatment and prevention of cholera. Chief among them is the use of oral rehydration therapy to treat cholera and other diarrheal diseases. Prior to the start of the USJCMSP, intravenous rehydration therapy had proven successful, but was impractical to administer—particularly to children with cholera—in impoverished, rural areas where the disease was endemic.[3] Two key figures who played a seminal role in the formation of

the U.S.– Japan Program, Drs. Colin MacLeod and Theodore Woodward,[4] had collaborated with Dr. Joseph Smadel of the NIH and others to establish centers in Asia for the study and treatment of cholera. One center was the Pakistan-SEATO Cholera Research Laboratory in Dacca, East Pakistan (now Dhaka, Bangladesh); the other was the Johns Hopkins International Center for Medical Research and training in Calcutta, India.[5] The USJCMSP Cholera Panels coordinated their early cholera research and treatment activities with these antecedent groups.[6]

"I went to Calcutta in 1962," said Dr. Carpenter. "My first job in Calcutta, and Bob's in Dhaka, was to see whether appropriate intravenous rehydration therapy, carefully administered, could be uniformly effective in both children and adults."[7] Dr. Carpenter then assisted Dr. Nathaniel Pierce, later Chair of the U.S. Cholera Panel, and Dr. Dilip Mahalanabis in designing the first trial of the glucose containing isotonic solution for oral rehydration in Calcutta in 1967. "The U.S.–Japan Program provided advice and support for the effort," he said. The results of these clinical trials demonstrated that the oral administration of an isotonic sugar/salt solution for treating cholera was as effective as giving the solution using orogastric tubes, a finding that made oral rehydration therapy feasible for treating cholera patients who lived in rural areas.[8]

Today, the use of oral rehydration therapy—a simple solution of sodium and potassium salts, glucose, and water—is a mainstay in preventing death from the severe, dehydrating diarrhea caused by infection with *Vibrio cholerae*. The treatment, if given quickly, reduces the cholera death rate from more than 40 percent[9] to less than 2 percent.[10] The development of oral rehydration therapy to treat cholera and other diarrheal diseases has been described as "potentially the most important medical advance" of the 20th century.[11, 12]

"At the time people didn't understand the importance of rehydration," said Dr. John La Montagne in a June 2004 interview. "It was not intuitive that you give isotonic saline to treat people with cholera." Also, the pervasive belief was that any solution administered orally would not be effective, due to the nature of the disease. When the USJCMSP was founded in 1965, the standard treatment for cholera was the intravenous administration of "16 to 20 liters of sterile fluids with correct electrolyte concentrations"[13] to each adult patient, a regimen difficult to offer to the thousands of cholera patients who lived in remote areas.

"I think that the [USJCMSP] cholera program progressed rapidly because the success of treating cholera stimulated more interest in other diarrheal diseases," said Dr. Carpenter. "The people involved in *E. coli* research were also involved—at some point—in the Cholera Panel. They demonstrated that *E. coli* not only worked much like cholera, but was probably the most common cause of travelers' diarrhea." A strain of *E. coli* that causes massive intestinal fluid loss produces a toxin similar to the cholera enterotoxin. Oral rehydration therapy is now widely used for *E. coli*-associated diarrhea, as well as for other non-cholera diarrheas.

"The cholera story – from the program point of view – is a shining example of a major problem that hadn't received much attention," said Dr. Adel Mahmoud. "The U.S.–Japan Program, by focusing on cholera [in 1965], addressed one of the major infectious diseases in the region. The Program allowed two major scientific countries to collaborate with each other and with scientists of the regions. It is an exercise in practical discovery that makes a difference in the health of the people in the region."

