

From the WHO position paper on cholera vaccines March 2010

key references with summaries

(followed by an extended reference list without summaries)

Epidemiology

Deen JL, von Seidlein L, Sur D, Agtini M, Lucas ME, Lopez AL, Kim DR, Ali M, Clemens JD. The high burden of cholera in children: comparison of incidence from endemic areas in Asia and Africa. PLoS Negl Trop Dis. 2008 Feb 20;2(2):e173.

BACKGROUND: Cholera remains an important public health problem. Yet there are few reliable population-based estimates of laboratory-confirmed cholera incidence in endemic areas around the world. **METHODS:** We established treatment facility-based cholera surveillance in three sites in Jakarta (Indonesia), Kolkata (India), and Beira (Mozambique). The annual incidence of cholera was estimated using the population census as the denominator and the age-specific number of cholera cases among the study cohort as the numerator. **FINDINGS:** The lowest overall rate was found in Jakarta, where the estimated incidence was 0.5/1000 population/year. The incidence was three times higher in Kolkata (1.6/1000/year) and eight times higher in Beira (4.0/1000/year). In all study sites, the greatest burden was in children under 5 years of age. **CONCLUSION:** There are considerable differences in cholera incidence across these endemic areas but in all sites, children are the most affected. The study site in Africa had the highest cholera incidence consistent with a growing impression of the large cholera burden in Africa. Burden estimates are useful when considering where and among whom interventions such as vaccination would be most needed.

Zuckerman JN, Rombo L, Fisch A. The true burden and risk of cholera: implications for prevention and control. Lancet Infectious Diseases, 2007, 7:521–530.

Cholera is a substantial health burden on the developing world and is endemic in Africa, Asia, South America, and Central America. The exact scale of the problem is uncertain because of limitations in existing surveillance systems, differences in reporting procedures, and failure to report cholera to WHO; official figures are likely to greatly underestimate the true prevalence of the disease. We have identified, through extensive literature searches, additional outbreaks of cholera to those reported to WHO, many of which originated from the Indian subcontinent and southeast Asia. Such underestimation of cholera can have important implications for decisions on provision of health interventions for indigenous populations, and on risk assessments for travellers. Furthermore, until recently, it has not been possible to implement public-health interventions in low-income countries to eliminate disease, and the prevention of cholera in travellers has been limited to restrictive guidelines. However, a vaccine against

cholera is now available that has proven efficacy and tolerability in mass vaccination campaigns in low-income countries, and among travellers.

Schwartz BS, Harris JB, Khan AI, Larocque RC, Sack DA, Malek MA, Faruque AS, Qadri F, Calderwood SB, Luby SP, Ryan ET. Diarrheal epidemics in Dhaka, Bangladesh, during three consecutive floods: 1988, 1998 and 2004. American Journal of Tropical Medicine and Hygiene, 2006,74:1067–1073.

We examined demographic, microbiologic, and clinical data from patients presenting during 1988, 1998, and 2004 flood-associated diarrheal epidemics at a diarrhea treatment hospital in Dhaka, Bangladesh. Compared with non-flood periods, individuals presenting during flood-associated epidemics were older, more severely dehydrated, and of lower socioeconomic status. During flood-associated epidemics, *Vibrio cholerae* was the most commonly identified cause of diarrhea, and the only diarrheal pathogen whose incidence proportionally increased in each epidemic compared with seasonally matched periods. Rotavirus was the second most frequently identified flood-associated pathogen, although the proportion of cases caused by rotavirus infection decreased during floods compared with matched periods. Other causes of diarrhea did not proportionally change, although more patients per day presented with enterotoxigenic *Escherichia coli*, *Shigella*, and *Salmonella* species-associated diarrhea during floods compared with matched periods. Our findings suggest that cholera is the predominant cause of flood-associated diarrheal epidemics in Dhaka, but that other organisms spread by the fecal-oral route also contribute.

Microbiological and clinical issues

Siddique AK, Nair GB, Alam M, Sack DA, Huq A, Nizam A, Longini IM Jr, Qadri F, Faruque SM, Colwell RR, Ahmed S, Iqbal A, Bhuiyan NA, Sack RB. El Tor cholera with severe disease: a new threat to Asia and beyond. Epidemiology and Infection, 2009, 14:1–6.

During epidemics of cholera in two rural sites (Bakerganj and Mathbaria), a much higher proportion of patients came for treatment with severe dehydration than was seen in previous years. *V. cholerae* O1 isolated from these patients was found to be El Tor in its phenotype, but its cholera toxin (CT) was determined to be that of classical biotype. Whether the observed higher proportion of severe dehydration produced by the El Tor biotype was due to a shift from El Tor to classical CT or due to other factors is not clear. However, if cholera due to strains with increased severity spread to other areas where treatment facilities are limited, there are likely to be many more cholera deaths.

Ansaruzzaman M, Bhuiyan NA, Safa A, Sultana M, McUamule A, Mondlane C, Wang XY, Deen JL, von Seidlein L, Clemens JD, Lucas M, Sack DA, Balakrish Nair G. Genetic diversity of El Tor strains of *Vibrio cholerae* O1 with hybrid traits isolated from Bangladesh and Mozambique. International Journal of Medical Microbiology, 2007, 297:443–449.

