2)a)

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 22/9/83

Principal Investigator J. CLEMENS

Trainee Investigator (if any) —

Application No. 83-035(P)

Supporting Agency (if Non-ICDDR,B) —

Title of Study Antibody responses to different [illegible] and 6-subunit + whole-cell

Project status: Pilot Study

( ) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

Circle the appropriate answer to each of the following (If Not Applicable write NA).

Source of Population:

(a) Ill subjects — Yes (No)
(b) Non-ill subjects — (Yes) No
(c) Minors or persons under guardianship — (Yes) No

Does the study involve:

(a) Physical risks to the subjects — Yes (No)
(b) Social Risks — Yes (No)
(c) Psychological risks to subjects — Yes (No)
(d) Discomfort to subjects — Yes (No)
(e) Invasion of privacy — Yes (No)
(f) Disclosure of information damaging to subject or others — Yes No

Does the study involve:

(a) Use of records, (hospital, medical, death, birth or other) — Yes (No)
(b) Use of fetal tissue or abortus — Yes (No)
(c) Use of organs or body fluids blood, milk — (Yes) No

Are subjects clearly informed about:

(a) Nature and purposes of study — (Yes) No
(b) Procedures to be followed including alternatives used — Yes No Not applicable
(c) Physical risks — Yes No NA
(d) Sensitive questions — Yes No NA
(e) Benefits to be derived — (Yes) No
(f) Right to refuse to participate or to withdraw from study — (Yes) No
(g) Confidential handling of data — (Yes) No
(h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — (Yes) No

5. Will signed consent form be required:
   (a) From subjects — (Yes) No
   (b) From parent or guardian (if subjects are minors) — (Yes) No
6. Will precautions be taken to protect anonymity of subjects — (Yes) No
7. Check documents being submitted herewith to Committee:

___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
✓ Protocol (Required)
✓ Abstract Summary (Required)
✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
✓ Informed consent form for subjects
✓ Informed consent form for parent or guardian
___ Procedure for maintaining confidentiality
___ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:

1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

Agree to obtain approval of the Ethical Review Committee for any changes involving the rights and welfare of subjects before making such change.

Principal Investigator

Trainee

25/9/83

SECTION I - RESEARCH PROTOCOL

1. Title: Antibody responses to different formulations of oral B-subunit + whole-cell cholera vaccine

2. Principal investigator: John Clemens, MD

   Co-investigator: Marianne Jertborn, MD, University of Goteborg

3. Starting date: October 1983

4. Completion date: March 1984

5. Total direct cost: $ 3,000.00

6. Abstract summary:

This study is designed to answer practical important questions relating to the formulation of a promising new oral cholera vaccine consisting of cholera B-subunit and heat- and formalin-killed classical and El Tor vibrios (B-subunit/whole cell vaccine). The study will be conducted as a 2 x 3 factorial design, with patients randomized to one of six different groups: 1) placebo (vitamin A solution) together with bicarbonate as given in earlier ICDDR,B studies, i.e. mixed with 100-150 cc of .75% sodium bicarbonate solution and swallowed 2-3 minutes after the volunteers have drunk 100-150 cc of the same sodium bicarbonate solution alone; 2) placebo given only with 100-150 cc of the sodium bicarbonate solution concurrently, without an earlier dose of sodium bicarbonate; 3) moderate dose vaccine (1 mg B-subunit/1x10" killed V. cholerae organisms) with antecedent and concurrent sodium bicarbonate, as noted in 1); 4) moderate dose vaccine with only concurrent sodium bicarbonate, as noted in 2); 5) high dose vaccine (5 mg B-subunit/3x10" killed V. cholerae organisms) with antecedent and concurrent sodium bicarbonate, as noted in 1) and 3); and 6) high dose vaccine with only concurrent sodium bicarbonate, as noted in 2) and 4). We will compare the serum antitoxin and vibriocidal antibody responses after a first and second immunization with each of these formulations of vaccine in children 2-8 years and in lactating women. We will also assess breast mild antitoxin and antibacterial responses in lactating women. The results obtained will be of critical importance for designing a subsequent major field trial with the B-subunit/whole-cell vaccine.

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective:

The long range goal is to develop effective vaccination against cholera. The objective of this pilot study is specifically to determine the antibody responses to the B-subunit and whole cell components of a combined B-subunit/whole cell oral cholera vaccine tested in different formulations: as a liquid moderate-dose vaccine together with and also preceded by sodium bicarbonate; as a liquid moderate-dose vaccine together with but not preceded by bicarbonate; as a liquid high-dose vaccine with antecedent and concurrent sodium bicarbonate; and as a liquid high-dose vaccine with only antecedent sodium bicarbonate. The outcome of these studies will be decisive for the formulation of vaccine to be used in a field trial of B-subunit/whole cell cholera vaccine planned for 1984.

2. Background:

Clinical cholera is known to give rise to immunity in convalescents from the disease of a magnitude and duration exceeding that observed in persons who have been immunized with the conventional parenteral whole cell cholera vaccine. There is now substantial evidence to support the view that an oral vaccine capable of evoking a mucosal antibacterial as well as antitoxic immune response in the gut would have a much greater chance than the hitherto used parenteral vaccines to confer more solid and long-lasting protection. Since five years ICDDR,B and the University of Goteborg in Sweden have collaborated on the development of an oral cholera vaccine with the above sought-for properties. Very promising results have been obtained with a combined vaccine consisting of a mixture of cholera B-subunit and whole cell vaccine (B+WCV). These results and data from studies in North American volunteers showing that orally administered B+WCV confers significant protection against challenge with a high dose of live, virulent *V. cholerae* were recently reviewed at a Consultation convened by the

Diarrhoeal Diseases Control Programme of the World Health Organization in Washington, DC, USA, 10-11 June 1983. The purpose of the Consultation was to discuss the development and field testing of an oral cholera vaccine at ICDDR;B. Participants included the vaccine developers, members of the ICDDR,B Board of Trustees, the Director of the ICDDR,B, members of the Steering Committee of the WHO Diarrhoeal Diseases Control Programmes Scientific Working Group on Bacterial and Enteric Infections, outside experts and WHO Secretariat.

