INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
Mail : ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh
Phone : 871751-60, Telex : 675612 ICDD BJ
Fax : 880-2-883116, 886050, 871568, 871689, Cable : Cholera Dhaka

CENTRE
FOR HEALTH AND
POPULATION RESEARCH

Memorandum

19 October 1999

To : Dr. Firdausi Qadri
Laboratory Sciences Division

From : Professor Mahmudur Rahman
Chairman, Ethical Review Committee

Sub : Protocol # 99-033

This has reference to your memo of 18th October 1999 attaching a modified copy protocol # 99-033 entitled "Phase II and immunogenicity studies of the enterotoxigenic *Escherichia coli (ETEC)* vaccine in Bangladeshi children". I am pleased to inform you that the protocol is hereby approved upon your appropriate addressing of the issues raised by the Committee in its meeting held on 6th October 1999.

Thanking you and wishing you success in running the said study.

copy:- Division Director
Laboratory Sciences Division

INTERNATIONAL CENTRE FOR
DIARRHOEAL DISEASE
RESEARCH, BANGLADESH

Memorandum

Date : 18/10/99

To : Professor Mahmudur Rahman
Chairperson, ERC

From : Firdausi Qadri F. Qadri
LSD

Subject: Resubmission of Protocol # 99-033 entitled " Phase II safety and immunogenicity studies of the enterotoxigenic Escherichia coli (ETEC) vaccine in Bangladeshi children"

Changes have been made in the protocol as was suggested as follows:

1) Information regarding adverse effects has been cited in the section on background information (page 7)

2) On the face sheet, minor persons under guardianship has been circled as "yes"

3. Dr. M. A. Salam is no longer a co-investigator but is in charge of the safety monitoring of the vaccine (page 11).

We hope we have been able to address the comments suitably.

A copy of the protocol is being resubmitted for further consideration.

Thank you.

Copy:- Division Director
Laboratory Sciences Division

Date : 18\10 99

To : Professor Mahmudul Rahman
Chairperson, ERC

From : Tridasai Ghosh
LSD

Subject: Resubmission of Protocol # 99-033 entitled " Phase II safety and immunogenicity studies of the enterotoxigenic Escherichia coli (ETEC) vaccine in Bangladeshi children"

Changes have been made in the protocol as was suggested as follows:

1) Information regarding adverse effects has been cited in the section on background information (page 7)

2) On the face sheet, minor persons under guardianship has been circled as "yes"

3. Dr. M. A. Salam is no longer a co-investigator but is in charge of the safety monitoring of the vaccine (page 11).

We hope we have been able to address the comments suitably.

A copy of the protocol is being resubmitted for further consideration.

Thank you.

Copy:- Division Director
Laboratory Sciences Division

Attachment 1 Date:

APPROVED COPY

(FACE SHEET) **ETHICAL REVIEW COMMITTEE, ICDDR,B.**

Principal Investigator: FIRDAUSI QADRI

Application No. 99033

Title of Study: Phase II Safety and Immunogenicity studies of the enterotoxigenic Escherichia coli (ETEC) vaccine in Bangladeshi children

Trainee Investigator (if any):

Supporting Agency (if Non-ICDDR,B)

Project Status: RRC approved

[ ] New Study

[ ] Continuation with change

[ ] No change (do not fill out rest of the f

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

1. Source of Population:
   - (a) Ill subjects — Yes No
   - (b) Non-ill subjects — ~~Yes~~ No
   - (c) Minor or persons under guardianship — Yes No

2. Does the Study Involve:
   - (a) Physical risk to the subjects — Yes ~~No~~
   - (b) Social risk — 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~~

3. Does the Study Involve:
   - (a) Use of records (hospital, medical, death or other) — Yes ~~No~~
   - (b) Use of fetal tissue or abortus — Yes ~~No~~
   - (c) Use of organs or body fluids — ~~Yes~~ No

4. Are Subjects Clearly Informed About:
   - (a) Nature and purposes of the study — ~~Yes~~ No
   - (b) Procedures to be followed including alternatives used — ~~Yes~~ No
   - (c) Physical risk — ~~Yes~~ No
   - (d) Sensitive questions — Yes No
   - (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 Require
   - (a) From subjects
   - (b) From parents or guardian (if subjects are minor)

6. Will precautions be taken to protect anonymity of subjects

7. Check documents being submitted her Committee:
   - ___ Umbrella proposal - Initially su overview (all other requiremen submitted with individual studi
   - ✓ Protocol (Required)
   - ✓ Abstract Summary (Required)
   - ✓ Statement given or read to subj of study, risks, types of questio and right to refuse to participat (Required
   - ___ ...formed consent form for subj
   - ✓ Informed consent form for pare
   - ___ Procedure for maintaining conf
   - ✓ Questionnaire or interview sch

* If the final instrument is not comp review, the following information included in the abstract summary

1. A description of the areas to be co questionnaire or interview which considered either sensitive or whi constitute an invasion of privacy
2. Example of the type of specific qu asked in the sensitive areas
3. An indication as to when the quest be presented to the Committee for

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

Firdausi Qadri
Principal Investigator

Trainee

Principal Investigator: Last, first, middle _____

International Centre for Diarrhoeal Disease Research, Bangladesh

RESEARCH PROTOCOL

FOR OFFICE USE ONLY
Protocol No: 99033 Date:
RRCApproval: Yes/ No Date:
ERC Approval: Yes/No Date:

1. Title of Project (Do not exceed 60 characters including spaces and punctuations)
Phase II safety and immunogenicity studies of the enterotoxigenic *Escherichia coli* (ETEC) vaccine in Bangla children