The success of oral rehydration therapy for cholera, progress in the development of cholera vaccines, and the recognition that other diarrheal diseases also posed severe health problems in Asia led to changes in the Cholera Panels. In June 1970, the Joint Cholera Panels expanded their guidelines to include cholera-like diarrheal diseases such as those caused by a pathogenic strain of *Escherichia coli*. In July 1987, the USJCMSP Joint Committee held its 23rd meeting at NIH in Bethesda, Maryland, and changed the name of the Cholera Panels, as the report explained:

> After hearing the reports of the Joint Panels/Boards, the Committee was convinced that research progress had been sparked by the application of new technology to the solution of old problems. The Committee was likewise pleased by the progress made by the Cholera Panels….In view of the significance of diarrheal diseases other than

> cholera, research by the panels including new vaccine development has been extended to other enteropathogenic bacterial diarrheas. To better indicate the scope of the panels' activities, the Joint Committee approved a change in the name to "Cholera and Related Diarrheal Diseases Panel."[14]

Members of the newly renamed Cholera and Related Diarrheal Diseases Panels also revised and expanded their guidelines. The expanded goals of the Panels came about because of the realization that many diarrheal diseases share pathogenic mechanisms and that the strategies for developing vaccines, based on recognized antigens, were also closely related. In 2001, the Panels were renamed again and became the Cholera and Other Bacterial Enteric Infections Panels.

The research activities of the Panels on Cholera and Other Bacterial Enteric Infections have broadened considerably since the original Cholera Panels were established in 1965. At their annual meetings in 2002 and 2003, the U.S. and Japanese Cholera Panels reported progress in understanding the pathogenesis, endemicity, epidemiology, vaccinology, enteric co-infections, and antibiotic resistance of cholera and other bacterial enteric infections. The USJCMSP continues to support the International Centre for Diarrhoeal Disease Research, Bangladesh, which has been built with assistance from Japan, Bangladesh, and NIAID, as well as other organizations. Specific projects include a successful field trial of the cholera vaccine Peru-15 in children (leveraged with the International Vaccine Institute), evaluation of the immunogenicity of the cholera toxin coregulated pilus, understanding the biology and significance of phage in cholera endemicity and emergence, a field trial demonstrating the effectiveness of a sari cloth water filter in preventing cholera, genomics analysis of cholera gene expression during disease, and detailed epidemiology studies that scale from bacterial culture to cutting-edge satellite-based observations.

The following is a list of important science advances in research on cholera and other bacterial enteric infections to which USJCMSP scientists have contributed. The list is adapted from information in the USJCMSP five-year reports, as well as information supplied by Haruo Watanabe, a member of the Japanese Panel on Cholera and Related Diarrheal Diseases from 1991–1995, and Chair of the (renamed) Japanese Cholera and Other Bacterial Enteric Infections Panel from 1995 to the present; and Robert Hall, U.S. Secretariat, Cholera and Other Bacterial Enteric Infections Panel from 2002–present.

Cholera and Other Bacterial Enteric Infections Panels
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[Science Advances: 1965 - 2000]

- Conducted clinical trials of oral rehydration therapy for cholera at the Cholera Research Laboratory in Dacca, East Pakistan (now Dhaka, Bangladesh), and in Calcutta, India. USJCMSP scientists provided advice and support for the trials, which demonstrated that glucose-electrolyte fluids administered orally (called oral rehydration therapy or ORT) can reduce mortality associated with cholera to less than 2 percent, even in refugee populations in impoverished rural areas. [See also discussion above.]
- Determined the molecular structure of cholera toxin. In the early 1970s, American scientists purified and characterized the structure of cholera toxin, the major virulence factor of *Vibrio cholerae*. The toxin is a large protein molecule (84 kilodaltons) that can be dissociated into two types of subunits, the A subunit (28 kD) and B subunit (11,000 kD). The five B subunits associated into a larger complex of 56 kD that was quickly recognized as being a natural toxoid that could induce toxin-neutralizing antibodies. Japanese scientists quickly showed that both antibacterial and antitoxin immunity could protect experimental animals from a lethal challenge of *V. cholerae*. These studies set the stage for development of several types of cholera vaccines in both countries.
- Recognized and helped control new cholera outbreaks in South and Central America, Asia, and Africa. USJCMSP scientists also demonstrated the effectiveness of a whole cell/B subunit vaccine in preventing cholera in exposed populations from Peru in 1993–1995.
- Helped develop and field test an oral, whole-cell/B subunit vaccine for cholera that provided significant protection for three years in rural Bangladesh. A genetically engineered live

oral vaccine is now being field-tested in Indonesia. USJCMSP scientists helped develop a model for government-industry cooperation for the production and testing of new genetically engineered oral vaccines.

