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Summary of Cholera Vaccine Workshop

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A workshop on cholera vaccines was held at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) 12-14 October 1993. Sponsored by ICDDR,B and supported by the United States Agency for International Development (USAID) and the Swedish Agency for Research Cooperation with Developing Countries (SAREC), the workshop was attended by 25 investigators from 9 countries (a list of the participants is included). On the first day, the participants visited Matlab, the rural field station of the ICDDR,B, which is about 45 km south-east of Dhaka. This study area has been used for 6 different cholera vaccine field trials, dating back to 1963. The last cholera vaccine field trial to be done there began in 1985, and involved the testing of two oral non-living cholera vaccines (whole cells with or without added B-subunit of cholera toxin). In the demographic surveillance area where these field trials were done, there are approximately 200,000 persons under fortnightly surveillance, and demographic records have been kept since 1966. A diarrhoea treatment field hospital of approximately 70 beds with a clinical laboratory supports the diarrhoea studies that are being done in the community. This visit was of particular importance to the participants, since this is a likely place to test the new generation of cholera vaccines.

On the second day, selected participants presented data on the epidemiology, laboratory, and clinical features of the past and present worldwide cholera problem with an emphasis on Latin America, Bangladesh, and India, and on the newly described *V. cholerae* O139 synonym Bengal. This was followed by presentations on the development of the new generation of cholera vaccines, both killed and live, and information on their safety and immunogenicity. Finally data from an ongoing cholera vaccine field trial in Indonesia were presented.

The third day was spent discussing issues related to the development and testing of new cholera vaccines, with a particular emphasis on the possibility of doing the field testing in Matlab.

The presentations given during the second day and the areas of consensus reached during the third day are presented here in more detail.

Epidemiological and clinical studies of *V. cholerae* O1 and *V. cholerae* O139. Dr. AK Siddique presented data on the occurrence of cholera in Bangladesh during the past 8 years. Both classical and El Tor strains of *V. cholerae* O1 have been responsible for outbreaks of cholera in the southern part of the country until this past December (1992), when a large outbreak of cholera due to a *V. cholerae* non-O1 was found. This organism, later identified and named as *V. cholerae* O139 Bengal, was responsible for an estimated 170,000 cases and nearly 2,000 deaths over the next 6- month period in the southern part of the country. The unusual features of the epidemic were 1) only *V. cholerae* O139 organisms were identified (in 69% of patients); no *V. cholerae* O1 organisms were cultured, 2) the cholera illness (63%), as well as deaths, (78%) were predominantly in persons more than 15 years of age. This contrasts markedly with previous epidemics in this area in which the comparable figures for cases and fatalities in adults were 41% and 38% respectively. This suggested that adults were not protected against the new cholera strain.

Dr. Md Yunus then presented data on cholera in Matlab over the past 27 years. During the period from 1966 to 1992, the overall attack rate for *V. cholerae* O1, classical and El Tor, among the 200,000 persons under surveillance varied from 0.5-8.0 per 1000 per year. Children aged 2-4 years had the highest attack rates (7/1000) as indicated by hospitalization. There had been no trend toward decreasing attack rates over the years. In addition to the regular yearly spring and fall epidemic periods, there had been longer 8-year cycles of rising and falling attack rates. In the past year an increasing percentage of O1 organisms were resistant to multiple antibiotics, including tetracycline. El Tor had been the predominant organism for the past approximately 8 years.

V. cholerae O139 appeared in Matlab in mid-February 1993, resulting in a large epidemic of cholera which peaked in March-April. (A fall cholera epidemic in which both O1 and O139 organisms are being isolated, is presently underway.) During the peak period, 55% of cultures were positive for *V. cholerae* O139. Most of the hospitalized patients were adults over the age of 15 years; the attack rates (3.5/1000/9 months) were also highest in this age group. Children under 5 years of age had an attack rate of 3.1/1000/9 months. All of the O139 isolates were susceptible to tetracycline. Patients came from all areas of Matlab, and there was no family clustering of patients. A family and neighborhood follow-up study of *V. cholerae* O139 index patients showed a secondary

infection rate of 18%, with a symptomatic: asymptomatic ratio of 1:5; none of the secondary cases, however, developed clinical cholera.