2a. Name of the Principal Investigator(s) (Last, Middle, First).	2b. Position / Title	2c. Qualification
Qadri Firdausi	Senior Scientist	PhD

3. Name of the Division/ Branch / Programme of ICDDR,B under which the study will be carried out.
LSD

4. Contact Address of the Principal Investigator 4a. Office Location: Immunology Section	4b. Fax No: 871686 4c. E-mail: fqadri@icddrb.org 4d. Phone / Ext: 871751/2413

5. Use of Human Subjects	5a. Use of Live Animal	5b. If Yes, Specify Animal Species
Yes [X]	Yes [ ]	
No [ ]	No [ ]	

6. Dates of Proposed Period of Support *(Day, Month, Year - DD/MM/YY)*	7. Cost Required for the Budget Period *7a. Ist Year ($): 59,356* *2nd Year ($): 37,591*
ASAP	7b. Direct Cost ($) 96,957 (overhead 24,227). Total Cost ($)

8. Approval of the Project by the Division Director of the Applicant

The above-mentioned project has been discussed and reviewed at the Division level . The protocol has been revise is approved.

V. I. MYRTHAN — Name of the Division Director

Signature

9/9/99 — Date of Approval

9. Certification by the Principal Investigator	10. Signature of PI
I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.	Firdausi Qadri Date: 8/9/99

Principal Investigator: Last, first, middle _____

PROJECT SUMMARY: Describe in concise terms, the hypothesis, objectives, and the relevant background of the project. Describe concisely the experimental design and research methods for achieving the objectives. This description will serve as a succinct and precise and accurate description of the proposed research is required. This summary must be understandable and interpretable when removed from the main application. **(TYPE TEXT WITHIN THE SPACE PROVIDED).**

Principal Investigator
Qadri Firdausi

Project Name Phase II safety and immunogenicity studies of a killed oral enterotoxigenic *Escherichia coli* (ETEC) vaccine

Total Budget $ 96, 957 Beginning Date ASAP Ending Date 2 years from starting date

Acute diarrhoeal diseases are a major health problem in developing countries. Infection with enterotoxigenic *Escherichia coli* (ETEC) is one of the most common causes of diarrhoea among children in developing countries and it also contributes to malnutrition. The prevalence of toxin types and colonization factors (CFs) was recently studied in fresh samples (n=4662) obtained from a 2% routine surveillance of diarrheal stools for two years from September 1996 to August 1998. It was observed that the prevalence of ETEC was 14% with over 70% isolated in children 0-5 years age range, of whom 93% were in the 0-3 years age range. The study showed that the isolation of ETEC increased in the children from as early as 3 months of age. Thus of all incidences more than 65% can be expected to occur in children < 3 years of age.

The infection could by prevented by a safe and immunogenic vaccine that is protective in children. One such vaccine undergoing evaluation is the oral inactivated ETEC vaccine consisting of recombinant cholera toxin B subunit (BS) and a mixture of formalin- inactivated whole cell ETEC bacteria expressing colonization factors (BS-CFA ETEC vaccine). The vaccine has been tested in a number of countries in phase-I safety and immunogenicity trails in adults (Svennerholm et al., 1997). These initial vaccine trials have been extended in Egypt to larger field based phase II and phase III studies. In addition Phase III trials of vaccine efficacy are ongoing which include United States travellers to Latin America (WHO, 1999). The vaccine is being extensively studied in Egypt where it has been tested in adults as well as in children in the 2-12 years old age group (Savarino et al. 1998, 1999) and is now being tested in 6-18 months old children (WHO, 1999). A phase I study carried out in Bangladeshi children in the 3-10 year range has shown that the vaccine is safe and immunogenic (Qadri et al. , manuscript submitted). Thus a phase-II safety and immunogenicity study now needs to be carried out in Bangladesh in children from 6 months to 3 years of age. Based on the results of this study the vaccine can subsequently be extended to smaller children (i.e. from 3 months of age to 3 years) in a larger phase III, protective efficacy trial.

Principal Investigator: Last, first, middle _____

KEY PERSONNEL (List names of all investigators including PI and their respective specialties)

Name	Professional Discipline/ Specialty	Role in the Project
1 Dr. Firdausi Qadri	Immunologist/ Immune response studies in patients and vaccines	PI of study
2 Prof. Ann Mari Svennerholm	Immunologist/ Develpment of vaccine and vaccine related work	Expert adivice on vaccine
3 Prof. V.I.Mathan	Epidemiologist and gastroenterologist	Advice and support
4 Dr. Abdullah-Hel Baqui	Senior Epidemiologist	Design and support in the field based study
5. Dr. Md. Yunus	Epidemiologist	Design and support
6. Prof. R. B. Sack	Microbiologist/Epidemiologist	Advice and support
7. Prof. D.A. Sack	Microbiologist/Epidemiologist	Advice and support

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be tested:

Concisely list in order, in the space provided, the hypothesis to be tested and the Specific Aims of the proposed study. Provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

One of the strategies for the prevention of ETEC diarrhoea is the design of a suitable and protective vaccine. Children who suffer most from the consequences of the repeated episodes of the disease will benefit most from such a vaccine. A candidate vaccine undergoing evaluation is the oral inactivated ETEC vaccine consisting of recombinant cholera toxin B sub-unit (BS) and a mixture of formalin-inactivated whole cell ETEC bacteria expressing colonization factors (the BS-CFA ETEC vaccine). The vaccine has been found to be safe and immunogenic in Phase I studies carried out in Sweden, Egypt and Bangladesh. A phase I, safety and immunogenicity study in children in Bangladesh has shown that the vaccine is safe and gives rise to significant antibody responses. A phase II study in children in Egypt has shown that the vaccine is sufficiently safe and immunogenic in children to justify proceeding to evaluation in infants and to phase III, safety and efficacy studies. We hypothesize that the vaccine should be suitable for children in Bangladesh. In order to test the hypothesis, the vaccine needs to be tested in a larger group of children in the field setting, initially in a phase II trial. This information is needed before testing the vaccine in studies on the protective efficacy of the vaccine in the pediatric age group in the field.