This Consultation summarized the information about the B-subunit/whole cell vaccine as follows. Also included is a follow-up letter of support for a trial of B subunit/Whole cell vaccine by Dr M. Merson, Programme Manager, Diarrhoeal Diseases Control Programme, World Health Organization.

2.2 B-subunit/whole-cell vaccine

The present composition of this vaccine (Table 1) is designed to provide sufficient amounts of the most suitable antigens currently available for evoking intestinal mucosal antitoxic and antibacterial immunity to protect against V. cholerae. Experiments in rabbits have shown that antitoxic and antibacterial antibodies act synergistically in protecting against experimental cholera. Protective antitoxic antibody is mostly directed against the B-subunit, whereas antibacterial immunity is mainly against the cell-wall lipopolysaccharide (LPS). Antibodies against heat-labile antigens, such as haemagglutinins, may also contribute to protection. All these various antigens are included in the vaccine. The vaccine is intended for oral use because current knowledge about intestinal immunology, supported by experiments in humans with this vaccine, indicate that the oral route is superior to the parenteral route for stimulating the formation of secretory IgA (SIgA) antibody in the gut mucosa and local immunological memory. The procedures for preparation and characterization of the vaccine are well defined, thus assuring good batch-to-batch reproducibility.

Vaccine safety: Initial studies in Sweden and Bangladesh demonstrated that the vaccine, given orally, caused no detectable side-effects. In the studies in Sweden, 10 adult volunteers received one or two doses of vaccine containing 0.5 mg of B-subunit and 5×10^{10} organisms of the heat-killed classical Inaba and Ogawa vaccine components (Table 1). In no instance were any local or systemic side-effects observed within one month after immunization. In subsequent studies, 30 adult Swedes and 16 Bangladeshi women were given two doses of vaccine, each containing 0.5-2.5 mg of B-subunit and 5×10^{10} of the heat-killed classical Inaba and Ogawa vibrios. Surveillance for side reactions was performed for 10 days after each immunization by a physician or a health worker; in no instance did these oral immunizations give rise to any detectable side-effects.

In more recent studies in Bangladesh, one or two doses (given one day apart) of 1 mg or 5 mg of B-subunit alone were given orally to about 1 200 family contacts (aged 1-70 years) of patients with cholera to evaluate its possible effectiveness as a toxin-receptor blocking agent. No local or systemic side-effects were observed.

Mucosal immunogenicity and serum antibodies in volunteers: At the ICDDR,B and in Sweden, mucosal immunogenicity studies have provided information on the ability of the vaccine to stimulate mucosal antibody in the

intestine of volunteers. The whole-cell vaccine used in these studies consisted of the heat-killed classical Inaba and Ogawa vaccine components (2.5×10^{10} organisms of each per oral dose) of the presently proposed vaccine (Table 1). Three groups (I-III) of 8-9 healthy Bengali women were given 2 oral or 2 intramuscular doses of vaccine 28 days apart and were compared with one group (9 persons) of cholera convalescents given a single oral vaccination. The vaccine doses given are indicated in Table 2. Five minutes before the oral immunization 100 cc of 0.1 M $NaHCO_3$ solution was given to neutralize gastric acidity; the oral vaccine was then administered in 100 cc of the same solution. Intestinal lavage was performed and fluid specimens were examined 0, 3, 9, and 28 days after each immunization, or (for cholera convalescents) 9 and 28 days after the onset of disease. Antitoxin and antibacterial antibodies in the lavage fluids were measured by an ELISA test using purified cholera toxin and LPS, respectively, as solid phase antigens. Total IgA was also determined by ELISA to permit the expression of all titres in relation to total IgA.

The results showed that a single peroral administration of 2.5 mg of B-subunit induced a significant increase in antitoxin titre in most recipients (Figure 1 and Table 3). Two immunizations with a 0.5 mg dose of B-subunit also induced a significant mucosal antitoxin response in most instances. Although intramuscular injections also induced a rise in the antitoxin IgA titre in many cases, the duration of the response was significantly shorter than after oral administration. The study thus showed the ability of B-subunit, administered orally, to stimulate intestinal mucosal antitoxin responses in Bangladeshi adults.

Measurements of the intestinal mucosal antibacterial antibody response revealed that although clinical cholera induced a substantially increased titre of intestinal IgA antibody to *V. cholerae* LPS in most recipients, increases occurred less frequently and were of lesser magnitude following a single oral or intramuscular vaccination with whole-cell vaccine (Figure 2 and Table 3). However, following the second oral administration of the whole-cell vaccine, intestinal antibacterial responses were induced that were comparable to those following disease in 12 out of 13 vaccinees. Both the magnitude and the duration of the mucosal response attained after two oral administrations were superior to those obtained by the 2-dose parenteral immunization regime.

As its final objective, this study explored the extent to which immunization could induce (or, in the naturally-primed Bangladeshi volunteers, perhaps boost) immunological memory for a mucosal response. Oral immunization with combined vaccine appeared to be as effective as clinical cholera in preparing the intestine for a local IgA antibody response to restimulation by cholera antigens. Both the antitoxin and the anti-LPS responses were seen within 3 days after the second immunizations, which was earlier than in the case of the initial vaccinations (Figs. 1 and 2). As regards the mucosal IgA antitoxin, a rapid response was also seen in many volunteers who had been vaccinated with B-subunit 15 months earlier, suggesting that antitoxic memory was long-lasting.