Dr. D Mahalanabis summarized the experience of cholera in the ICDDR,B Dhaka hospital over the past 10 years. The hospital routinely cultures a systematic 4% sample of all persons presenting for treatment. During the past 10 years, approximately 100,000 patients with cholera were seen, which represents about 14% of all patients seen. The average number of diarrhoea patients seen was between 60,000 and 90,000 per year. This year, however, with the appearance of the O139 vibrio, the number had already exceeded 100,000 and was expected to reach about 130,000 by the end of the year.

The O139 epidemic in Dhaka began in late January following a large gathering of Muslim pilgrims nearby; both an early spring and now a fall epidemic are being seen. Approximately 27% of patients were positive for O139 vibrios during the first four months of 1993. Mostly persons over 15 years of age (71%) were being seen. The patients were primarily of the urban poor. The clinical disease was indistinguishable from cholera due to O1 vibrios and seemed to be more severe in adults than in children. In adults, 83% required initial intravenous rehydration. Preliminary studies showed a close association between blood group O and susceptibility to *V. cholerae* O139, as has been seen with El Tor cholera, but not classical.

Dr. Allen Ries described the Latin American experience with the 7th pandemic strain of cholera, *V. cholerae* O1 biotype El Tor. After a hiatus of 96 years, cholera returned to Latin America in January 1991, with explosive epidemics in several countries, particularly Peru and Ecuador. In the first 6 weeks of the epidemic in Peru, there were over 65,000 cases and 350 deaths reported; by this time approximately 40% of the population had serological evidence of infection. Cholera quickly spread to other South and Central American countries; to date the Caribbean islands, however, have been spared. By the end of the second year of the outbreak, nearly 750,000 cases had been reported in Latin America.

Several modes of transmission were identified during these outbreaks: consuming municipal water, river water, ice, street vendor foods and beverages, leftover rice, uncooked seafood, cooked crabs, and unwashed fruits and vegetables. Protective factors included boiling water, adding citrus juice to water, and having soap in the home. Many of these findings were quickly applied as preventive measures. In this completely non-immune population, an efficacious vaccine applied in advance of the epidemic wave could possibly have decreased the morbidity and mortality of cholera in Latin America.

Dr. RB Sack discussed some of the features of the past 7 cholera pandemics which have occurred over the last 170 years. Only the serogroups of the cholera vibrios causing the last 3 pandemics (5-7) are known; it is certainly possible that earlier pandemics could have been caused by organisms other than O1. The length of the pandemics varied from as little as 6 years to the present one, which is now 30 years old. This suggests that pandemics can move relatively rapidly, even without the means of modern rapid transportation. The El Tor vibrio replaced the "classical" vibrio in India in one year (1963), but in Bangladesh, the two biotypes remained together for over 25 years before the "classical" strains disappeared (1991).

The O139 strain seems to be replacing the O1 vibrios at a fairly rapid rate, both in the environment and in the population, and has spread very rapidly during the past year since its recognition. Because O139 is a new serogroup, and there is no serogroup-specific immunity in the population, the spread of the vibrio should be even more rapid than with El Tor, where the same serogroup of the pandemic strain was similar to the one it was replacing. *V. cholerae* O139 is likely to spread around the world as the next pandemic strain.

Microbiological studies of *V. cholerae* O139. Dr. MJ Albert discussed microbiological and cross-protection studies with *V. cholerae* O139. These organisms are similar to *V. cholerae* O1 in most respects, except for their serogroup. Of particular note, they agglutinate chicken and human O-group red cells; they are resistant to polymyxin B and to vibriostatic compound (O/129) and to Mukherjee's phages for both biotypes of O1; they are uniformly susceptible to tetracycline. They possess pili and are invasive in HEp-2 cells. All isolates produce cholera toxin, as indicated by all conventional assays. Restriction fragment length polymorphisms of cholera toxin A gene (*ctx*A) of the isolates with the enzyme *Bgl*I revealed two patterns suggesting the existence of multiple copies of the gene, but ribotyping showed that all isolates belonged to a single pattern suggesting the clonal nature of these isolates.