Vibrio cholerae O1 strains that are hybrids between the classical and El Tor biotypes were isolated during two consecutive years (2004-2005) from diarrheal patients in Mozambique. Similar variants isolated in Bangladesh and recently isolated El Tor strains were analyzed for genetic diversity. Pulsed-field gel electrophoresis (PFGE) analysis using the restriction enzyme NotI, resulted in 18-21 bands showed five closely related PFGE patterns that were distributed similarly in both years (2004-2005) among the 80 strains tested in Mozambique. Overall based on the PFGE patterns the hybrids indicated an El Tor lineage. The restriction patterns of whole-chromosomal DNA grouped the hybrid strains from Mozambique into a separate cluster from Bangladeshi clinical and environmental hybrid strains. A high molecular weight band of 398kb that contain *rstR* allele of the classical type was detected from all hybrid strains, which was absent in all conventional classical and El Tor strains. This band could be designated as a marker for the hybrid strains. This study suggests that hybrid strains from Mozambique are closely related to each other, different from Bangladeshi hybrid strains that are diverse in nature and all hybrid strains differed markedly from conventional classical and El Tor strains.

Nair GB, Qadri F, Holmgren J, Svennerholm AM, Safa A, Bhuiyan NA, Ahmad QS, Faruque SM, Faruque AS, Takeda Y, Sack DA. Cholera due to altered El Tor strains of *Vibrio cholerae* O1 in Bangladesh. *J Clin Microbiol.* 2006 Nov;44(11):4211-3.

We determined the types of cholera toxin (CT) produced by a collection of 185 *Vibrio cholerae* O1 strains isolated in Bangladesh over the past 45 years. All of the El Tor strains of *V. cholerae* O1 isolated since 2001 produced CT of the classical biotype, while those isolated before 2001 produced CT of the El Tor biotype.

Sack DA, Sack RB, Nair GB, Siddique AK. Cholera. *Lancet*, 2004, 363:223–233.

Intestinal infection with *Vibrio cholerae* results in the loss of large volumes of watery stool, leading to severe and rapidly progressing dehydration and shock. Without adequate and appropriate rehydration therapy, severe cholera kills about half of affected individuals. Cholera toxin, a potent stimulator of adenylate cyclase, causes the intestine to secrete watery fluid rich in sodium, bicarbonate, and potassium, in volumes far exceeding the intestinal absorptive capacity. Cholera has spread from the Indian subcontinent where it is endemic to involve nearly the whole world seven times during the past 185 years. *V. cholerae* serogroup O1, biotype El Tor, has moved from Asia to cause pandemic disease in Africa and South America during the past 35 years. A new serogroup, O139, appeared in south Asia in 1992, has become endemic there, and threatens to start the next pandemic. Research on case management of cholera led to the development of rehydration therapy for dehydrating diarrhoea in general, including the proper use of intravenous and oral rehydration solutions. Appropriate case management has reduced deaths from diarrhoeal disease by an estimated 3 million per year compared with 20 years ago. Vaccination was thought to have no role for cholera, but new oral vaccines are showing great promise.

Cholera vaccines

Sur D, Lopez AL, Kanungo S, Paisley A, Manna B, Ali M, Niyogi SK, Park JK, Sarkar B, Puri MK, Kim DR, Deen JL, Holmgren J, Carbis R, Rao R, Nguyen TV, Donner A, Ganguly NK, Nair GB, Bhattacharya SK, Clemens JD. Efficacy and safety of a modified killed-whole-cell oral cholera vaccine in India: an interim analysis of a cluster-randomised, double-blind, placebo-controlled trial. *Lancet*. 2009 Nov 14;374(9702):1694-702.

BACKGROUND: Oral cholera vaccines consisting of killed whole cells have been available for many years, but they have not been used extensively in populations with endemic disease. An inexpensive, locally produced oral killed-whole-cell vaccine has been used in high-risk areas in Vietnam. To expand the use of this vaccine, it was modified to comply with WHO standards. We assessed the efficacy and safety of this modified vaccine in a population with endemic cholera. **METHODS:** In this double-blind trial, 107 774 non-pregnant residents of Kolkata, India, aged 1 year or older, were cluster-randomised by dwelling to receive two doses of either modified killed-whole-cell cholera vaccine (n=52 212; 1966 clusters) or heat-killed *Escherichia coli* K12 placebo (n=55 562; 1967 clusters), both delivered orally. Randomisation was done by computer-generated sequence in blocks of four. The primary endpoint was prevention of episodes of culture-confirmed *Vibrio cholerae* O1 diarrhoea severe enough for the patient to seek treatment in a health-care facility. We undertook an interim, per-protocol analysis at 2 years of follow-up that included individuals who received two completely ingested doses of vaccine or placebo. We assessed first episodes of cholera that occurred between 14 days and 730 days after receipt of the second dose. This study is registered with ClinicalTrials.gov, number NCT00289224. **FINDINGS:** 31 932 participants assigned to vaccine (1721 clusters) and 34 968 assigned to placebo (1757 clusters) received two doses of study treatment. There were 20 episodes of cholera in the vaccine group and 68 episodes in the placebo group (protective efficacy 67%; one-tailed 99% CI, lower bound 35%, $p < 0.0001$). The vaccine protected individuals in age-groups 1.0-4.9 years, 5.0-14.9 years, and 15 years and older, and protective efficacy did not differ significantly between age-groups ($p = 0.28$). We recorded no vaccine-related serious adverse events. **INTERPRETATION:** This modified killed-whole-cell oral vaccine, compliant with WHO standards, is safe, provides protection against clinically significant cholera in an endemic setting, and can be used in children aged 1.0-4.9 years, who are at highest risk of developing cholera in endemic settings. **FUNDING:** Bill & Melinda Gates Foundation, Swedish International Development Cooperation Agency, Governments of South Korea, Sweden, and Kuwait.