- Made important discoveries about the mechanism of pathogenesis of cholera toxin. USJCMSP scientists discovered that cholera toxin catalyzes the adenosine diphosphate ribosylation of the regulatory G protein of adenylate cyclase, thereby activating it, and identified ganglioside GM1 as the cholera toxin receptor. They also found that cholera toxin is encoded by a filamentous bacteriophage (CTX), and discovered the transcriptional regulation of the cholera toxin gene. The toxin co-regulated pilus (TCP) and cholera toxin of *Vibrio cholerae* use interconnected regulatory mechanisms.
- Determined the molecular basis for the emergence of toxigenic strains of *V. cholerae*. Shortly after cloning the LT and CT genes, USJCMSP scientists conducted a molecular analysis of environmental and clinical isolates of *V. cholerae* and established that toxigenicity was a genetic property of primarily clinical isolates. Eventually, the scientists showed that toxigenic strains acquire two chromosomal segments; one encodes toxin co-regulated pilus (TCP), an essential intestinal colonization factor, while the other encodes cholera toxin. The researchers also demonstrated that the second toxin-encoding chromosomal element is the genome of a filamentous bacteriophage that uses TCP as its receptor. Genetic analysis further established that the transcriptional regulation of the cholera toxin and TCP genes is interconnected by common regulatory mechanisms.
- Discovered and characterized a novel pandemic strain of cholera, *Vibrio cholerae* O139. In India in 1992, Japanese scientists led by Dr. Yoshifumi Takeda (Chair of the Japanese Delegation 2001–present) in collaboration with Dr. G. B. Nair and his coworkers of National Institute of Cholera and Enteric Diseases, Calcutta, India, isolated a novel serotype of *V. cholerae* called O139, which produced cholera toxin and caused pandemics of cholera in Asia. The genetic structure and virulence factors of *V. cholerae* O139 were similar to those of *V. cholerae* O1, although a morphological analysis (in electron microscopy using freeze-substitution technique) revealed that all the strains of the O139 serogroup had a thin fibrous, capsule-like structure on the outside of the outer membrane. A mutant strain of O139 that lacked the capsule synthesis activity had lost this surface layer. The O139 strains were more resistant to serum-killing activity than was the O1 strain. Also, antibodies against *V. cholerae* O139 did not cross-react with those of *V. cholerae* O1.
- Identified the toxins of *Escherichia coli* that are similar to the Shiga toxin of *Shigella dysenteriae* type 1. Members of the Panels on Cholera and Other Bacterial Enteric Infections and their colleagues showed that *E. coli* heat labile toxin (LT), which is nearly identical to cholera toxin; permanently activates the ubiquitous cell signal adenylate cyclase in gut epithelial cells; and thereby causes massive intestinal fluid loss. They also developed new assays for enterotoxins, and gained a greater understanding other disorders of fluid transport, including cystic fibrosis.
- Conducted a molecular analysis of *Shigella* virulence. Japanese members of the Panel on Cholera and Other Bacterial Enteric Infections conducted a thorough molecular analysis of *Shigella*-cell invasion, bacterial cell-to-cell transmission, and *Shigella* gene regulation. Invasion into the epithelium of the target cell depends on the type III secretion system (TTSS), which is encoded by the *mxi-spa* and *ipa* genes on a virulence plasmid. Ipa proteins and other effector proteins are secreted into the cytoplasm of epithelial cells through TTSS, and they modulate host cellular signals after binding to cytoskeleton-associated molecules. The VirG protein is involved in bacterial movement in the cytoplasm, which binds to N-WASP and accumulates actin molecules. The expression of TTSS is tightly regulated by CpxA-R, a two-component system that senses cell-envelope stresses and external conditions, and by down-stream regulators, VirF and InvE (VirB) proteins.
- Increased understanding of the genetic diversity of enterohaemorrhagic *Escherichia coli* (EHEC) genomes and showed its usefulness for epidemiological analysis. Japanese Panel Members determined the entire genome sequence of the Sakai strain and, after comparing it to EHEC O157 strains, revealed extensive genomic diversity among O157 strains, which is caused mainly by the insertion of bacteriophages and subsequent genetic rearrangement. The genomic diversity can be used to help identify linkages among *E. coli* O157 strains, and can also be used for molecular epidemiological analyses and the early detection of O157-associated outbreaks.