Cross-protection studies were done by immunizing adult rabbits orally with either O139 or O1 vibrios. The live vaccine strain CVD103-HgR, derived from *V. cholerae* Inaba 569B, was used for the O1 immunization. Two oral doses were used to immunize 12 rabbits with either strain; 3 weeks post-immunization they were bled and challenged by the RITARD procedure. Half of the rabbits in each of the groups were challenged with either homologous or heterologous live virulent organisms. At the time of challenge, all immunized rabbits had high levels of cholera toxin antibodies, and antibodies to the LPSs of the corresponding immunizing strains. Homologous protection (no diarrhoea in 12 of the 12 immunized rabbits) was demonstrated, but no heterologous protection (12 of 12 animals developed severe diarrhoea) was seen. Control unimmunized rabbits all developed severe watery diarrhoea. This study shows that there was no cross-protection induced by infection with these strains, and that anti-toxic immunity was not sufficient for protection. These studies indicated that the relevant antigenic components from both serogroups need to be included in any future vaccine formulations.

Dr. Y Takeda described the spread of *V. cholerae* O139 in India and the characterization of the strains. This organism was first responsible for a large outbreak of cholera-like illness in Madras, India, in October 1992, and within a month was isolated from other parts of India, including Madurai, Vellore, and Calcutta. In mid-February, an explosive outbreak of cholera occurred in

Calcutta. Shimada and coworkers assigned these strains (which were simultaneously occurring in Bangladesh) to a new serogroup O139, with a synonym Bengal. This serogroup has now spread also into Thailand, Nepal, Pakistan, Malaysia, and western China.

Biochemical and physiological characterization of 165 strains showed that they formed a homogeneous group with identical responses, that were similar in most respects to that exhibited by the O1 serogroup. Serologically they were not agglutinated by O1 polyclonal or monoclonal antibodies, or antisera against the other 137 non-O1 serogroups. With the exception of serogroup O22, none of the other non-O1 serogroups agglutinated with antisera prepared against O139. Therefore antisera prepared against O139 had to be absorbed with an O22 serogroup strain to remove cross-reacting agglutinins. Serological similarity was shown among strains isolated from widely separated geographical areas.

All O139 strains produced cholera toxin, as measured by the bead-ELISA, and hybridized with DNA probes specific for CT and ZOT genes. To further analyze the similarity between CT of O1 and O139, they cloned and sequenced the CT gene from a strain of O139 and found the nucleotide sequence of the cloned CT gene to be identical to that of an El Tor strain. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNAs of different isolates of O139 showed that the restriction fragment length polymorphism pattern exhibited by all the O139 isolates were identical, suggesting the pandemic spread of a single clone.

Laboratory development of candidate cholera vaccines. Dr. J Holmgren presented information on the development and testing of an oral, B subunit-killed whole cell cholera vaccine containing antigens from both *V. cholerae* O1 and O139. Several isolates of O139 vibrios were characterized; all strains produced high levels of cholera toxin, had abundant expression of MSHA fimbria, produced the TcpA subunit, but not detectable amounts of TCP fimbriae or TCP surface antigen. These studies suggest that O139 is very similar to El Tor; in further support of this similarity, CT isolated from O139 was found to be structurally and immunologically indistinguishable from El Tor CT as tested with monoclonal antibodies; sequencing of CTB genes revealed a complete identity with that of El Tor O1. Polyclonal rabbit antisera to live, heat-killed, or formalin-killed O139 strains agglutinated all O139 strains, exhibited vibriocidal activity, and were protective against challenge with live O139 organisms in rabbit ileal loop tests.

Based on these results, in collaboration with the Swedish Bacteriology Laboratory (SBL vaccines), Stockholm, a new oral B-subunit-bivalent O1/O139 whole cell cholera vaccine intended to give protection against both O1 and O139 cholera is being developed. The vaccine is the same as the previous one, but has in addition 5×10^{10} formalin-killed O139 vibrios of a strain stably expressing much LPS and MSHA, but only minimal amounts of soluble hemagglutinin/protease. Studies are now planned to test this new vaccine for safety, mucosal IgA immunogenicity, and ultimately protection, in volunteers.

Dr. J Kaper described the construction of the present live attenuated cholera vaccine, CVD 103-HgR, as well as that of an O139 vaccine candidate. The latter was constructed utilizing recombinant DNA techniques on a wild type *V. cholerae* O139 strain AI-1837, isolated from a patient with clinical cholera in Bangladesh, which was also shown to cause cholera in volunteers. Sequences encoding all known toxins of *V. cholerae*, including CT, Zot, Ace, and the hemolysin/cytolysin (*hly*A) as well as all copies of the RS1 site-specific transposon were deleted. The *ctx*B gene, encoding the B-subunit of cholera toxin, was cloned into the mutated *hly*A locus along with sequences encoding mercury resistance. Expression of the *ctx*B gene is under the control of the natural *ctx* promoter. The attenuated O139 vaccine candidate was designated CVD 112 and will soon be tested in volunteers.