Tackett CO, Sack DA. Cholera vaccines. In: Plotkin SA, Orenstein WA, Offit P, eds. *Vaccines*, 5th ed. Philadelphia, PA, WB Saunders Company, 2008:127-138 (Book chapter).

Mahalanabis D, Lopez AL, Sur D, Deen J, Manna B, Kanungo S, von Seidlein L, Carbis R, Han SH, Shin SH, Attridge S, Rao R, Holmgren J, Clemens J, Bhattacharya SK. A randomized, placebo-controlled trial of the bivalent killed, whole-cell, oral cholera vaccine in adults and children in a cholera endemic area in Kolkata, India. PLoS One. 2008 Jun 4;3(6):e2323.

OBJECTIVES: An effective vaccine against cholera has been used for public health purposes in Vietnam since the 1990s. This vaccine was reformulated to meet WHO requirements. We assessed the safety and immunogenicity of the reformulated bivalent (*Vibrio cholerae* O1 and O139) killed whole cell oral vaccine in a cholera endemic area in Kolkata, India. **DESIGN:** Double-blind, randomized, placebo controlled trial. **SETTING:** The trial was conducted in the clinical trial ward of the Infectious Diseases Hospital in Kolkata, India. **PARTICIPANTS:** The participants were 101 healthy adults (males and non-pregnant females) aged 18-40 years and 100 healthy children (males and non-pregnant females) aged 1-17 years. **INTERVENTIONS:** Participants were randomized to receive either the bivalent killed whole cell oral cholera vaccine or placebo (killed oral *Escherichia coli* K12). **OUTCOME MEASURES:** For safety: proportion of subjects with adverse events during the duration of study participation. For immunogenicity: Proportion of subjects who had a ≥ 4 -fold rise in serum vibriocidal antibody titers 14 days after the second dose of vaccine or placebo. **RESULTS:** Adverse reactions were observed with similar frequency among vaccine and placebo recipients in both age groups. Among adults 4% of vaccine and 8% of placebo recipients and among children 4% of vaccine and 2% of placebo recipients had at least one adverse event within 28 days of the first dose of the vaccine. Following immunization, 53% of adult and 80% of children vaccinees showed a ≥ 4 fold rise in serum *V. cholerae* O1 vibriocidal antibody titers. A less pronounced response to *V. cholerae* O139 vibriocidal antibody titers post-immunization was noted among vaccinees. **CONCLUSIONS:** We found the vaccine to be safe and immunogenic in a cholera-endemic area in India. **TRIAL REGISTRATION:** ClinicalTrials.gov NCT00119197.

Longini IM Jr, Nizam A, Ali M, Yunus M, Shenvi N, Clemens JD. Controlling endemic cholera with oral vaccines. PLoS Med. 2007 Nov 27;4(11):e336.

BACKGROUND: Although advances in rehydration therapy have made cholera a treatable disease with low case-fatality in settings with appropriate medical care, cholera continues to impose considerable mortality in the world's most impoverished populations. Internationally licensed, killed whole-cell based oral cholera vaccines (OCVs) have been available for over a decade, but have not been used for the control of cholera. Recently, these vaccines were shown to confer significant levels of herd protection, suggesting that the protective potential of these vaccines has been underestimated and that these vaccines may be highly effective in cholera control when deployed in mass immunization programs. We used a large-scale stochastic simulation model to investigate the possibility of controlling endemic cholera with OCVs. **METHODS AND FINDINGS:** We construct a large-scale, stochastic cholera transmission model of Matlab, Bangladesh. We find that cholera transmission could be controlled in endemic areas with 50% coverage with OCVs. At this level of coverage, the model predicts that there would be an 89% (95% confidence interval [CI] 72%-98%) reduction in cholera cases among the unvaccinated, and a 93% (95% CI 82%-99%) reduction overall in the entire population. Even a more modest coverage of 30% would result in a 76% (95% CI 44%-95%) reduction in cholera incidence for the population area covered. For

populations that have less natural immunity than the population of Matlab, 70% coverage would probably be necessary for cholera control, i.e., an annual incidence rate of ≤ 1 case per 1,000 people in the population. CONCLUSIONS: Endemic cholera could be reduced to an annual incidence rate of ≤ 1 case per 1,000 people in endemic areas with biennial vaccination with OCVs if coverage could reach 50%-70% depending on the level of prior immunity in the population. These vaccination efforts could be targeted with careful use of ecological data.

Anh DD, Canh do G, Lopez AL, Thiem VD, Long PT, Son NH, Deen J, von Seidlein L, Carbis R, Han SH, Shin SH, Attridge S, Holmgren J, Clemens J. Safety and immunogenicity of a reformulated Vietnamese bivalent killed, whole-cell, oral cholera vaccine in adults. *Vaccine*. 2007 Jan 22;25(6):1149-55.

Vietnam currently produces an orally administered, bivalent (O1 and O139) killed whole-cell vaccine and is the only country in the world with endemic cholera to use an oral cholera vaccine in public health practice. In order to allow international use, the vaccine had to be reformulated to meet World Health Organization (WHO) requirements. We performed a randomized, placebo controlled, safety and immunogenicity studies of this reformulated vaccine among Vietnamese adults. One hundred and forty-four subjects received the two-dose regimen and 143 had two blood samples obtained for analysis. We found that this reformulated oral killed whole-cell cholera vaccine was safe, well tolerated and highly immunogenic.