[Science Advances: 2000 – 2004]

- Demonstrated that thermostable direct hemolysin (TDH) is not the only virulence factor of *Vibrio parahaemolyticus* (Vp), and determined the entire genome sequence of a clinical isolate of *V. parahaemolyticus*.[15] *V. parahaemolyticus* is a gram-negative marine bacterium that is phylogenetically related to *V. cholerae*, and is typically associated with shellfish. Recently, certain serotypes of *V. parahaemolyticu* have caused a pandemic of gastroenteritis. Vp-TDH is usually produced by Kanagawa phenomenon-positive *V. parahaemolyticus*, which causes hemolysis, and is essential for the virulence of *V. parahaemolyticus*. Japanese members of the Panel on Cholera and Other Bacterial Enteric Infections sequenced the genome of a clinical strain of *V. parahaemolyticus* (RIMD2210633) and found that it consists of two circular chromosomes (3288558 and 1877212 base pairs in length). A comparison of the genome of *V. parahaemolyticus* with that of *V. cholerae* showed many rearrangements within and between the two chromosomes.

The researchers identified genes for the type III secretion system (TTSS) of *V. parahaemolyticus*, which is also important for its virulence. In addition to its role in the pathogenicity of *V. parahaemolyticus*, the TTSS functions as a critical virulence factor in bacteria that cause diarrhea (e.g., *Shigella, Salmonella*, and enteropathogenic *Escherichia coli*). Additionally, the new results suggest that *V. parahaemolyticus* and *V. cholerae* establish infections in eukaryotic cells using different mechanisms. *V. parahaemolyticus* uses the TTSS, probably for protein secretion as it interacts with cells, whereas *V. cholerae* does not. The involvement of the TTSS in *V. parahaemolyticus* infections may help explain the pathogen's ability to trigger clinical symptoms such as inflammatory diarrhea.

- Determined that quorum-sensing plays a role in regulating the transitions between the biofilm (sessile) and free-swimming (planktonic) forms of *V. cholerae*.[16] Members of the U.S. and Japanese Panels on Cholera and Other Bacterial Enteric Infections studied *V. cholerae* excreted by cholera patients using microarray analyses. The researchers deduced the environmental signals, including quorum sensing, which help regulate the virulence of *V. cholerae* and allow its transition from environmental forms to the pathogenic forms that cause disease in humans. In quorum sensing, cholera bacteria use extracellular signaling molecules (autoinducers), which allow *V. cholerae* to monitor their cell population density and develop or dissociate complex surface-associated communities called biofilms. The timing of these transitions is critically important to the cycling of cholera through the environment and the human host. The new data therefore suggest how the quorum-sensing regulation of the formation of *V. cholerae* biofilm communities may help regulate the *V. cholerae* infection cycle.

- Made progress in developing candidate cholera vaccines. Peru-15, a non-toxigenic derivative of *V. cholerae* El Tor Inaba, was found to be well tolerated in volunteers. On challenge, only 1 of 24 vaccinees showed mild diarrheal symptoms. Protection against severe and moderate diarrhea was 100 percent.[17] In another trial of a candidate cholera vaccine, volunteers in Bangkok, Thailand, fed with *V. cholerae* O139 showed that the dose required to evaluate vaccine efficacy was 1–2 logs greater in Thai than in American volunteers.[18] These results emphasize the importance of host factors in endemic areas.