The SIgA antitoxin and anti-LPS antibody responses in intestinal lavage specimens following one or two immunizations with similar doses of combined vaccine were also studied in adult Swedish volunteers. Significant titre increases of both antibodies were observed in most vaccinees, but they were about 50% lower than those observed in Bangladeshi volunteers.

Challenge studies: Studies of vaccine efficacy were recently performed at the Center for Vaccine Development in Baltimore, USA. Nineteen adult volunteers received 3 oral doses of combined vaccine at 2-week intervals. Each dose of vaccine consisted of 5 mg of B-subunit and 5×10^{10} heat-killed classical Inaba, 5×10^{10} heat-killed classical Ogawa and 1×10^{11} formalin-treated El Tor Inaba organisms - i.e., the first three components of the whole-cell vaccine (Table 1). Three hours prior to ingesting vaccine, the volunteers ingested 300 mg of cimetidine; one minute before vaccination, they took 2 g sodium bicarbonate in 150 ml of distilled water. Antibody responses in jejunal fluid and serum were measured after each immunization. Five weeks after the last immunization, the susceptibility of 11 vaccinees to oral challenge with 2×10^6 live El Tor V. cholerae organisms (strain N16961) administered with 2 g sodium bicarbonate was compared with that of unimmunized control volunteers.

No notable adverse reactions were observed in any vaccine recipients. The results of the challenge studies (Table 4) showed that the vaccine gave significant protection against an ID_{100} challenge dose with live vibrios: 4 of 11 vaccinated persons as compared with 7 of 7 controls developed diarrhoeal illness after challenge (64% vaccine efficacy, $p = 0.01$). The vaccination afforded complete protection against severe disease; no vaccinee had diarrhoea exceeding 1 litre, while 4 of 7 controls had diarrhoeal stools of 2 litres or more (Table 4). The vaccine had no effect on the rate of excretion of V. cholerae organisms, though the quantity of excreted organisms was slightly less in the vaccinated group (Table 5).

Seventeen of 19 vaccine recipients manifested significant rises in serum vibriocidal antibody (measured against Inaba serotype). There was no relationship between serum vibriocidal responses and protection against diarrhoea among the 11 vaccinees who participated in the challenge study. Three of 4 vaccinees who developed illness and all 7 protected vaccinees had significant rises in vibriocidal antibody during and after vaccination.

All 19 vaccinees exhibited significant serum antitoxin responses following vaccination and 13 vaccinees (68%) had significant rises in intestinal SIgA antitoxin measured after only two doses of vaccine. There was no correlation between serum or intestinal antitoxin responses and protection.

Measurements of intestinal antibody to Inaba LPS and outer membrane proteins are in progress. Preliminary work has revealed significant rises in SIgA anti-LPS in intestinal fluids from about half of the vaccinees.

Possible field trial: On the basis of these results consideration can now be given to a field trial of the B-subunit/whole-cell vaccine. The main purpose of such a trial should be to determine the protective eifficacy of the vaccine against cholera and the duration of protection. However, given the particular nature of the vaccine, consideration should also be given to detecting any possible cross protection afforded against Escherichia coli LT disease by the B-subunit component of the vaccine. The opportunity should also be taken to study the protection of infants via "immune milk" from vaccinated mothers, as well as prevention of the carrier state (asymptomatic infection).

3. FIELD TEST FACILITIES AT ICDDR,B

The ICDDR,B has the capabilities for field testing a cholera vaccine. The part of the field area in Matlab Thana that is fully covered by the health services has a population of 80 000 and is immediately available for such a field trial. Additional field areas may be made available, if needed, provided sufficient resources are forthcoming. It was felt that in designing such a trial, it would be important to ensure that each cell is large enough to provide definitive information about the efficacy of the vaccine tested.

4. FUTURE STUDIES WITH B-SUBUNIT/WHOLE-CELL VACCINE - CONCLUSIONS AND RECOMMENDATIONS

The following are the conclusions and recommendations of the Group regarding future studies to be undertaken with the B-subunit/whole-cell vaccine, based on its review of the above information:

(1) The Group considers that the B-subunit/whole-cell vaccine offers a novel approach to protection against cholera. It notes that the vaccine has been shown to be unquestionably safe and immunogenic (i.e., has the capability to evoke antibodies) in volunteers in Bangladesh, Sweden and the USA. The Group also recognizes that it is difficult to predict the efficacy of the vaccine in a population in which cholera is endemic based on the efficacy observed in American adult volunteers (64%); in naturally primed individuals protection is in fact likely to be greater than in unprimed volunteers.

(2) The Group recommends that the highest priority be given to the following three studies:

(a) the whole-cell vaccine (with three strains) that has been tested in combination with the B-subunit in American volunteers should be tested alone for its immunogenicity and efficacy in these volunteers. If its efficacy is found to be poor, consideration should be given to excluding a test of the whole-cell vaccine component given alone in any subsequent field trial.

(b) the acceptability of a tablet formulation for administration of the B-subunit/whole-cell vaccine should be evaluated in a sample of infants and young children in Bangladesh and Sweden.

(c) if a tablet formulation is found to be acceptable, liquid and tablet formulations of the combined vaccine should be compared for immunogenicity in children and adults in Bangladesh and Sweden. In this study the vaccine should be administered using the same schedule and dosage as would be followed in any subsequent field trial.