Sack DA. Herd protection and herd amplification in cholera. *Journal of Health, Population and Nutrition*, 2006, 24(1):1-5. (No summary)

Hill DR, Ford L, Lalloo DG. Oral cholera vaccines: use in clinical practice. *Lancet Infect Dis*. 2006 Jun;6(6):361-73.

Cholera continues to occur globally, particularly in sub-Saharan Africa and Asia. Oral cholera vaccines have been developed and have now been used for several years, primarily in traveller populations. The licensure in the European Union of a killed whole cell cholera vaccine combined with the recombinant B subunit of cholera toxin (rCTB-WC) has stimulated interest in protection against cholera. Because of the similarity between cholera toxin and the heat-labile toxin of *Escherichia coli*, a cause of travellers' diarrhoea, it has been proposed that the rCTB-WC vaccine may be used against travellers' diarrhoea. An analysis of trials of this vaccine against cholera (serotype O1) shows that for 4-6 months it will protect 61-86% of people living in cholera-endemic regions; lower levels of protection continue for 3 years. Protection wanes rapidly in young children. Because the risk of cholera for most travellers is extremely low, vaccination should be considered only for those working in relief or refugee settings or for those who will be travelling in cholera-epidemic areas and who will be unable to obtain prompt medical care. The vaccine can be expected to prevent 7% or less of cases of travellers' diarrhoea and should not be used for this purpose.

Thiem VD, Deen JL, von Seidlein L, Canh do G, Anh DD, Park JK, Ali M, Danovaro-Holliday MC, Son ND, Hoa NT, Holmgren J, Clemens JD. Long-term effectiveness against cholera of oral killed whole-cell vaccine produced in Vietnam. *Vaccine*. 2006 May 15;24(20):4297-303.

We assessed the long-term protection afforded by a killed whole-cell oral cholera vaccine produced in Vietnam. A mass immunization of children and adults with the killed whole-cell oral cholera vaccine was undertaken in half of the communes of Hue, Vietnam, in 1998; the remaining communes were immunized in 2000. No cholera was observed in Hue until 2003, when an outbreak of El Tor cholera made it possible to conduct a case-control study. The overall vaccine effectiveness 3-5 years after vaccination was 50% (9-63%). This low-cost, easily administered vaccine should be considered as a tool for the control of cholera.

Ali M, Emch M, von Seidlein L, Yunus M, Sack DA, Rao M, Holmgren J, Clemens JD. Herd immunity conferred by killed oral cholera vaccines in Bangladesh: a reanalysis. *Lancet*. 2005 Jul 2-8;366(9479):44-9.

BACKGROUND: Decisions about the use of killed oral cholera vaccines, which confer moderate levels of direct protection to vaccinees, can depend on whether the vaccines also provide indirect (herd) protection when high levels of vaccine coverage are attained. We reanalysed data from a field trial in Bangladesh to ascertain whether there is evidence of indirect protection from killed oral cholera vaccines. **METHODS:** We analysed the first year of surveillance data from a placebo-controlled trial of B subunit-killed whole-cell and killed whole-cell-only oral cholera vaccines in children and adult women in Bangladesh. We calculated whether there was an inverse, monotonic trend for the relation between the level of vaccine coverage in a residential cluster and the incidence of cholera in individual vaccine recipients or placebo recipients residing in the cluster after controlling for potential confounding variables. **FINDINGS:** Vaccine coverage of the targeted population ranged from 4% to 65%. Incidence rates of cholera among placebo recipients were inversely related to levels of vaccine coverage (7.01 cases per 1000 in the lowest quintile of coverage vs 1.47 cases per 1000 in the highest quintile; $p < 0.0001$ for trend). Receipt of vaccine by an individual and the level of vaccine coverage of the individual's cluster were independently related to a reduced risk of cholera. Moreover, after adjustment for the level of vaccine coverage of the cluster, vaccine protective efficacy remained significant (55% [95% CI 41-66], $p < 0.0001$). **INTERPRETATION:** In addition to providing direct protection to vaccine recipients, killed oral cholera vaccines confer significant herd protection to neighbouring non-vaccinated individuals. Use of these vaccines could have a major effect on the burden of cholera in endemic settings.

Lucas ME, Deen JL, von Seidlein L, Wang XY, Ampuero J, Puri M, Ali M, Ansaruzzaman M, Amos J, Macuamule A, Cavailler P, Guerin PJ, Mahoudeau C, Kahozi-Sangwa P, Chaignat CL, Barreto A, Songane FF, Clemens JD. Effectiveness of mass oral cholera vaccination in Beira, Mozambique. *N Engl J Med*. 2005 Feb 24;352(8):757-67.

BACKGROUND: New-generation, orally administered cholera vaccines offer the promise of improved control of cholera in sub-Saharan Africa. However, the high prevalence of human immunodeficiency virus (HIV) infection in many cholera-affected African populations has raised doubts about the level of protection possible with vaccination. We evaluated a mass immunization program with recombinant cholera-toxin B subunit, killed whole-cell (rBS-WC) oral cholera vaccine in Beira, Mozambique, a city where the seroprevalence of HIV is 20 to 30 percent. **METHODS:** From December 2003 to January 2004, we undertook mass immunization of nonpregnant persons at least two years of age, using a two-dose regimen of rBS-WC vaccine in Esturro, Beira (population 21,818). We then assessed vaccine protection in a case-control study during an outbreak of El Tor Ogawa cholera in Beira between January and May 2004. To estimate the level of vaccine protection, antecedent rates of vaccination were compared between persons with culture-confirmed cholera severe enough to have prompted them to seek treatment and age- and sex-matched neighborhood controls without treated diarrhea. **RESULTS:** We assessed the effectiveness of the vaccine in 43 persons with cholera and 172 controls. Receipt of one or more doses of rBS-WC vaccine was associated with 78 percent protection (95 percent confidence interval, 39 to 92 percent; $P=0.004$). The vaccine was equally effective in children younger than five years of age and in older persons. A concurrently conducted case-control study designed to detect bias compared persons with treated, noncholeraic diarrhea and controls without diarrhea in the same population and found no protection associated with receipt of the rBS-WC vaccine. **CONCLUSIONS:** The rBS-WC vaccine was highly effective against clinically significant cholera in an urban sub-Saharan African population with a high prevalence of HIV infection. Copyright 2005 Massachusetts Medical Society.