Specific Aims:

Describe the specific aims of the proposed study. State the specific parameters, biological functions/ rates/ processes that will be assessed by specific methods (TYPE WITHIN LIMITS).

A. Determine the safety of the oral BS-CFA ETEC vaccine in healthy Bangladeshi children aged 6 months to 3 years in a randomized double-blind placebo-controlled trial.

- **B.** Determine the immunogenicity of the vaccine in a subset of the children by studying.

1. the antibody secreting cell (ASC) responses to the vaccine components (CFs and BS) prior to vaccination and after each of the two vaccine doses.

2. the antibody response in plasma and faeces to the vaccine components prior to vaccination and after each of the two vaccine doses.

3. the cellular response to the vaccine components by analyzing the cytokine responses in plasma, faeces and stimulated peripheral blood mononuclear cells.

Background of the Project including Preliminary Observations

Describe the relevant background of the proposed study. Discuss the previous related works on the subject by citing specific references. Describe logically how the present hypothesis is supported by the relevant background observations including any preliminary results that may be available. Critically analyze available knowledge in the field of the proposed study and discuss the questions and gaps in the knowledge that need to be fulfilled to achieve the proposed goals. Provide scientific validity of the hypothesis on the basis of background information. If there is no sufficient information on the subject, indicate the need to develop new knowledge. Also include the **significance and rationale** of the proposed work by specifically discussing how these accomplishments will bring benefit to human health in relation to biomedical, social, and environmental perspectives. (DO NOT EXCEED 5 PAGES, USE CONTINUATION SHEETS).

Burden of disease due to ETEC diarrhoea

It has been estimated that ETEC infections alone cause approximately 210 million episodes of diarrhoea and over 380,000 deaths annually in children under 5 years of age (WHO, 1996, 1999). The peak of incidence of ETEC diarrhoea in endemic countries occurs in the first year of life, with a declining incidence with age thereafter. Of the 7-8 episodes of diarrhoea annually in children, in the developing world, at least 2-4 attacks may be due to ETEC (Black, 1993). As a result, ETEC diarrhoea may contribute to growth retardation and death in these children. In addition, it is responsible for over 50% of diarrhoea in travellers to ETEC endemic regions of the world. A high proportion of diarrhoeal illness in military personnel is also caused by ETEC (Wolf *et al.*, 1997). The clinical symptoms of the disease are watery diarrhoea often accompanied by abdominal cramps, malaise and low grade fever. It may last 3-7 days and the disease spectrum can range from mild diarrhoea to dehydrating cholera-like symptoms, which is seen in about 5% of cases (Black, 1986).

The pathogenicity of ETEC is due to their ability to colonize the small intestine and to produce one or both of two types of toxins, the heat stable enterotoxin (ST) (Field *et al.*, 1978) and/or the heat labile enterotoxin (LT)

(Svennerholm and Holmgren, 1978). These bacteria also possess a variety of surface located adhesins that anchor them to the intestinal mucosal receptors (Gaastra and de Graaf, 1982; Evans and Evans, 1998). This anchorage enables the bacteria to resist the flushing action of the intestinal peristalsis and the toxins are delivered close to the enterocytes. The adhesins are termed colonization factors (CFs) and may be fimbrial, fibrillar or non-fimbrial in structure. Some 20 adhesins have been identified on human ETEC strains (Gaastra and Svennerholm, 1996). Of these structures, CFA/I consists of a single subunit, whereas CFA/II may consist of three antigenically separate CS antigens, CS1, CS2 and CS3 (Smyth, 1982). Likewise, it has been found that CFA/IV may consist of CS4, CS5 and CS6 (Thomas *et al.*, 1985).

Epidemiological studies carried out in various geographical areas show a large variation in the prevalence of CFs in ETEC isolated in the different regions (Gaastra and Svennerholm, 1996). However, the 7 best characterized CFs are CFA/I, CS1, CS2, CS3 (CFA/II components), CS4, CS5 and CS6 (CFA/IV components). These have been found in 50-80% of clinical ETEC isolates worldwide (Giron *et al.*, 1995).
In some areas CFA/I and CFA/IV are common (Lopez-Vidal *et al.*, 1990; Gothefors *et al.*, 1994), whereas in others CFA/II may be more prevalent (Wolf *et al.*, 1993; Levine *et al.*, 1993). In a study carried out in Bangladesh from 1980 to 1982, it was observed that CFA/I and CFA/II were commonly present on ETEC (Gothefors *et al.*, 1985). However, in a study, that has been conducted recently in Bangladesh on the 2% surveillance patients with diarrhoea at ICDDR,B where screening for 12 different CFs was carried out, CFA/I, CFA/II and CFA/IV were detected in the highest frequencies in ETEC (Qadri *et al.*, manuscript submitted).