- Conducted genomics studies of enteric bacterial pathogens, including *V. cholera*, and identified potential critical immunogens and virulence factors, which may be useful for the development of vaccines and therapeutics. Within the past several years, the era of genomic-sequencing studies has opened for a host of bacterial enteric pathogens, including *H. pylori* (1999); *V. cholerae* (2000);[19] *Salmonella typhi, S. typhimurium,* and *E. coli* O157:H7 (2001); *C. jejuni* (2001); *S. flexneri* 2a (2002); and *V. parahaemolyticus* (2002). Genomic sequences nearing completion include *C. difficile*, *S. enterica* serovars Dublin, Pullorum, and Choleraesuis, *V. vulnificus*, and additional strains of these organisms. The cholera genome sequence showed that the majority of known genes are on the larger of its two chromosomes. The smaller chromosome possesses more hypothetical genes as well as the integron island, which may be important in *V. cholerae* virulence and antibiotic resistance.

- Discovered evidence that there may be a survival advantage to an Alzheimer's susceptibility gene, perhaps analogous to the balanced polymorphism of carrying a single gene for sickle cell hemoglobin and having increased resistance to malaria.[20] U.S. Panel member Richard Guerrrant presented the team's new findings at the December 2004 USJCMSP meeting held in Kyoto. The researchers previously reported that diarrheal diseases in the first two years of life in Brazilian urban shantytowns are associated with long-lasting cognitive development deficits and "childhood stunting," most noticeably in semantic fluency, functions that are also impaired in Alzheimer's dementia. Recently, they found a higher-than-normal frequency of the Apo-E4 allele (one of several genes associated with increased susceptibility to Alzheimer's) in children with lower diarrhea burdens. Among children with the heavier diarrhea burdens, those with an Apo-E4 allele performed significantly better on a coding subtest than did Apo-E4 negative children who had similar diarrhea burdens. Apo-E4 is relatively common in favela children, which may suggest a protective role of the Apo-E4 allele and help explain its persistence in human populations.

Footnotes — Cholera and Other Bacterial Enteric Infections Panels

[1] "Study Report on U.S. – Japan Cooperative Medical Science Programme: 1965." Japanese Committee; U.S.–Japan Cooperative Medical Science Programme, p. 31.

[2] Carpenter, CC. "Scientific Presentation—Cholera." Presented at the Hawaiian Conference, October 1956. U.S.–Japan Cooperative Medical Science Program. (Text provided by Dr. Carpenter)

[3] See - Phillipa RA. The pathophysiology of cholera. *Bull. World Health Org.* 1963;28:297; and Carpenter CC, Mitra PP, Sack RB, Dans PE, Wells SA, and Chaudhuri RN. Clinical evaluation of fluid requirements in Asiatic cholera. *Lancet* 1965;1:726.

[4] Dr. Colin Macleod was U.S. Delegation Chair from 1965–1972 [see Profile, p. v]; Dr. Theodore Woodward was a U.S. Delegation Member from 1965–1995 and is now Emeritus Member. During World War II, Woodward pioneered the use of chloromycetin to treat typhoid. Also, during his military service, Woodward met Joseph Smadel, who became associate director of NIH (1956–1960) and established the NIH Cholera Advisory Committee; Ian Bennett (U.S. Delegation Member 1967–1972, U.S. Delegation Chair 1972–1990), and Dr. MacLeod. The four, together with Fred Soper and others, also helped establish the South East Asia Treaty Organization (SEATO) Cholera Research Laboratory in Dacca, East Pakistan (now Bangladesh).

[5] Carpenter CC. The treatment of cholera: clinical science at the bedside. *J Infect. Dis* 1992;166:2–14.

[6] The United States–Japan Cooperative Medical Science Program. Five Year Report, 1965–1970. U.S. Department of State. Washington, D.C. U.S. Government Printing Office (Stock Number 4400-1370), p. 4.

[7] By April 1967, a collaborative group of researchers had begun clinical trials of oral rehydration therapy for cholera at the Cholera Research Laboratory in Dacca, East Pakistan (now Dhaka, Bangladesh); Manila, Philippines; and Calcutta, India. Dr. Colin MacLeod and others with the USJCMSP provided advice and support for the trials, which were the result of collaborations among Dr. Norbert Hirschhorn (Cholera Research Laboratory), Dr. Craig Wallace (Naval Medical Research Unit and The Johns Hopkins School of Medicine), Captain Robert Phillips (Naval Medical Research Unit), Dr. Charles Carpenter, Dr. Robert Gordon (NIH), and Dr. Nathanial Pierce.