Holmgren J, Bergquist C. Oral B-subunit killed whole-cell cholera vaccine. In: Levine MM et al, eds. New generation vaccines, 3rd ed. New York, Marcel Dekker, 2004:499-510. (Book chapter)

Trach DD, Cam PD, Ke NT, Rao MR, Dinh D, Hang PV, Hung NV, Canh DG, Thiem VD, Naficy A, Ivanoff B, Svennerholm AM, Holmgren J, Clemens JD. Investigations into the safety and immunogenicity of a killed oral cholera vaccine developed in Viet Nam. Bull World Health Organ. 2002;80(1):2-8.

OBJECTIVE: To evaluate a killed oral cholera vaccine produced in Viet Nam, and to compare the Vietnamese vaccine with one that is licensed internationally. **METHOD:** Two-dose regimens of a locally produced, bivalent, anti-O1, anti-O139 killed oral whole-cell cholera vaccine (biv-WC) and of a commercially available, monovalent (anti-O1) oral recombinant B subunit-killed whole-cell cholera vaccine (rBS-WC) were compared in two trials in Viet Nam. In the first trial, 144 adults were randomized to biv-WC with or without buffer, rBS-WC with buffer, or placebo without buffer. In the second, 103 children aged 1-12 years were randomized to biv-WC without buffer, rBS-WC with buffer, or placebo without buffer. **FINDINGS:** No regimen was associated with significant side-effects. In adults, ca 60% of recipients of either vaccine exhibited at least fourfold serum anti-O1 vibriocidal antibody responses and ca 40% of recipients of biv-WC demonstrated anti-O139 vibriocidal responses. Both anti-O1 (ca 90% in each vaccine group and anti-O139 (68% in the biv-WC group) vibriocidal responses occurred more frequently in children. The responses to biv-WC

were unaffected by the receipt of buffer. CONCLUSION: It was concluded that biv-WC was safe and immunogenic, that it could be administered without buffer, and that it could elicit robust immune responses even in children, for whom the risk of endemic cholera is highest.

Clemens JD et al. Misleading negative findings in a field trial of killed, oral cholera vaccine in Peru. *Journal of Infectious Diseases*, 2001,183:1306–1308. (No summary)

Taylor DN, Cárdenas V, Sanchez JL, Bégué RE, Gilman R, Bautista C, Perez J, Puga R, Gaillour A, Meza R, Echeverria P, Sadoff J. Two-year study of the protective efficacy of the oral whole cell plus recombinant B subunit cholera vaccine in Peru. *J Infect Dis*. 2000 May;181(5):1667-73.

The protective efficacy of an oral inactivated whole cell *Vibrio cholerae* plus recombinant B subunit cholera vaccine was determined against El Tor cholera among Peruvian children and adults (2-65 years old) in a randomized, double-blind manner. Study subjects received 2 doses of vaccine or placebo 2 weeks apart, followed by a booster dose 10 months later. Surveillance for cholera was performed actively, with 2 visits per week to each household, and passively, at a local hospital. Stool samples were collected during diarrhea episodes and were cultured for *V. cholerae*. A total of 17,799 persons received 2 doses of vaccine or placebo, and 14,997 of these persons received the booster dose. After 2 doses (first surveillance period), *V. cholerae* biotype O1 was isolated from 17 vaccinees and 16 placebo recipients, demonstrating vaccine efficacy (VE) of -4%. After 3 doses (second surveillance period), *V. cholerae* O1 was isolated from 13 vaccinees and 32 placebo recipients, demonstrating VE of 61% (95% confidence interval \pm CI, 28%-79%). In the second surveillance period, the VE for illness requiring hospitalization was 82% (95% CI, 27%-96%). VE was also higher for persons >15 years old (VE, 72%; 95% CI, 28%-89%).

Trach DD, Clemens JD, Ke NT, Thuy HT, Son ND, Canh DG, Hang PV, Rao MR. Field trial of a locally produced, killed, oral cholera vaccine in Vietnam. *Lancet*. 1997 Jan 25;349(9047):231-5.