Dr. J Mekalanos described the molecular analysis of virulence determinants carried by the O139 vibrio and the construction of vaccine prototypes. Results from studying several strains from several different geographical areas showed: 1) all O139 strains analyzed carried two or more copies of the CTX genetic element (and therefore *ctx*AB, *zot*, *cep*, and RS1) which were easily resolvable with the restriction enzyme *Xba*I and which are inserted in tandem at the same site occupied by the CTX element in El Tor strains, 2) all strains carried a characteristic 5- kilobase pair *Xba*I fragment that hybridized strongly to the *tcpA* probe, and 3) insertional inactivation of *toxR* in one of these strains resulted in elimination of cholera toxin expression. These studies suggest that the O139 strains are most closely related to El Tor, rather than classical strains of *V. cholerae*, and are distinct from other non-O1 vibrios.

A vaccine prototype has been constructed from the O139 strain (MO10, isolated in Madurai, India), using previously described methods that had been successfully applied to a 1991 isolate of an El Tor strain from Peru. Plasmid pAR62 was used to introduce a large deletion into the *ctx* region of the strain that removes the entire CTX genetic element (including *ctx*AB, *zot*, *cep*, and RS1 and attRS1 sequences); this strain was designated Bengal-2. Into the *rec*A gene of this strain was introduced an insertion that encodes cholera toxin B-subunit which was expressed at high level via the *htp*G heat shock promoter. This candidate vaccine will also be undergoing safety and immunogenicity testing.

Safety, immunogenicity, and protection studies of cholera vaccines. Dr. M Levine reviewed the safety, immunogenicity, and efficacy studies of the live oral cholera vaccine strain CVD 103-HgR. This vaccine strain was derived from an O1 classical Inaba strain, 569B, by deleting 94% of the gene encoding the A-subunit of cholera toxin and introducing a gene encoding resistance to mercury into the *hly*A locus of the chromosome. To date more than 4,000 subjects from 2-50 years of age around the world have participated in clinical trials, and the vaccine has been found to be free of side effects. At doses of 5×10^8 CFU in developed countries and 5×10^9 in developing countries, it gives rise to seroconversions (a four-fold or greater rise in vibriocidal antibodies) in

75%-90% of subjects after a single dose. Eight experimental challenge studies have been done, all of which show 100% protection against severe or moderate diarrhoea, irrespective of the serotype or the biotype of the challenge strain. Protection was evident as early as 8 days and has been shown to last for at least 6 months. The vaccine is presently undergoing a large field trial of efficacy in Indonesia. (See presentation of Dr. Ritchie.)

Dr. D Taylor presented information on the safety and immunogenicity of prototype live, oral attenuated vaccines against El Tor cholera. These strains, which were from Peru, Bangladesh, and Bahrain, had been produced by a deletion that removes all known toxins, an intestinal colonization factor (CEP) and RS1, and inserting the B-subunit of *ctx* into the *rec*A gene. (See previous presentation by Dr. Mekalanos.) A filamentous, motility-deficient mutant of a Peru strain, designated Peru-14, that gave about 3 times less colonization in the infant mouse model, was also selected for further study. Five candidate strains were fed to 3-6 volunteers each. All strains produced diarrhoea in some volunteers, although strain Peru-14 was best tolerated. (Two of 12 volunteers fed the Peru-14 strain had mild abdominal discomfort and loose stools.) All recipients excreted the ingested vaccine strain. Vibriocidal antibody rises were seen in essentially all volunteers. Eight volunteers were challenged 4 months after immunization with Peru-3 or Peru-5 strains, of which 7 were protected. This is compared to 3 of 3 unimmunized volunteers becoming ill ($p=0.02$).

These studies suggest that Peru-3 and its filamentous mutant Peru-14 are strains that efficiently colonize the gut and induce high titer vibriocidal antibodies at 10^6 organisms, and are potential vaccine candidates for protection against O1 El Tor cholera.