[8] Hirschorn N, Kinzie D, Sachar R, Northrup RS, Taylor JO, Ahmad SZ, Phillips RA. Decrease in net stool output in cholera during intestinal perfusion with glucose-containing solution. *N Engl J Med* 1968;279:176–81; and Pierce NF, Sack RB, Mitra RC, Banwell JG, Brigham KL, Fedson DS, Mondal A. Replacement of water on electrolyte losses in cholera by an oral glucose-electrolyte solution. *Ann Intern Med* 1969;70:1173-81.

[9] The United States–Japan Cooperative Medical Science Program. The First Five Years: 1965–1970. Abbreviated edition. U.S. Government Printing Office: 1971 (O-421-976) May 1971, p. 11.

[10] World Health Organization Initiative for Vaccine Research: Cholera. http://www.who.int/vaccine_research/diseases/cholera/en/.

[11] Carpenter, CC. The erratic evolution of cholera therapy: from folklore to science. *Clin. Ther.* 1990. 12:22–8.

[12] "Water with sugar and salt." *Lancet* 1978. 2 (8084): 300–301.

[13] The United States–Japan Cooperative Medical Science Program: The First Five Years, 1965–1970. Abbreviated edition. U.S. Government Printing Office: 1971 (O-421-976) May 1971, p. 11.

[14] The United States–Japan Cooperative Medical Science Program. Fifth Five-Year Report: 1985–1990. Department of State Publication 9761: Bureau of Oceans and International Environmental and Scientific Affairs, p. 2.

[15] Makino K, Oshima K, Kurokawa K, Yokoyama K, Uda T, Tagomori K, Iijima Y, Najima M, Nakano M, Yamashita A, Kubota Y, Kimura S, Yasunaga T, Honda T, Shinagawa H, Hattori M, and Iida T. Genome sequence of *Vibrio parahaemolyticus*: a pathogenic mechanism distinct from that of *V cholerae*. *Lancet*. 2003 Mar 1;361(9359):743–9; and Park KS, Ono T, Rokuda M, Jang MH, Iida T, and Honda T. Cytotoxicity and enterotoxicity of the thermostable direct hemolysin-deletion mutants of *Vibrio parahaemolyticus*. *Microbiol Immunol*. 2004;48(4):313–8.

[16] Zhu J and Mekalanos JJ. Quorum sensing-dependent biofilms enhance colonization in *Vibrio cholerae*. *Dev Cell* 2003 Oct;5(4):647–56.

[17] Cohen MB, Giannella RA, Bean J, Taylor DN, Parker S, Hoeper A, Wowk S, Hawkins J, Kochi SK, Schiff G, and Killeen KP. Randomized, controlled human challenge study of the safety, immunogenicity, and protective efficacy of a single dose of Peru-15, a live attenuated oral cholera vaccine. *Infect Immun* 2002 Apr;70(4):1965–70.

[18] Pitisuttithum P, Cohen MB, Phonrat B, Suthisarnsuntorn U, Bussaratid V, Desakorn V, Phumratanaprapin W, Singhasivanon P, Looareesuwan S, Schiff GM, Ivanoff B, and Lang D. A human volunteer challenge model using frozen bacteria of the new epidemic serotype, *V. cholerae* O139 in Thai volunteers. *Vaccine*. 2001 Dec 12;20(5-6):920–5.

[19] Heidelberg JF, Eisen JA, Nelson WC, Clayton RA, Gwinn ML, Dodson RJ, Haft DH, Hickey EK, Peterson JD, Umayam L, Gill SR, Nelson KE, Read TD, Tettelin H, Richardson D, Ermolaeva MD, Vamathevan J, Bass S, Qin H, Dragoi I, Sellers P, McDonald L, Utterback T, Fleishmann RD, Nierman WC, and White O. DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*. *Nature*. 2000 Aug 3;406(6795):477–83.

[20] Guerrant DI, Moore SR, Lima AAM, Patrick P, Schorling JB and Guerrant RL. APOE4 protects the cognitive development in children with heavy diarrhea burdens in northeast Brazil. *Ped Res.* 2004 (in press).