BACKGROUND: Several studies have shown that orally administered killed cholera vaccines are safe and protective in populations at risk of cholera in developing countries. However, these vaccines have not been adopted for use in developing countries because of their expense and limited efficacy in young children. We have tested an inexpensive, killed whole-cell cholera vaccine developed and produced in Vietnam. METHODS: The efficacy of the vaccine was assessed in a large-scale, open field trial in people at least 1 year old residing in 22,653 households in the central coastal city of Hue. Alternate households were assigned vaccine (67,395 people; two doses per person) or no vaccine (67,058 people). Surveillance for cholera was conducted in all Ministry of Health facilities serving this population. Analysis was by intention to treat. FINDINGS: During an outbreak of El Tor cholera 8-10 months after vaccination, 37 cases of cholera requiring inpatient care occurred among age-eligible people allocated to the vaccine group, and 92 cases among age-eligible people allocated to the no-vaccine group (protective impact 60% [95% CI 40-73]). Among the 51,975 people who received the complete two-dose vaccine regimen, the protective efficacy was 66% (46-79): in

this subset, the protective efficacy was similar for children aged 1-5 years (68%) and for older people (66%). INTERPRETATION: These findings suggest that oral killed whole-cell vaccines can confer substantial protection against El Tor cholera in young children, who are at highest risk of cholera in endemic settings. An inexpensive, locally produced, and effective oral cholera vaccine may be within reach of the limited health-care budgets of poor countries with endemic cholera, if our findings can be replicated in a randomised double-blind trial.

PIP: *Vibrio cholera* 01, El Tor biotype, entered Vietnam in 1964 and during 1990-94 an average of 3240 cases were reported annually with a case-fatality rate of about 1%. The efficacy of an inexpensive, killed whole-cell cholera vaccine developed in Vietnam was assessed in a large-scale, open field trial in the city of Hue. The vaccine contained *V. cholera* 01 constituents: heat-killed *V. cholera* Inaba, heat-killed *V. cholera* Ogawa, and formalin-killed *V. cholera* Inaba. All 134,453 residents, aged 1 year or older, of 22,653 households in 19 communes were eligible to take part in the trial. Alternate households were assigned vaccine (67,395 people; 77% received 2 doses per person) or no vaccine (67,058 people serving as controls) during December 1992 and January 1993 by 80 vaccination teams. Following the vaccination no cases of cholera were detected until late August 1993. Between August 20 and October 4, 1993, there were 129 cases of cholera requiring inpatient care among age-eligible participants. The isolates were 01 serogroup and Ogawa serotype. There were 37 cases of cholera in the vaccine group and 92 cases in the control group. The risk of cholera was 0.5/1000 and 1.4/1000, respectively. The protective impact was 60% (95% confidence interval [CI] 40-73; $p < 0.001$). Among 51,975 recipients of 2 vaccine doses the protective efficacy was 66% (CI 46-79; $p < 0.001$). The protective efficacy was similar for children 1-5 years old (68%) and for older people (66%). The protective efficacy was somewhat higher among the vaccinated living in homes with unclean water sources (74% vs. 62%). The protective efficacy was also higher against severe than against non-severe cholera (76% vs. 58%). Oral killed whole-cell vaccines can protect against El Tor type cholera in children who are highest risk. The government has lately added a killed *V. cholera* 0139 strain to the existing formulation. Phase 2 tests of safety are under way, and a large-scale, randomized, double-blind field trial will start in 1997.

van Loon FP, Clemens JD, Chakraborty J, Rao MR, Kay BA, Sack DA, Yunus M, Ali M, Svennerholm AM, Holmgren J. Field trial of inactivated oral cholera vaccines in Bangladesh: results from 5 years of follow-up. *Vaccine*. 1996 Feb;14(2):162-6.

To determine the protective efficacy (PE) of three doses of oral B subunit-killed whole cell (BS-WC) or killed whole cell-only (WC) vaccines against cholera, a clinical trial was conducted among 62285 children over 2 years and adult women in rural Bangladesh. During 5 years of follow-up, there were 144 cases of cholera in the BS-WC group (PE = 49%; $P < 0.001$), 150 in the WC group (PE = 47%; $P < 0.001$), and 283 in the K12 group. Protection by each vaccine was evident only during the first three years of follow-up; long-term protection of young children was observed only against classical but not El Tor cholera; 3-year protection against both cholera biotypes occurred among older persons, but at a higher level against classical cholera.

Concha A, Giraldo A, Castañeda E, Martínez M, de la Hoz F, Rivas F, Depetris A, Svennerholm AM, Sack DA. Safety and immunogenicity of oral killed whole cell recombinant B subunit cholera vaccine in Barranquilla, Colombia. Bull Pan Am Health Organ. 1995 Dec;29(4):312-21.

In January and February 1992, an assessment was conducted of the safety and immunogenicity of two doses of a new oral cholera vaccine prepared from the recombinant B subunit of the toxin and from killed whole cells (rBS/WC) in 1,165 individuals between the ages of 12 months and 64 years in Barranquilla, Colombia. This was a randomized, double-blind placebo-controlled study. Participants received two doses of either the vaccine or a placebo (killed *Escherichia coli* K12) over a two-week interval. Few symptoms were detected during the three days following administration of the initial dose and even fewer following the second. Sera obtained upon administration of the first dose and two weeks after administration of the second were tested for *Vibrio cholerae* 01 Inaba vibriocidal antibodies and antitoxins. Geometric mean titers (GMT) of vibriocidal antibodies were found to increase two-fold in subjects receiving the vaccine. In the paired samples taken from vaccinated subjects, two-fold or greater increases were observed in 44% and four-fold or greater increases were observed in 34%, as compared to similar increases in 9.2% and 2.2% of the sera taken from those receiving the placebo ($P < 0.05$). The GMTs of IgG and IgA antitoxins, as determined by ELISA, increased by factors of 4 and 3.2, respectively, in those receiving the vaccine, as compared to factors of 1.1 and 1.1 in those given the placebo ($P < 0.001$ for IgG, $P < 0.01$ for IgA). Approximately 80% of the paired samples from the vaccinated group showed an increase of both IgG and IgA antitoxins ≥ 1.5 , as compared to only about 20% of those in the placebo group ($P < 0.000001$). Belonging to the O blood group did not significantly affect the immune response. Children under age four tended to show a weaker vibriocidal antibody response and a stronger antitoxin response than older subjects. The two doses of oral vaccine were found to be safe and without attributable side-effects. The vibriocidal antibody and antitoxin responses were similar to those obtained previously with the conventional oral killed whole cell B subunit cholera vaccine.