Ongoing cholera vaccine trial. Dr. E Richie summarized the efficacy trial of the live, attenuated CVD 103-HgR vaccine being carried out presently in Indonesia. Cholera is endemic in North Jakarta, with attack rates estimated at 1-5 cases of cholera per 1000 population per year. This large (67,000 participants), randomized, double-blind, placebo-controlled trial aims to assess the efficacy of a single dose of the vaccine to prevent moderate to severe cholera. All vaccine recipients are between the ages of 2-41 years of both sexes, with the exclusion of pregnant women. Photo-identification cards are being used to assist in identification of the participants. Passive surveillance is being maintained at 8 hospitals and additional village health centers. Rectal swabs are being obtained at the surveillance sites, and transported in holding media to a central laboratory. In a subsample of 800 subjects, more detailed studies of reactogenicity and immunogenicity are being done.

To date 38,000 persons have been immunized; children between the ages of 2-5 years constituted 14% of the vaccinees. Two cases of cholera have been identified among study subjects. No *V. cholerae* O139 victims have yet been detected in Indonesia.

Cholera vaccine trial methods. Dr. J Clemens summarized lessons learned from previous cholera vaccine trials. In Bangladesh, cholera is known to be multi-focal, and therefore somewhat unpredictable. With the appearance of the new O139 vibrio, estimates of cholera attack rates by either O1 or O139 vibrios becomes even more difficult. Also noted is the filtering effect of enrolling subjects into a prospective trial, which can lead to marked differences in the risk of the enrolled from the source population. Since comprehensive active surveillance is impossible because of the large numbers needed for a cholera vaccine trial, it will be impossible to determine infection rates of cholera. Passive surveillance will only detect the more severe end of the clinical spectrum of disease, and therefore efficacy estimation will primarily reflect protection against severe illness. Mixed infections also make the definition of cholera in a field trial more problematic. It is important to address methods for assessing subgroup-specific efficacy in a statistically precise fashion before the field study is done, because vaccine protection will vary in patients of different ages, blood groups, and infectious agents (biotypes and serotypes, and now serogroups).

Issues of field testing of candidate cholera vaccines. The third day was spent discussing issues relating to the development and testing of the vaccine. This was done in the form of answering a series of questions; consensus was reached on most points.

1. **What antigens should the vaccine contain?** Since epidemics are presently being caused by both El Tor and O139 vibrios, a universal "cholera vaccine" will need to include antigens of both O1 and O139 vibrios. Both serotypes (Inaba and Ogawa) and biotypes (El Tor and classical) of O1 vibrios should be included, since protection may be serotype-related, and classical antigens may be more generally protective than El Tor antigens. The final vaccine will therefore have to be at least bivalent, and perhaps trivalent in order to include all the necessary antigens.

2. **Which vaccines should be tested?** It will probably not be possible to test more than two candidate vaccines in one trial. A killed and a live attenuated vaccine could be tested together. Although a cold chain is clearly necessary only for the live vaccine, it would need to be used to protect the blinding of the study. A live vaccine should contain a marker in order to verify that it is not causing disease.

3. **Can a placebo group be used?** Although a placebo group can be used outside Bangladesh (because there are no data on efficacy), inside Bangladesh a placebo group may not be possible. Rather, a control group could receive the oral whole cell vaccine, which has previously been shown to be effective in Matlab. This control group, however, may turn out to be, in essence, a placebo group if the O139 vibrio completely displaces El Tor in the study area.

4. **How many doses need to be given?** At least two doses will be necessary for the killed vaccine, while a single dose may be sufficient for the live vaccine. Both

vaccines will probably have to be given as boosters at intervals as yet unknown (probably several years) to maintain long term protection.

5. **What type of field trial can be done?** This will depend on the excretion patterns of the live vaccines being developed; if they are shed heavily into the environment, it will not be possible to do the usual randomized efficacy study, since there may be inadvertent immunization of control persons in contact with the vaccinees. In that case some larger groups of persons, located by geographic area, will need to be randomized, perhaps in an effectiveness, rather than an efficacy trial.

6. **What is the sample size needed?** This will, of course, depend on the type of trial being done, and the anticipated attack rate. Past trials in Matlab have been done with approximately 25,000 persons per vaccine group. The sample size should be large enough so the lower confidence limits of protection are no less than 40% for the predominant vibrio serogroup.

7. **What segment of the population should be vaccinated?** It is possible to consider all individuals at risk for the O139 vibrio. Although cholera is not a major problem under 2 years of age, it may be desirable to immunize at the earliest possible time. A live vaccine, however, may not survive well in breastfed infants. (No phase I studies have yet been done in children under the age of 2 years.) In endemic cholera, children between the ages of 2 and 4 years have the highest attack rates, and would therefore be given priority.