(3) The Group agrees that there is sound justification for the undertaking of a field trial with the B subunit/whole-cell vaccine at ICDDR,B following completion of the above studies, and recommends that a proposal to this effect be submitted for consideration by the appropriate authorities at ICDDR,B, the Bangladesh Medical Research Council, and the Government of Bangladesh. Such a trial should include at least two groups -- one receiving B-subunit/whole-cell vaccine and the other a beneficial placebo (to be determined). A third group receiving

the whole-cell vaccine should also be included unless the study in American volunteers (2a above) shows it to be ineffective. All the vaccines in the trial should be given in a total of 3 doses administered at appropriate intervals (to be decided) in the formulation (tablet or liquid) determined by acceptability and immunogenicity studies (see 2b and 2c above). The whole-cell vaccine should contain a formalin-killed classical Ogawa V. cholerae strain recently isolated in Bangladesh in place of the formalin-killed V. cholerae classical Ogawa strain (Cairo 50) (see Table 1). Each dose of vaccine should be the same in all age-groups and should contain 1 mg of B-subunit and/or 1×10^{11} killed V. cholerae organisms. The trial lots of vaccine should meet all WHO and national (French, Swedish, and Bangladeshi) requirements and should be tested for safety and immunogenicity in a sample of the field trial population before undertaking the trial.

(4) The Group recommends that, at the earliest possible date, studies be undertaken in animals to ensure that the immunogenicity of the individual whole-cell and B-subunit vaccine components remains stable when the two components are mixed and stored for a 3-month period. A range of temperatures (4-28°C) should be used in these studies.

(5) The Group considers that studies should, if possible, be undertaken in American volunteers to assess the immunogenicity (and efficacy) of the tablet formulation and dosage used (if it is used) in the field trial.

(6) The Group hopes that with this schedule a vaccine field trial can be undertaken by ICDDR,B by the autumn of 1984. It emphasizes that the mode of preparation and dosage of the vaccine component(s) recommended for this trial have been selected to maximize the likelihood of protection. If the vaccine tested should prove to be sufficiently protective, subsequent trials will be necessary to define the optimal formulation and dosage schedule at the lowest cost for its use as a public health tool.

TABLE 1

PROPOSED B-SUBUNIT/WHOLE-CELL VACCINE

B-subunit component:

Oral cholera toxin B-subunit
1 mg/ml in 1 ml of PBS buffer
Institut Mérieux, Lyon, France

Whole-cell vaccine component:

Oral inactivated whole-cell cholera vaccine
1×10^{11} cells in 8 ml of PBS buffer
The National Bacteriological Laboratory, Sweden

Contents

- heat-killed *V. cholerae* classical Inaba (strain Cairo 48)	2.5×10^{10} organisms
- heat-killed *V. cholerae* classical Ogawa (strain Cairo 50)	2.5×10^{10} organisms
- formalin-killed *V. cholerae* El Tor Inaba (strain Phil 6973)	2.5×10^{10} organisms
- formalin-killed *V. cholerae* classical Ogawa (Cairo 50)	2.5×10^{10} organisms
- PBS	8 ml
- Merthiolate^{R}	0.1 mg

TABLE 2

IMMUNIZATION PROTOCOL FOR COMBINED CHOLERA B-SUBUNIT/WHOLE-CELL VACCINE

Immunization group	Immunization: Initial Route	Initial B-sub (mg)	Initial WCV (vibrios)	Second Route	Second B-sub (mg)	Second WCV (vibrios)
Cholera Convalescents	Clinical cholera			PO	0.5	5×10^{10}
I	PO	2.5	5×10^{10}	PO	0.5	5×10^{10}
II	PO	0.5	5×10^{10}	PO	0.5	5×10^{10}
III	IM	0.15	6×10^{9}	IM	0.15	6×10^{9}

TABLE 3

FREQUENCY OF ANTITOXIN (ANTI-CT) AND ANTIBACTERIAL (ANTI-LPS) RESPONSES IN INTESTINAL IgA AMONG CHOLERA CONVALESCENT AND VACCINE RECIPIENTS

Immunization group		Responders to: Initial immunization anti-CT	anti-LPS	Second immunization anti-CT	anti-LPS
Cholera Convalescents		8/9*	8/9	7/9	8/9
I	PO + PO	7/8	4/6	8/8	5/6
II	PO + PO	3/8	4/8	5/7	7/7
III	IM + IM	6/9	4/8	4/7	4/6

*Number of volunteers with a >2-fold rise in ELISA IgA antibody/total IgA in intestinal lavage in relation to day 0.

TABLE 4

CLINICAL RESPONSE OF VACCINEES (THREE ORAL DOSES OF B-SUBUNIT/WHOLE-CELL VIBRIO CHOLERAE VACCINE) AND CONTROLS TO CHALLENGE WITH 2×10^6 EL TOR INABA V. CHOLERAE

	N	No. with Diarrhoea	Mean Incubation Period (h)	Diarrhoeal Stool Volume (l)			Mean number of Diarrhoeal stools
				Mean	Median	Range	
Controls	7	7*	28.8	3.5**	2.9+	0.3-7.7	13.8++
Vaccinees	11	4*	48.6	0.7**	0.7+	0.4-1.0	6.8++

* $p = 0.01$ (Fisher's Exact Test), 64% vaccine efficacy

** $p < 0.05$ (t test)

+ NS (Mann Whitney U test)

++ $p < 0.05$ (t test)

TABLE 5

BACTERIOLOGIC RESPONSE OF VACCINEES (THREE ORAL DOSES OF B-SUBUNIT/KILLED WHOLE-CELL *VIBRIO CHOLERAE* VACCINE) AND CONTROLS TO CHALLENGE WITH 10^6 EL TOR INABA *V. CHOLERAE*