PIP: In a randomized, double-blind, placebo-controlled study in January and February 1992, the safety and immunogenicity of two doses of a new oral cholera vaccine was assessed. The vaccine was prepared from the recombinant B subunit of the toxin and from killed whole cells (rBS/WC) in 1165 individuals between the ages of 12 months and 64 years in Barranquilla, Colombia. Participants received two doses of either the vaccine or a placebo (killed *Escherichia coli* K12) over a 2-week interval. Few symptoms were detected during the 3 days following administration of the initial dose and even fewer following the second one. Sera obtained upon administration of the first dose and 2 weeks after administration of the second dose were tested for *Vibrio cholera* 01 Inaba vibriocidal antibodies and antitoxins. Geometric mean titers (GMTs) of vibriocidal antibodies were found to increase two-fold in subjects receiving the vaccine. In the paired samples taken from vaccinated subjects, two-fold or greater increases were observed in 44% and four-fold or greater increases were observed in 34%. In comparison, similar increases were found only in 9.2% and 2.2% of the sera taken from those receiving placebo ($p .05$). The GMTs of IgG and IgA antitoxins, as determined by ELISA, increased by factors of 4 and 3.2, respectively, in those receiving the vaccine as compared with factors of 1.1 and 1.1, respectively, in those given the placebo ($p .001$ for IgG and $p .01$ for IgA). Approximately 80% of the paired samples from the vaccinated group showed an increase of both IgG and IgA antitoxins ≥ 1.5 as compared with only about 20% of those in the placebo group ($p .000001$). Belonging to the O blood group did not significantly affect the immune response. Children under the age of 4 years tended to show a

weaker vibriocidal antibody response and stronger antitoxin response than did older subjects. The two doses of oral vaccine were found to be safe and without attributable side effects.

Begue RE, Castellares G, Ruiz R, Hayashi KE, Sanchez JL, Gotuzzo E, Oberst RB, Taylor DN, Svennerholm AM. Community-based assessment of safety and immunogenicity of the whole cell plus recombinant B subunit (WC/rBS) oral cholera vaccine in Peru. *Vaccine*. 1995 May;13(7):691-4.

Every year since its introduction in 1991, there have been epidemics of cholera in Lima, Peru. Vaccination is one approach to the control of cholera. A pilot study was conducted to assess the safety and immunogenicity of a whole cell plus recombinant B subunit (WC/rBS) cholera vaccine in Lima, Peru. Five hundred and forty-one volunteers aged 2-65 years received two doses two weeks apart of WC/rBS vaccine or *Escherichia coli* K12 placebo administered in bicarbonate buffered water. Symptoms were monitored on all subjects and blood was collected from 102 persons before the first dose and two weeks after the second dose. Mild post-vaccination gastrointestinal symptoms were reported with equal frequency for both the vaccine and placebo recipients. Among 51 vaccines, 49% had a twofold or greater increase in serum vibriocidal titers (GMT = 78; range < 1:10 to 1:5120); and 92% and 82% developed a twofold or greater serum anti-cholera toxin IgG and IgA response, respectively. Persons with elevated prevaccination vibriocidal titers had a decreased response to the WC/rBS. Age and blood group did not affect the immune response. The WC/rBS vaccine was safe and immunogenic in a group of native Peruvians.

Sanchez JL, Vasquez B, Begue RE, Meza R, Castellares G, Cabezas C, Watts DM, Svennerholm AM, Sadoff JC, Taylor DN. Protective efficacy of oral whole-cell/recombinant-B-subunit cholera vaccine in Peruvian military recruits. *Lancet*. 1994 Nov 5;344(8932):1273-6.

The cholera epidemic in South America has reinforced the need for safe and effective oral vaccines. In a randomised, double-blind, placebo-controlled efficacy trial among 1563 Peruvian military recruits we have investigated the protective efficacy of an oral inactivated whole-cell/recombinant-B-subunit (WC/rBS) cholera vaccine. Participants were given two oral doses of cholera vaccine or *Escherichia coli* K12 placebo, with an interval of 7-14 days. 1426 (91%) subjects received the two prescribed doses and were followed up for a mean of 18 weeks (median 21 weeks). After vaccination, *Vibrio cholerae* O1 El Tor Ogawa was isolated from 17 subjects with diarrhoea. 16 of the cholera cases occurred 2 weeks or longer after the second dose of vaccine (14 placebo recipients, 2 vaccinees). We also detected 14 symptomless infections (11 [7 placebo recipients, 4 vaccinees]) 2 weeks or longer after the second dose. The vaccine had significant protective efficacy against cholera (86% [95% CI 37-97], $p < 0.01$) but not against symptomless infection (42% [-96 to 85]). All cholera cases were in people of blood group O, who made up 76% of the study population ($p < 0.01$). Two doses of WC/rBS vaccine, given 1 to 2 weeks apart, provide rapid, short-term protection against symptomatic cholera in adult South Americans, who are predominantly of blood group O. Long-term efficacy studies in Peruvian adults and children are under way.