8. **Should Matlab be used for such a trial?** There was consensus that Matlab represents a unique site with its well-kept demographic surveillance system in a population of 200,000, its known cholera seasons, and its past experience gained in conducting cholera vaccine trials. It is not known, however, at this time, whether O1 or O139 vibrios (or both) will be circulating when the vaccines are ready for testing. There was some concern that since cholera vaccines had been used there in the past (8 years ago the last cholera vaccines were given) this might interfere with a new trial. This was felt, however, not to be a significant limitation, since by the time a trial could be done, no children under 10 years of age (perhaps the major target group) would have received a cholera vaccine, and the O139 vibrio was, in any case, new to the entire population.

9. **What type of surveillance should be used?** Although active surveillance would be most accurate in detecting cholera infections, it is impossible to carry out on a large scale. Therefore, passive institutional surveillance, which will detect moderate to severe illness, is the best choice. In Matlab this can be easily done with the existing hospital and health sub-centers.

10. **How soon will vaccines be available for field testing?** It was estimated that no new vaccines would be ready for field testing for at least 6 months (killed vaccines) and 12 months (living vaccines.) For practical reasons it seems that it will be approximately 18 months before a large (3-cell) field trial could be initiated.

11. **Assuming a trial will be done in Matlab, what can be done there in the interim to prepare for a trial?** It was thought that 1) the family, neighborhood contact study could be expanded, to determine rates of asymptomatic infection in the community, 2) geographic information systems (GIS) analysis (already begun) will define the clustering of cases, 3) more systematic clinical information from young children with cholera could be obtained to determine the range of clinical severity, 4) an ongoing serosurvey could be conducted in the population to determine the widespread exposure to O139 vibrios, 5) other entry points for delivery of the vaccine, such as schools, EPI encounters, could be explored, and 6) data collection on age-specific attack rates for both O1 and O139 cholera will be continued.

12. **What are some of the sensitivities of doing such a trial?** Persons in Matlab have never received any benefit (in a public health sense) from the previous six cholera vaccine trials. It was felt that if a vaccine was found effective in the trial, it should be made available to all the Matlab residents.

    The use of a genetically-engineered live vaccine should not be a problem in Bangladesh; there is a precedent for using live vaccines (BCG, polio, measles). It is important, however, that the vaccines used have no known side effects.

13. **Can the candidate vaccines be produced in Bangladesh?** It is important that any vaccine found effective be able to be provided to the country at a low cost. This could best be done by producing the vaccine locally, if facilities and expertise were available. A cost of ten U.S. cents/dose was felt to be a reasonable target cost.

14. **How should the vaccine be packaged for the trial?** It was felt that the vaccine should be tested in the form that it will ultimately be used (single or multiple-dose packaging.) This is particularly important because packaging makes up the largest part of the cost of the vaccine.

15. **Who will fund such a trial?** This type of trial clearly will be expensive. A number of funding agencies might be interested in supporting the testing of a universal cholera vaccine. These include: from the US-AID, NIH, Armed Forces; from the United Nations, WHO, UNICEF; from Europe, EEC; the Government of Bangladesh (through bilateral funding), and the manufacturers of the vaccines. A consortium of funding agencies will probably be needed.

CONCLUSIONS AND RECOMMENDATIONS

The International Workshop on Cholera Vaccine held at the ICDDR,B in October 1993 brought together 25 leading investigators active in the area of cholera vaccine development and testing. Following selected reviewers of the epidemiology and immunology of cholera, and discussions of past and ongoing studies of cholera vaccines, the participants formulated some general guidelines for further development of cholera vaccines. Because of the appearance of *V. cholerae* O139 Bengal as a cause of epidemic cholera in India and Bangladesh, it was agreed that newly developed cholera vaccine should contain protective antigens of both *V. cholerae* O1 and O139. The advantages of killed vs live attenuated oral candidate vaccines were discussed and it was felt that both types could be field-tested in a single trial, which would of necessity require a large sample size. The trial would ideally be done in an area where both vibrio serogroups were known to be causing cholera. Matlab was felt to be a suitable place for such a trial, although it was recognized that new vaccine candidates will not be available for field testing for at least another one and a half years, and it is not known what *V. cholerae* serogroups will be circulating at that time. The participants strongly endorsed the continued development of cholera vaccines as a potential useful public health tool.

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