Protection from ETEC disease

For ETEC, which are noninvasive, antibodies that can be induced locally in the gut are believed to be protective (Levine, 1990). Both antibacterial (mainly directed against CFs) and antitoxic antibodies act synergistically to provide protection (Ahren and Svennerholm, 1982). Protective immunity is dependent on the stimulation of the mucosal immune system and generation of secretory IgA antibodies in the gut-associated lymphoid tissue. Studies in animal models and human volunteers suggest that primary ETEC infections can protect against re-infections (Levine *et al.*, 1984; Svennerholm *et al.*, 1990). Natural ETEC infection also appears to be protective. This is evident since the highest incidence of the disease is in the age group of 6-36 months and the rate of illness decreases with age, suggesting the development of protective immunity (Black *et al.*, 1986). In a prospective study recently conducted in ICDDR,B, the highest incidence of ETEC was in children less that 5 years of age. About 74% of ETEC were isolated from diarrhoeal children less than 5 years of age, of whom 93% were up to 3 years of age (Qadri *et al.*, manuscript submitted). It was observed that the incidence of ETEC diarrhoea increased from 3 months of age.

ETEC diarrhoea also gives rise to specific antibody secreting cell (ASC) responses which can be detected in the peripheral blood as well as in the small intestinal biopsies. An increase in CF and toxin-specific ASC responses were seen very early after onset of illness. The peripheral blood B cell responses are believed to be derived from the GALT, which after circulating in the blood, home-back to the mucosa for antibody secretion (Czerkinsky *et al.*, 1987). Therefore, these cells appear to be good markers of the immune response of the GALT to locally presented antigens. Determination of the specific B cell responses in peripheral blood has been found to be a reliable proxy measure of the mucosal immune response in the gut. Antibody production and B cell function are dependent on T cells which control the proliferation and differentiation of B cells and is, therefore, dependent on signaling by cytokines.

T cell responses have been studied to the BS-CFA ETEC vaccine in adults (Wenneras *et al.*, 1994). Immunization of Swedish volunteers resulted in increases of IFN-gamma but not IL-2. Of the studies carried

out on adult vaccinees and patients in endemic regions, preliminary results indicate that a wide array of cytokines may be induced although there seems to be less of IFN-gamma being detected *in vitro* (Qadri *et al.*, manuscript in preparation). However, the response seen in adults is that of a secondary nature and studies have not been carried out in children who have been vaccinated. Such a study needs to be carried out in order to better understand which cytokines are involved in the induction of the antibody responses.

ETEC infection is spread through water or food contaminated by faeces from infected persons. The disease can effectively be prevented if clean water and food and good sanitary conditions are made available in the developing countries. However, since this is not be possible to accomplish in the near future, the other alternative for the prevention of the disease would be the availability of a vaccine that is cheap and effective. The vaccine should be easily administered to infants and children, who are the most vulnerable to infections.

Since ETEC causes considerable morbidity and mortality the development of a safe and effective vaccine has been given a high priority (Svennerholm *et al.*, 1997, WHO, 1999). An ideal ETEC vaccine should be given orally and contain a combination of CFs and the heat-labile toxin antigen derived antigen. Some ETEC vaccine candidates that have been considered include: (a) live bacteria expressing the major CFs but lacking the toxin genes (Levine, 1990); (b) those composed of purified CFs (Evans *et al.*, 1984) ; Levine, 1990); (c) colicin E2-inactivated toxigenic and CFA-carrying ETEC strain (Evans *et al.*, 1988), live vaccine containing attenuated *Salmonella* strain in which genes of common CFs have been introduced (Giron *et al.*, 1995); and (e) the killed ETEC vaccine containing recombinant cholera toxin B subunit (BS) and a mixture of formalin-killed ETEC strains expressing CFs (BS-CFA ETEC vaccine). The killed vaccine is the only vaccine that is undergoing extensive studies in adults in both developed and developing countries and that has reached the stage of phase-II clinical trials. The vaccine has been found to be safe and immunogenic in adult Swedish volunteers (Wenneras *et al.*,1992; Ahren *et al.*, 1993). Phase III trials of protective efficacy are ongoing in travellers from the USA going to Latin America and those from Europe going to Kenya (WHO, 1999). It has been tested in about 40 adult Bangladeshi volunteers and found to be safe and immunogenic, giving rise to ASCs in the circulation and in the gut as well as systemic and local antibodies in the gut (Qadri *et al.*, submitted; Wenneras *et al.*, submitted). The vaccine has also been found to be safe and immunogenic in children 3-10 years of age (Qadri *et al.*, Manuscript submitted). The vaccine has also undergone testing in other countries, including Egypt (Savarino *et al.*, 1998) and a phase-II study has been completed in children, 2-12 years of age (Savarino *et al.*,1999) . Studies carried out in Sweden and Egypt have found the vaccine to be safe. In these studies, adverse events were noted in about 2-6% of recipients which included nausea, mild abdominal cramps, 1-2 loose stools per day during the 3 day observation period. These effects were seen in the group given vaccine and placebo. Based on the safety and immunogenicity studies in Egypt, recently, testing has been initiated in children 6-18 months of age (Savarino *et al.*, personal communication, WHO, 1999).