Peltola H, Siltonen A, Kyronseppa H, Simula I, Mattila L, Oksanen P, et.al. Prevention of travellers' diarrhoea by oral B-subunit/whole-cell cholera vaccine. Lancet 1991/Nov 23; 338(8778):1285-89.

307 tourists received two oral doses of BS-WC, whereas 308 controls received a placebo before departure. A research team went out with tourists and a laboratory for enteric pathogens was set up on location. A faecal specimen was taken from 100 randomly selected subjects before departure, from all travellers with diarrhoea, and routinely after return. Enteropathogenic bacteria were not isolated from any of the pre-departure specimens but were present during or after the holiday in 47% of tourists with travellers' diarrhoea, and in 14% of those without diarrhoea. BS-WC induced a 52% protection ($p = 0.013$) against diarrhoea caused by ETEC. The protection was better for mixed infections (65%, $p = 0.016$). The protective efficacy against a combination of ETEC and any other pathogen was 71% ($p = 0.02$), and that against ETEC plus *Salmonella enterica* even better at 82% ($p = 0.01$).

Clemens JD, Sack DA, Harris JR, Van Loon F, Chakraborty J, Ahmed F, Rao MR, Khan MR, Yunus M, Huda N, et al. Field trial of oral cholera vaccines in Bangladesh: results from three-year follow-up. Lancet, 1990 , 335 : 270-273.

The protective efficacy (PE) of B subunit killed whole-cell (BS-WC) and killed whole-cell-only (WC) oral cholera vaccines was assessed in a randomised double-blind field trial among children aged 2-15 years and women over 15 years in rural Bangladesh. Among the 62 285 subjects who received three doses of BS-WC, WC, or *Escherichia coli* K12 strain placebo, cumulative PE at 3 years of follow-up was 50% for BS-WC and 52% for WC. PE was similar against severe and non-severe cholera, but was significantly lower in children who were vaccinated at 2-5 years (26% for BS-WC; 23% for WC) than in older persons (63% for BS-WC; 68% for WC). Among persons vaccinated at 2-5 years, protection at 4-6 months of follow-up was similar to that for older persons, but rapidly waned thereafter and was not evident during the third year of follow-up. In contrast, persons vaccinated at older ages were protected even in the third year of follow-up (PE 40% for BS-WC; 62% for WC). PE was substantially higher against classical cholera (58% for BS-WC; 60% for WC) than against El Tor cholera (39% and 40%).

Clemens JD, Sack DA, Harris JR, Chakraborty J, Khan MR, Stanton BF, Ali M, Ahmed F, Yunus M, Kay BA, et al. Impact of B subunit killed whole-cell and killed whole-cell-only oral vaccines against cholera upon treated diarrhoeal illness and mortality in an area endemic for cholera. Lancet. 1988 Jun 18;1(8599):1375-9.

The impact of B subunit killed whole-cell (BS-WC) and killed whole-cell-only (WC) oral cholera vaccines was assessed in a randomised double-blind trial in rural Bangladesh. 62,285 children aged 2-15 years and women aged over 15 ingested three doses of one of the vaccines or placebo. During the first year of follow-up there was a 26% reduction of all visits for treatment of diarrhoea in the BS-WC group and a 22% reduction in the WC group. The reduction of all admissions for fatal or severely dehydrating diarrhoea was 48% in the BS-WC group and 33% in the WC group. Overall mortality rates were 26% lower in the BS-WC

group and 23% lower in the WC group during the first year, and reductions of mortality were observed only in women vaccinated at ages over 15 years. However, no differences in cumulative mortality were evident at the end of the second year of surveillance.

Cost-effectiveness

Jeuland M, Cook J, Poulos C, Clemens J, Whittington D; DOMI Cholera Economics Study Group. Cost-effectiveness of new-generation oral cholera vaccines: a multisite analysis. Value Health. 2009 Sep;12(6):899-908.

OBJECTIVES: We evaluated the cost-effectiveness of a low-cost cholera vaccine licensed and used in Vietnam, using recently collected data from four developing countries where cholera is endemic. Our analysis incorporated new findings on vaccine herd protective effects. **METHODS:** Using data from Matlab, Bangladesh, Kolkata, India, North Jakarta, Indonesia, and Beira, Mozambique, we calculated the net public cost per disability-adjusted life year avoided for three immunization strategies: 1) school-based vaccination of children 5 to 14 years of age; 2) school-based vaccination of school children plus use of the schools to vaccinate children aged 1 to 4 years; and 3) community-based vaccination of persons aged 1 year and older. **RESULTS:** We determined cost-effectiveness when vaccine herd protection was or was not considered, and compared this with commonly accepted cutoffs of gross domestic product (GDP) per person to classify interventions as cost-effective or very-cost effective. Without including herd protective effects, deployment of this vaccine would be cost-effective only in school-based programs in Kolkata and Beira. In contrast, after considering vaccine herd protection, all three programs were judged very cost-effective in Kolkata and Beira. Because these cost-effectiveness calculations include herd protection, the results are dependent on assumed vaccination coverage rates. **CONCLUSIONS:** Ignoring the indirect effects of cholera vaccination has led to underestimation of the cost-effectiveness of vaccination programs with oral cholera vaccines. Once these effects are included, use of the oral killed whole cell vaccine in programs to control endemic cholera meets the per capita GDP criterion in several developing country settings.

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*this list is extracted from Background paper on the integration of oral cholera vaccines into global cholera control programmes. Ad-hoc vaccine working group (http://www.who.int/entity/immunization/sage/1_Background_Paper_Cholera_Vaccines_FINALdraft_13_oct_v2.pdf, accessed March 2010).

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