	N	No. with *V. cholerae* in stool	No. with Positive Direct Cultures	No. of Volunteers Positive within 24 hours after challenge	Geometric mean* No. of *V. cholerae* per gram of stool
Controls	7	7	7	6	1.0×10^8
Vaccinees	11	10	9	6	3.2×10^6

*Mean of highest number of *V. cholerae* detected in stools of 7 vaccinees and 9 controls

FIGURE 1

IgA ANTITOXIN ANTIBODY RESPONSES IN INTESTINAL LAVAGE AFTER IMMUNIZATION WITH COMBINED B-SUBUNIT/WCV AFTER CLINICAL CHOLERA

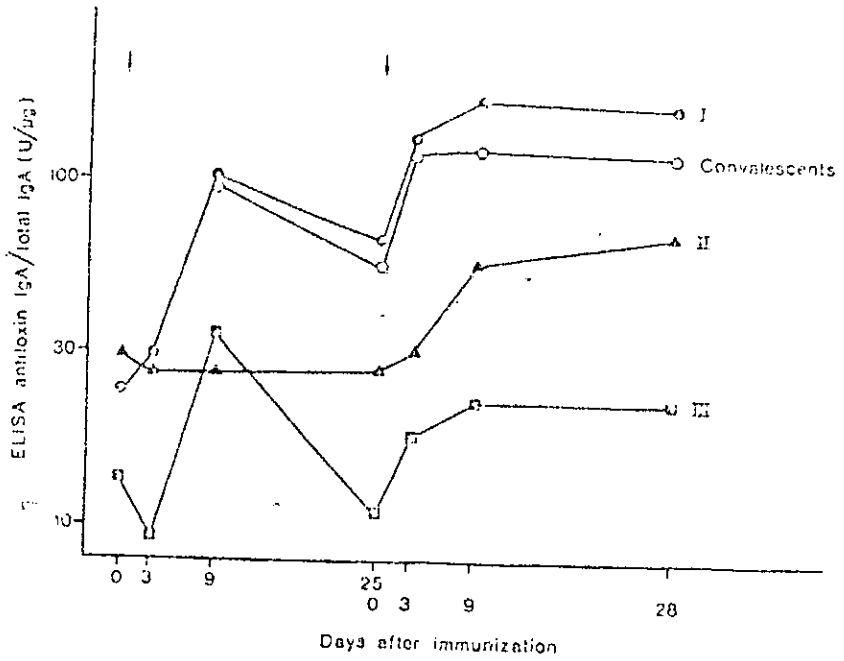
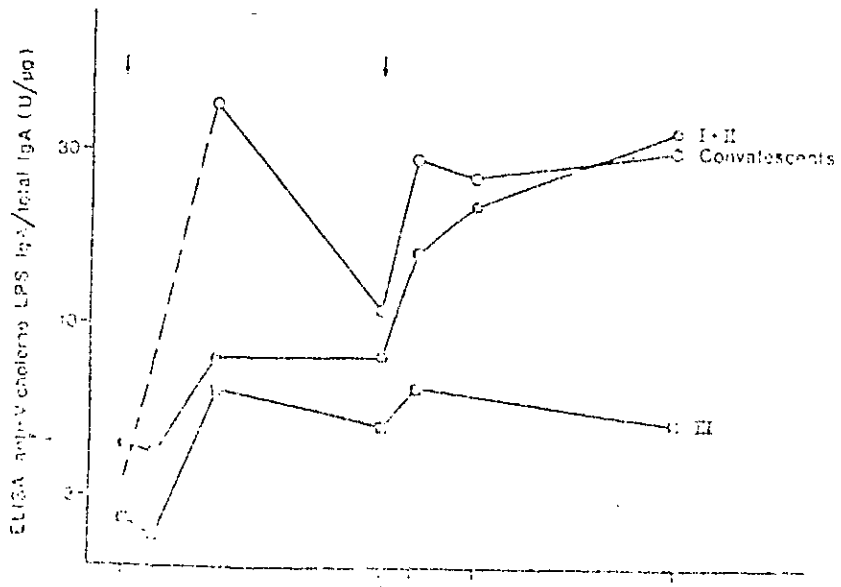


FIGURE 2

IgA ANTIBODY RESPONSE TO V. CHOLERAE LPS IN INTESTINAL LAVAGE AFTER IMMUNIZATION WITH COMBINED B-SUBUNIT/WCV AND AFTER CLINICAL CHOLERA



Dear Bucky:

At its meeting last week our Bacterial Enteric Infections Steering Committee formally approved our collaboration in a field trial of the B-subunit/Whole cell cholera vaccine at ICDDR,B.

In making this decision the Committee requested that I bring the following points to your attention:

1. The Committee had considerable concern about the use of a tablet to administer the vaccine to choldren below 5 years of age. It was aware that paediatric societies in the United States and Sweden have expressed concern about the safety (not efficacy) of biologicals given in the form of tablets of any size to young children and requested us to obtain more precise information in this regard. In addition, the Committee thought you might wish to obtain the opinion of the Bangladesh Paediatric Society (or of leading paediatricians in the country) on this matter as soon as possible. Unless we are assured that there is no safety issue involved in the administration of tablets, the Committee would prefer that their use not be considered in the trial or any further evaluation of the vaccine in Bangladesh.

2. As you and I had discussed earlier, the Committee felt that an attempt should be made as early as possible to organize a meeting between representatives from ICDDR,B, the Government of Bangladesh, BMRC, WHO, and perhaps also the vaccine manufacturers to discuss the trial. In preparation for such a meeting it would be best to have a draft protocol for both the "pre-test" and the final trial. In this regard, we would appreciate knowing whom you have designated as the Principal Investigator for the trial and when you feel such a draft protocol could be available. Such a meeting could be held here or in Bangladesh, but

as it would take a minimum of 4 to 6 weeks to organize, we would like to know as soon as possible, what your plans are in this regard.