Research Design and Methods

Describe in detail the methods and procedures that will be used to accomplish the objectives and specific aims of the project. Discuss the alternative methods that are available and justify the use of the method proposed in the study. Justify the scientific validity of the methodological approach (biomedical, social, or environmental) as an investigation tool to achieve the specific aims. Discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Point out safety procedures to be observed for protection of individuals during any situations or materials that may be injurious to human health. The methodology section should be sufficiently descriptive to allow the reviewers to make valid and unambiguous assessment of the project. **(DO NOT EXCEED TEN PAGES, USE CONTINUATION SHEETS).**

d) **Research Plan, Research design and Methods**

Study Design

This vaccine study will be a randomized, double blinded, placebo controlled trial. Children will be stratified by age (in two age bands, 6 months to 18 months, > 18 months to 3 years) before randomization. In each group each subject will be assigned a sequential number at the time of initial dosing, corresponding to sequentially numbered and randomized set of two single-dose vials of study agent. The codes will be broken after all clinical and laboratory evaluation have been completed.

Selection of study subjects

Before entering into the study children in the different age groups will be screened from the slums and history recorded (Appendix III). Although 158 children need to be recruited in the two age groups, more will be screened to obtain study subjects which meet the inclusion criteria. Previous experience in similar studies (Protocol No.98-001) has shown that around 30 to 40% more have to be screened.The children will be carefully examined by a physician. A detailed history of the volunteer of previous immunizations, nutritional status, recent illness (as outlined below) that can compromise the immune system, family background etc. will be taken (Appendix III). Healthy male and female children will be recruited from the urban slums.

Children will be excluded from enrollment if interviewers find: (a) a history of chronic gastrointestinal disorder, (b) diarrhoeal illness in the past 2 weeks (diarrhoea being defined as passing of ≥ 3 loose or liquid stools in 24 hours (c) febrile illness in the preceding week, or (d) other serious chronic illness (e) had antibiotic treatment atleast 7 days prior to the enrollment in the study (f) children suffering from second or third degree of protein energy malnutrition (PEM).

A routine stool microscopic examination and culture for common enteric pathogens including ETEC will be carried out three days prior to vaccination. Children whose stools are postive for ETEC will not be vaccinated since this will interfere in the evaluation of the response to the vaccine When immunization for the second dose of the vaccine will be carried out (14 days after the first dose) children who have fever or diarrhoea will be excluded from the study.

The nutritional level of the children will be monitored using anthropometric measurements (weight-for-age, and weight-for-length/height). Children below > -2SD of the NCHS will not be enrolled. We plan to recruit children in the 6 month -3 year range for immunization.

Sample size calculation

Sample size calculation (N) has been carried out using the formula shown below at a power of 80% and significant difference level at 95%. The number to be recruited for the safety studies on the vaccine has been derived using side-effects data obtained in a similar study (Savarino *et al.*, 1999). Here p_1 = 1% and p_2 =11%, (ETEC vaccinees = p_1,; placebo control = p_2, d= p_1-p_2). Therefore:

$$N = \frac{(Z\alpha/2 + Z\beta)^2 (2\bar{pq})}{d^2}$$

A sample size of 106 is calculated for each of the age groups (6 months to 18 years, >18 month to 3 years) in the study population. Considering a 20% prevalence of ETEC and taking into consideraton a 15% adjustment of dropout, the number is 126. However since the prevalence of diarrhoea in the population in the

children in this age group is about 25% (Baqui et al. 1993), and this is taken into consideration, 158 children will be recruited in each age group. The subset of study subjects to be studied for the immune response studies from the groups above have similarly been obtained by using conservative estimations (data for the response obtained to the CF antigen CS4, to which the frequency of response was the lowest). Here $p_1 = 40\%$ and $p_2 = 11\%$., a sample size of 40 and with adjustment for 15 % dropout, the number to be recruited is 46 in each age group for both the study groups.

Baseline data

Baseline data from volunteers will be collected during the week prior to vaccination by home visits (Appendix III). The laboratory specimens will be collected prior to vaccination on the day of immunization. The laboratory assays will include blood for immunological assays and stool for culture and routine examination for ova and parasites and immunogical assays. These data will serve as baseline values for the study subjects.

Safety evaluation

The primary end point for evaluating safety will be defined as the occurrence of any of the following diarrhoea, vomiting or abdominal cramps of at least moderate grade. Diarrhea will be defined as three or more loose or liquid stools in any 24-h period. A measured oral or rectal temperature above 38°C will be considered a fever.

Vaccine and placebo composition, allocation and administration

The ETEC BSA-CF ETEC vaccine is produced by SBL Vaccin AB (Stockholm, Sweden) One 4 ml dose contains 1.0 mg recombinantly produced CTB plus $2x10^{10}$ formalin inactivated bacteria of each of the following ETEC strains: SBL101 (O78:H12; CFA/I; ST^+); SBL104 (O25:H42; CS4+CS6); SBL 105 (O167:H5; CS5+ CS6; ST^+); SBL106 (06:H16; CS1); and SBL107 (OR:H6;CS2+CS3). SBL101 and SBL105 are washed prior to formulation to remove any heat stable enterotoxin (ST) activity. The placebo dose will consist of $1x10^{11}$ heat-killed *E. coli* K-12 bacteria (SBL vaccin).

Healthy boys and girl will be recruited in the study from the urban suburbs around Dhaka city. Children will be excluded from enrollment if interviewers find a history of chronic gastrointestinal disorder, diarrhoea in the past two weeks,febrile illness in the preceding week or some other serious chronic illness. Subjects will be given two doses of the study agent 2 weeks apart and will not be allowed to eat or drink for about 60 min before and after each dose. They will however be allowed to drink water during this period if necessary. Previous experience with the ETEC vaccine in Egypt has shown that in children 6-18 months of age this was suitable.