We are pleased that our Steering Committee has given us the go-ahead to proceed with the trial and look forward to your early reply.

Best wishes.

Yours sincerely,

Dr M.H. Merson
Programme Manager
Diarrhoeal Diseases Control Programme

B. SPECIFIC AIM

The specific aim of the proposed study is to compare the immunogenicity of several different formulations or administrations of the oral B-subunit/whole cell cholera vaccine by measuring the antitoxin and vibriocidal antibody responses in serum and in breast milk after a first and a second oral immunization with the different vaccine formulations.

C. METHODS OF PROCEDURE

1. Subjects:

Approximately 180 subjects will be recruited to participate in the study. These subjects will be selected from the two major targets of a cholera vaccine program: young children and young women. Ninety of the subjects will be children aged 2-8 years and 90 will be lactating women. These persons will be recruited in the Matlab intense surveillance area and be healthy persons without any known recent history of cholera or other severe watery diarrhoea.

The subjects, or in the case of children their parents or other legal guardian, will be fully informed about the purpose of the study, the procedures involved and possible side-effects in relation to the immunization and sampling of specimens. Informed consent will be required from everyone participating in the study - a copy of the information sheet is enclosed. Before entering the study the subjects will be interviewed as to their present health status and history of diarrhoeal disease, and they will also be examined by a physician.

2. Immunizations:

The subjects will be divided into 6 equally-sized, age-matched study groups and immunized with the different formulations of B-subunit/whole cell cholera vaccine as shown in Table 1:

Because tablet formulations of the vaccine may not be acceptable for young children, the present study addresses a major logistic issue relevant to the administration of the liquid preparation: Is it necessary to administer a "priming" dose of sodium bicarbonate several minutes before ingestion of the vaccine, or is it sufficient to administer sodium bicarbonate concurrently as a vehicle for ingesting the vaccine? A related question concerns the dosage of the vaccine preparation, since the dosage of the vaccine suggested by the WHO Consultation is lower than the dosage used to determine whether the vaccine prevents clinical illness in volunteer studies by Dr. Myron Levine. We therefore will compare the regimen suggested by the WHO Consultation with the higher dose vaccine used in volunteer studies. Thus, the study will simultaneously evaluate whether a simpler regimen of sodium bicarbonate, which may have greater applicability for large-scale public health measures, is effective, and whether a higher dose vaccine preparation can substantially augment immunologic responses to vaccination.

Although the main effect of the orally administered B-subunit/whole cell vaccine would be to stimulate gut mucosal antibodies, the vaccine has also been found to result in antibody titer rises in serum. Studies in volunteers in Bangladesh, Sweden and USA have shown significant titer rises in serum of cholera antitoxin as well as vibriocidal antibodies after oral immunization with B-subunit/whole cell vaccine. Furthermore, in a large family study in Matlab R. Glass and coworkers demonstrated that the serum antitoxin response following oral immunization with B-subunit related both to the dose of B-subunit used and the age of the vaccine recipients. These data suggest that measurements of serum, antitoxin, and vibriocidal antibodies in adequately sized groups of children and adults immunized with the different formulations of cholera B-subunit/whole cell vaccine should be useful to identify differences in immunogenicity between the different formulations of vaccine, if present. As a supplement to these serological measurements, breast milk antitoxic and vibriocidal antibodies, which have been found to parallel gut mucosal antibodies, will be evaluated in lactating women.

Table 1

Group	Treatment	Route	Number of immunizations
I	Moderate dose vaccine* in 100-150 ml of .75% sodium bicarbonate solution, preceded 3-5 minutes earlier by 100-150 ml of .75% sodium bicarbonate without vaccine.	P O	2 (21 days apart)
II	Placebo (100,000 international units of Vitamin A), given with .75% sodium bicarbonate as in I.	P O	2 (21 days apart)
III	High dose vaccine+, given with .75% sodium bicarbonate as in I and II.	P O	2 (21 days apart)
IV	Moderate dose vaccine in 100-150 ml of water together with 1g tablet of sodium bicarbonate and without preceding sodium bicarbonate before vaccine ingestion.	P O	2 (21 days apart)
V	Placebo (100,000 international units of Vitamin A) given with sodium bicarbonate as in IV.	P O	2 (21 days apart)
VI	High dose vaccine, given with sodium bicarbonate as in IV and V.	P O	2 (21 days apart)

* Moderate dose vaccine = 1 mg cholera B-subunit and 1x10" heat - or formalin-killed V. cholerae.

+ High dose vaccine = 5 mg cholera B-subunit and 3x10" heat - or formalin-killed V. cholerae.

Immunizations will be supervised by a physician.

3. Vaccines:

The B-subunit/whole cell cholera vaccine that will be used for these studies has been prepared jointly by Institut Merieux, France and the Swedish National Bacteriological Laboratory. In its liquid form, given together with bicarbonate according to the regimen to be used for group I, the vaccine has been extensively tested in Bangladeshi, Swedish and North American volunteers and found to be entirely safe and immunogenic. Group IV will receive the same formulation of vaccine, the only difference being that a commercially available sodium

bicarbonate tablet will be dissolved in water together with the vaccine; this modification could not conceivably be associated with any increased risk of side-effects other than the possibility of reduced immunogenicity because of less effective neutralization of gastric acidity. Experience with the high dose vaccine similarly has given evidence of no side effects. Finally, the dosage of vitamin A to be used as a placebo is that recommended by the World Health Organization for children and breast-feeding postpartum mothers in the prevention of vitamin A deficiency in areas where the deficiency occurs.