Immediately before use individual doses of the vaccine will be mixed with the reconstituted bicarbonate buffer. For children 18 months -3 years of age , the vaccine (4 ml) will be dissolved in 30 ml (total 7 teaspoon) of a raspberry flavored bicarbonate buffer [the buffer is prepared by adding 100 ml water to a sachet of bicarbonate buffer; Recip AB, Stockholm of which 30 ml will be used to prepare the vaccine dose].

For younger children the vaccine will be administered in 15 ml of the buffer (about 4 teaspoon) [prepared by adding 75 ml water to a sachet of the above buffer of which 15 ml will be used] and given to the child. This is the procedure followed for immunization of children in the different age groups in Egypt and has been found suitable since in most cases the total dose of the vaccine was ingested. Informed consent will be taken from the parent/guardian of the children for the safety studies or for the safety and for permission to draw blood (Appendices Ia, Ib, IIa, IIb).

Study subjects taking atleast 90% of the vaccine dose will be kept enrolled in the study but the data will be analyzed separately as well as with the others to see differences in the immune response.

Safety Monitoring of the Vaccine

On each study day (Days 0,7,14 and 21) clinical history and physical examination will be carried out (Appendix IV). The safety and reactogenicity of the vaccine will be monitored in the subjects by observing for 30 minutes after each dose for occurrence of immediate adverse experiences. On 3 consecutive days after each dose parents will be interviewed for 24 hour recall of symptoms by the health workers and the physician by home visits each day. All side-effects will be recorded in reaction surveillance forms for local and systemic reactions (Appendix V). Reported symptoms will be graded as mild, moderate and severe. In case of serious adverse effects the data will be entered in a separate form (Appendix V). The study subject will be hospitalized at the CRSC of ICDDR,B under supervision of the clinician monitoring the safety of the vaccine.

Sample collection

Stool: Stool will be collected prior to vaccination (3 days prior to initial immunization) and subsequently at follow-up visits 7 days after intake of the first as well as 7 days after intake of the second dose of the vaccine. Routine examination of stools will be carried out to detect parasites. Bacteriological examination will be carried out to exclude the presence of enteric pathogens (WHO, 1987). Five lactose-fermenting colonies will be tested for colonization factor expression by dot blot immunoassay techniques and for the enterotoxins LT and ST. Stools will be also be tested prior to intake of the second dose and tested similarly. The stools will also be tested to exclude the presence of ETEC by assaying for LT and ST using the GM1-ELISA technique (Svennerholm and Holmgren, 1978; Svennerholm *et al.*, 1986). Stools will also be collected for preparing faecal extracts for antibody and cytokine assays. Faecal extracts will be prepared (Qadri *et al.*, 1997) and stored at -70°C until tested.

Blood: Venous blood will be collected from the vaccinees prior to immunization and 7 days after intake of each dose of the vaccine. About 3 ml blood will be collected from vaccinees in the age group ranging from 2-3 years of age, in younger children (<18 months) 1.5 ml of blood samples will be collected. For the children in the 6 months to 18 months of age since the volume of blood will be less, these will only be used only for antibody assays. Total and differential counts of blood and hematocrit will be carried out in the clinical laboratory of ICDDR,B. Cytokine responses will be studied in the serum and in the feces. In older children, ASC responses and cytokine assays with stimulation with CF antigens will be carried out.

Mononuclear cells and plasma:

Blood will be centrifuged on Ficoll-paque for separation of plasma and mononuclear cells (MNC) for the ASC assays. Plasma samples will be stored at -70°C for cytokine assays or at -20°C for antibody assay. The MNC will be used for the antibody secreting cell responses.

Detection of antibody secreting cells (ASCs)

The Ficoll-separated MNCs will be assayed for total and ETEC -specific numbers of ASC by the two-colour ELISPOT technique (Czerkinsky *et al.*, 1988; Wenneras *et al.*, 1992). Purified vaccine-specific CF-ASC response of the IgA/IgM isotype (CFA/I and, CS1) (Evans *et al.*, 1979,Klemm *et al.*, 1985) and recombinant CTB-ASC response of the IgA/IgM isotype (Sanchez *et al.*, 1989) will be used. A significant response will be determined if the vaccine specific ASC response as ≥2 fold increase over baseline value of ASC/10^7 MNC in the baseline sample. Only ASC levels > 10 ASCs per 10^7 MNCs in the post immunization specimen will be considered a responder.

CF- and CTB-specific ELISA

Pre- and post-immunization serum or plasma samples from vaccinees will be tested for the presence of IgM and IgA antibodies specific for vaccine-associated CFA/I and IgG and IgA antibodies to CTB will be carried out using purified antigens and methods described earlier (Ahren *et al.*, 1993). A ≥2-fold increase in end-point titer between pre- and post immunization titer specimens will be used to signify seroconversion. Vaccine-specific IgA antibodies will also be determined in faecal extracts. For this purpose the vaccine specific response will be expressed as the number of units per microgram and will be calculated by dividing the titers (in units per ml) by the total IgA concentration (in μg per ml). A twofold or greater increase in the specific titers between the pre-immune and post-immunization stage samples will be considered a significant response.

Stimulation of MNC from peripheral blood and detection of cytokines

Separated MNC ($1x10^6$ MNC) will be stimulated with purified CFA/I (Wenneras *et al.*, 1994) and the culture supernatant tested for cytokine production. The cytokines that will be studied include both Th1 (IFN-gamma), Th2 types (IL-4, IL-5) as well as the proinflammatory type (TNF-α) using ELISA and reverse transcriptase-PCR techniques (James, 1993; Klappworth *et al.*, 1995; Jung *et al.*, 1995). These samples will be frozen at -70°C until testing is carried out.