4. Clinical specimens:

Serum samples will be prepared from 100 µl blood obtained by a finger stick on the day for the first immunization, and on day 21 and 42 thereafter. Following initial separation of the serum, it will be diluted 1:10 with physiological saline and 3 aliquots will be made and frozen. The milk will be collected from lactating mothers by manual expression into a fecal cup according to the same schedule as the serum collections. The milk will then be centrifuged at 10,000xg for 10 minutes and the middle layer will be aspirated, aliquotted into 3 aliquots, and frozen.

5. Laboratory assays:

The serum specimens collected will be tested for antitoxin and antibacterial antibodies by the following methods:

a. Antitoxin antibodies of the IgG immunoglobulin class will be determined with the ELISA IgG anti-cholera toxin assay;

b. Antibacterial antibodies will be determined by a micro-titer vibriocidal assay. Both the serum sampling procedures and the immunological methods are well established and more or less routinely practised at ICDDR,B.

Antitoxic and antibacterial IgA antibodies in breast milk will be assayed with an ELISA assay, measuring antibodies against cholera-toxin and purified V. cholerae lipopolysaccharide, respectively. Total IgA in breast milk

will be measured with the immunobead ELISA method. The relative proportion of secretory IgA in relation to total IgA will be measured in all milk specimens. This will be done by testing the samples for specific IgA - and SC - containing antibodies before and after passage through an affinity column with anti-SC antibodies covalently coupled to Sepharose. The column will remove the secretory IgA antibodies; while non-secretory IgA passes unbound through the column.

6. Data analyses:

Multivariate models will be used to evaluate the separate effects of sodium bicarbonate dosage and vaccine dosage upon immunological response. This will be accomplished in two ways. First, we will express vaccine responses dimensionally as absolute geometric mean titers (or percent titers for breast milk), and we will use two-way analysis of variance to assess the independent contributions of bicarbonate and vaccine dosage. Second, we will express vaccine responses categorically as the presence or absence of a significant (> 2 fold increase in titer) change between pre- and post-immunization measurements. We will then employ logistic regression to assess the independent effects of bicarbonate and vaccine dosage. Both of these analyses will be performed on data from the entire pool of all study subjects, and on children and adults separately to discern any independent effect of age on immunologic response. The analyses will also be done separately as serclogical responses after the first immunization, since this might be a more sensitive test of immunogenicity of the different formulations due to the absence of immunologic memory from an earlier immunization which might hide differences when immunization is repeated. Nonetheless it might be very useful to measure antibody responses also to a second immunization: for instance, the group of children might respond poorly in serum to a first immunization with each vaccine formulation so that any differences in immunogenicity between the formulations could only be determined after the second immunization in this age group. Based upon our previous results this situation is unlikely to happen with regard to antitoxin antibodies, but is difficult

to predict with regard to vibriocidal antibodies which to date have only been studied in adult volunteers after immunization with the B-subunit/whole cell-cholera vaccine.

D. SIGNIFICANCE

These studies will be critical with regard to the formulation of the new oral cholera vaccine consisting of purified B-subunit and killed cholera vibrios. If the results support the view that a liquid form of vaccine with a simpler administration than that previously used works, that is to mix the vaccine together with a dissolvable bicarbonate tablet in water and giving this mixture without any prior intake of bicarbonate to neutralize gastric acidity, the vaccine studied in the field trial will offer substantial practical advantages over the B-subunit/whole cell vaccines used in earlier studies. Moreover, if substantially greater responses are observed with the high-dose vaccine -- or if the easier bicarbonate regimen evokes satisfactory responses only with the high dose regimen -- the choice of a higher dose than suggested by the WHO Consultation may be desirable. These data thus will be critical for the ultimate design of a field trial with the new vaccine and also have implications for the development of other enteric vaccines.

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SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

Name	Position	% of effort	Annual salary	Project requirements Taka	Dollars
Dr. John Clemens	Investigator				
Dr. M.U. Khan	Co-Investigator				
Dr. Marianne Jertborn	Co-Investigator				
Field Physician Matlab		3 months 50%			
Field Supervisor (M.R. Khan)		3 months 50%			
2 fiels workers (Health asstt.)		3 months 100%			
Lab. Asstt in Matlab		2 pers.months			
Lab. staff, Immunology		2 pers.months			

2. SUPPLIES AND MATERIALS

Reagents(antisera conjugates, enzyme etc.)	Tk. 12,000/-
Miscellaneous supplies Matlab/Dhaka and xerox facilities	Tk. 7,500/-

3. EQUIPMENT

None

4. PATIENT HOSPITALIZATION

None

5. OUTPATIENT CARE

None

6. ICDDR,B TRANSPORT

Speed boat costs 6 weeks, 4 h/day x 400 Taka/h	Tk. 36,000/-
Transport Dhaka - Matlab 6 trips x 1000 Taka/h	Tk. 6,000/-

	Taka	Dollar

7. TRAVEL AND TRANSPORTATION OF PERSONS

None

8. TRANSPORTATION OF THINGS

$ 200/-

9. RENT, COMMUNICATION AND UTILITIES

None

10. PRINTING AND REPRODUCTION

Forms, Stencils, xerox — Tk. 1500/-

Publication costs — $ 300/-

11. OTHER CONTRACTUAL SERVICES

None

12. CONSTRUCTION, RENOVATION AND ALTERATION

None

Approximate total $ 3,000.00

B. BUDGET SUMMARY

Category	Taka	Dollar
1. Personnel services		
2. Supplies	19,500	
3-5. Nil items	-	-
6. ICDDR,B Transport	42,000	-
7. Travel and Transportation of persons	-	-
8. Transportation of things		200
9. Rent, Communication and Utilities	-	-
10. Printing and reproduction	1,500	300
11-12. Nil items	-	-
	Tk. 63,000/-	$ 500

Approximate total $ 3,000/-

CONSENT FORM

The International Centre for Diarrhoeal Disease Research, Bangladesh is carrying out research to determine the immune response to a promising candidate oral cholera vaccine. This oral vaccine, which is composed of a non-toxic part of the cholera toxin molecule called the B-subunit and of slightly modified common whole cell vaccine has no harmful activity. We would like you and/or your child to participate in this study, the purpose of which is to determine which of several different formulations of this vaccine is easier to administer and gives the best immune response. If you do decide to participate you can expect the following :

1. We will give you 2 doses of liquid to drink. The liquid will either be the vaccine or a compared agent, which will be Vitamin A, a substance known to benefit children directly and through breast feeding.