Safety Monitoring Committee

All subjects participating in the study will be included for post-dosing safety analysis (those that have completed intake of both doses or have only taken one dose). A data safety and monitoring committee will be setup for this project. Dr. M.A. Salam, Clinical Scientist from Clinical Sciences Division, ICDDR,B will be responsible for safety monitoring of the vaccine and in setting up the committee.

Facilities Available

Describe the availability of physical facilities at the place where the study will be carried out. For clinical and laboratory-based studies, indicate the provision of hospital and other types of patient's care facilities and adequate laboratory support. Point out the laboratory facilities and major equipment that will be required for the study. For field studies, describe the field area including its size, population, and means of communications. **(TYPE WITHIN THE PROVIDED SPACE).**

Existing field, hospital, laboratory and office facilities are adequate and are outlined:

Field site for study

The Public Health and Sciences Division of ICDDR,B has the basic information of demography and health indicators of urban slums in Dhaka. This study will be done in a sample of urban slum population which will be found suitable by the epidemiologist in the study.

Clinical facilities

Although this is a field based study, any clinical facility needed in the study will be provided by the clinicians in the Clinical Research and Service Centre of the ICDDR,B hospital. As a benefit all children will be given a month's supply of multivitamin supplement at the end of the study.

Laboratory facilities
Existing laboratory facilities are adequate. An ELISA Reader is needed which has been budgeted for the study. Replacement of a computer is needed in the laboratory which will be used for data input, hook-up to the ELISA Reader as well as for word processing.

Data Analysis

Describe plans for data analysis. Indicate whether data will be analyzed by the investigators themselves or by other professionals. Specify what statistical softwares packages will be used and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to monitor further progress of the study. (TYPE WITHIN THE PROVIDED SPACE).

Methods to ensure data quality:

To ensure the quality of data, the study site will be visited by the investigators for random checks. In addition a 5% sample of study subjects will be re-interviewed within one day to re-assess the original interview.

Data management
To maintain consistency the data forms will be reviewed by the investigators. This will be carried out to ensure the completeness and accuracy of the data. If necessary additional home visits will be made to recheck entries or fill up missing entries. After editing, data will be entered in an appropriate data entry program that will be suitable for the study.

Data analysis

Baseline characteristics of the vaccine and placebo groups will be examined for group comparability. Any baseline difference will be controlled for during data analysis. The data for the two age groups will be handled together and will also be analyzed separately to evaluate the responses.
The frequency distribution will be examined to assess the distribution of data. If the data is not normally distributed, decisions about data transformation and on appropriateness of statistical tests will be made.
The data from the vaccine and placebo group will be compared to compare the safety of the vaccine by assessing the association with adverse events in each group.
The data from the two groups will also be compared for assessing the immunogenicity of the vaccine. This will be done for the ASC, plasma antibody and faecal antibodies using criteria described above for defining a response in the study subjects.
All subjects receiving at least one dose of study agent will be included in the postdosing safety analysis. The profile likelihood method will be used to assess differences in the proportions of primary outcomes in vaccinees versus placebo recipients after each dose (Newcombe, 1998). For individual symptoms, proportions will be compared with Fisher's Exact test. This test will also be used to assess differences in the occurrence of ASC responses and antibody seroconversion between the two groups after each dose. The number of antigen specific ASC after each dose will be compared between treatment groups by the median test. Within the vaccine group, the one sample rank sum Wilcoxon test will be used to assess whether number of ASC and plasma antibody titer fold increases are boosted after each successive dose. All statistical tests will be interpreted in a two-tailed fashion.

Ethical Assurance for Protection of Human Rights

Describe in the space provided the justifications for conducting this research in human subjects. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how subject's rights are protected and if there is any benefit or risk to each subject of the study.

Signed consent will be obtained from parents for participation of the children in the study for vaccination and specimen collection (Appendix I and II). All data related to side-effects of each dose of the vaccine will be examined by a safety monitoring committee. The reaction surveillance will be carried out for 3 days after each dose of the vaccine and recorded systematically (Appendix-IV).

Use of Animals

Describe in the space provided the type and species of animal that will be used in the study. Justify with reasons the use of particular animal species in the experiment and the compliance of the animal ethical guidelines for conducting the proposed procedures.

Not needed for the study.

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however exercise judgment in assessing the "standard" length.

Bibliography

Papers and manuscripts from ongoing studies

Qadri F, Wenneras C, Bardhan PK, Hossain J, Albert MJ, Sack RB, Svennerholm A-M. B cell response to enterotoxigenic *Escherichia coli* (ETEC) in vaccinees and patients after oral immunization and infection (submitted).

Rudin A, Wiklund G, Qadri F, Wenneras C, Svennerholm A-M. Infection with colonization factor antigen I expressing enterotoxigenic *Escherichia coli* may boost immune responses against heterologous CFAs in primed subjects. Epidemiol Infect 119:391-393.

Wenneras C, Qadri F, Bardhan PK, Sack RB, Svennerholm A-M. Intestinal antibody responses to enterotoxigenic *Escherichia coli* in vaccinees and patients after oral immunization and infection (in press, Infection and Immunity).

Qadri F, Hossain J, Begum YA, Jonson G, Azim T, Sack RB, Albert MJ, Svennerholm A-M. Immune response to the mannose sensitive hemagglutinin (MSHA) in patients with cholera caused by *Vibrio cholerae* serogroups O1 and O139. 8th International Congress of Mucosal Immunology. Clin Immunol Immpath 1994; 76:S105, P615.