2. We will collect samples of blood from a finger prick at 3 times during the study, the first 2 samples being taken on the day for the first and second immunization which are given with an interval of 3 weeks and the third sample 2 weeks after the second immunization.

3. If you are currently breast feeding, we will also ask you to express a small cupful of breast milk for study at these times.

4. You do not have to join the study. If you decide not to join, you will still be eligible for care at ICDDR,B. You may also decide to withdraw after entering the study and this will not affect any medical care you might require now or later on.

5. Any member of your family developing diarrhoea during the study will be treated with oral rehydration saline.

6. Your medical records will be kept confidential.

7. We will take care of any complications arising from vaccination

I agree to cooperate with the study on my own/my child's behalf

Signature _____

Signature of staff :

LTI _____

Date _____

সম্মতি পত্র

একটি নতুন ওরাল কলেরা ভেক্সিনের রোগ-প্রতিরোধী ক্ষমতা আছে কি না তা নির্ধারণের জন্যে আন্তর্জাতিক উদরাময় ব্যাধি গবেষণা কেন্দ্র, বাংলাদেশ (আই সি ডি ডি আর,বি) গবেষণা চালাচ্ছে। এই ওরাল ভেক্সিন কলেরা টক্সিন অণুর বিষক্রিয়া মুক্ত অংশ (যাকে বলা হয় বি-সাবইউনিট) এবং সামান্য পরিবর্তিত সাধারণ সমগ্র কোষ ভেক্সিনের সমন্বয়ে গঠিত - যার কোন ক্ষতিকর ক্রিয়া নেই। ভেক্সিনের কয়েকটি ভিন্ন ফর্মুলেশনের কোনটি সহজে প্রয়োগ করা যায় এবং সর্বশ্রেষ্ঠ রোগ-প্রতিরোধী ক্ষমতা প্রদর্শন করে তা নির্ণয়ের উদ্দেশ্যে আমরা এই নিরীক্ষায় আপনার এবং/অথবা আপনার শিশুর অংশগ্রহণ কামনা করি। যদি আপনি অংশগ্রহনের সিদ্ধান্ত নেন তাহলে নিম্নলিখিত পদক্ষেপগুলো আশা করতে পারেন ঃ

১ আমরা আপনাকে দুই ডোজ তরল পদার্থ পান করতে দেবো। এই তরল পদার্থ হবে হয়তো ভেক্সিন নতুবা একটি তুলনীয় বস্তু যাতে থাকবে ভিটামিন এ, যা সরাসরি অথবা সুন্যপানের মাধ্যমে শিশুকে সুফল দেবে।

২ নিরীক্ষাকালে আংগুলে সুঁই ফুটিয়ে আমরা তিনবার রক্তের নমুনা সংগ্রহ করবো, যার প্রথম দু'টি নমুনা নেয়া হবে প্রথম ও দ্বিতীয় প্রতিষেধক প্রয়োগের দিনে (যার মধ্যে ৩ সপ্তাহের ব্যবধান থাকবে), এবং তৃতীয় নমুনাটি নেয়া হবে দ্বিতীয় প্রতিষেধক প্রয়োগের ২ সপ্তাহ পরে।

৩ যদি সাম্প্রতিককালে আপনি শিশুকে সুন্যদানরত থাকেন তাহলে উল্লিখিত সময়গুলোতে আপনাকে আমরা অনুরোধ করবো পরীক্ষার জন্যে ছোট এক কাপ ভর্তি বুকের দুধ বের করে দিতে।

৪ আপনাকে নিরীক্ষায় অংশ নিতে হবেই এমন কোন কথা নেই। যদি আপনি অংশগ্রহন না করার সিদ্ধান্ত নেন তবুও আপনি আই সি ডি ডি আর, বি-তে চিকিৎসা ও সেবা পাবেন। আপনি নিরীক্ষায় অংশগ্রহন করার পরও তা প্রত্যাহার করে নিতে পারেন এবং তাতে বর্তমানে বা ভবিষ্যতে আপনার প্রয়োজনীয় চিকিৎসা ও সেবায় কোন ত্রুটি হবে না।

৫ নিরীক্ষাকালে আপনার পরিবারের কোন সদস্য যদি উদরাময় ব্যাধিতে আক্রান্ত হন তাহলে খাওয়ার স্যালাইন দিয়ে তার চিকিৎসা করা হবে।

৬ আপনার চিকিৎসার রেকর্ড গোপন রাখা হবে।

৭ প্রতিষেধক প্রয়োগের ফলে যে কোন জটিলতা দেখা দিলে আমরা তার যত্ন নেবো।

আমি নিজে/আমার সন্তানের পক্ষে এই নিরীক্ষায় সহযোগিতা করতে সম্মত।

স্বাক্ষর----------

বাম বৃদ্ধাংগুলির ছাপ----------

তারিখ----------

কর্মচারীর স্বাক্ষরঃ