Qadri F, Hossain J, Chowdhury A, Azim T, Salam MA, Khan AM, Sack RB, Albert MJ. Antigen-specific antibody secreting cells in peripheral blood due to *Vibrio cholerea* O139 (W20/34). 12th European Immunology Meetings, Barcelona, June, 1994.

Qadri F, Wenneras C, Mannoor K, Salam MA, Albert MJ, Svennerholm A-M. Mannose-sensitive haemagglutinin (MSHA)-specific antibody response and cytokine production in peripheral blood and the gut in cholera.9th International Congress of Muccosal Immunology, 27-31 January, 1997., Sydney, Australia. Immunol Cell Biol 1997; 75(Suppl 1):A130.

Wenneras C, Qadri F, Bardhan P, Sack RB, Czerkinsky C, Svennerholm A-M. Antibody and cytokine response determined in duodenal biopsy extracts after oral immunization of human volunteers with a prototype vaccine to enterotoxigenic *Escherichia coli*. 12th European Immunology Meeting, 14-17 June, 1994, Barcelona, Spain. p.500.

Qadri F., Ahmed F., Karim M., Wenneras C, Begum Y. A., Salam M.A., Albert MJ, McGhee JR. Lipopolysaccharide and cholera toxin specific subclass response of B cell responses in cholera. 1999. Clin Diag. Lab Imm. 6 (November issue), 000-000.

Qadri F, Das SK, Faruque ASG, Fuchs GJ, Albert MJ, Sack RB, Svennerholm A-M. Prevalence of toxin types and colonization factors in enterotoxigenic *Escherichia coli* isolated during a two year period from diarrheal patients in Bangladesh. J Clin Microbiol (in press)

Wenneras C, Qadri F, Bardhan P, Sack RB, Svennerholm A-M. Intestinal immunity to enterotoxigenic *Escherichia coli* (ETEC) infection and vaccination. 9th International Congress of Mucosal Immunology, 27-31 January, 1997, Sydney, Australia. Immunol Cell Biol 1997; 75(Suppl 1):A64.

Wenneras C, Qadri F, Czerkinsky C, Bardhan P, Sack RB, Sevenerholm A-M. Intestinal immune responses in patients infected with enterotoxigenic *E. coli*. 8th International Congress of mucosal immunology, 17-20 July, 1995, San Diego, USA. Clin Immunol Immunopath 1995; 76:S103,601.

Qadri F, Makela H, Holmgren J, Albert MJ, Mannoor K, Kantele A, Saha D, Salam MA, Kantele JM. Enteric infections in an endemic area induce a circulating antibody-secreting cell response with homing potentials to both mucosal and systemic tissues. J Infect Dis 1998, 1594-1599.

Qadri F, Jonson G, Begum YA, Wenneras C, Albert MJ, Salam MA, and Svennerholm A-M. Immune response to the mannose-sensitive hemagglutinin in patients with cholera due to *Vibrio cholerae* O1 and O139. Clin Diagn Lab Immunol 1997; 4, 429-434

Qadri F, Wenneras C, Albert MJ, Hossain J, Mannoor K, Begum YA, Mohi, G, Salam MA, Sack RB, and Svennerholm A-M, Comparison of the immune responses inpatients infected with *Vibrio cholerae* O139 and O1. Infection and Immunity, 65, 3571- 3576

References

Ahren M, Svennerholm A-M. Synergistic protective effect of antibodies against *Escherichia coli* enterotoxin and colonization factor antigens. Infect Immun 1982; 38:74-79.

Ahren C, Wenneras C, Holmgren J, Svennerholm A-M. Intestinal antibody response after oral immunization with a prototype cholera B subunit colonization factor antigen enterotoxigenic *Escherichia coli* vaccine. Vaccine 1993; 11:929-934.

Baqui AH, Islam R, Begum N, Nurani S, Qaiyum MA, Husain JB, Yousuf A, Arifeen SE

Urban Panel Survey-Dhaka, Characteristics of sampled population, demographic events, fertility regulation, and sources of MCH-FP services 1995, ICDDR,B Scientific Report No. 81

Baqui A. H., N.Paljor, D.R. Silimperi. 1993. The prevention and treatment of diarrhoea in Dhaka urban slums. ICDDR,B Working Paper No. 32.

Black RE. The epidemiology of cholera and enterotoxigenic *Escherichia coli* diarrhoeal diseases.In: Holmgren J, Lindberg A, Mollby R, editors. Development of vaccines and drugs against diarrhea. Lund: 11th Nobel Confeence, Stockholm, Student Literature, 1986: 23-32.

Black RE. Epidemiology of diarrhoeal diseases: implication for control by vaccines. Vaccine 1993; 11:100-106.

Czerkinsky C, Prince SJ, Michalek SM, Jackson S, Russell MW, Moldovenu Z, McGhee JR, Mestecky J. IgA antibody producing cells in peripheral blood after antigen ingestion: Evidence for a common mucosal immune system in humans. Proc Natl Acad Sci USA 1987; 84:2449-2453.

Czerkinsky CZ, Moldoveanu Z, Mestecky J, Nilsson L-A, Ouchterlony O. A novel two colour ELISPOT assay. I. Simultaneous detection of distinct types of antibody secreting cells. J Immunol Method 1988; 115:31-37.

Evans DJ Jr, Evans DG. Determinants of microbial attachment and their genetic control. In: Farthing MJG, Keusch GT, editors. Enteric infection: mechanisms, manifestations and management. London: Chapman and Hall Medical Publishers, 1988: 22-26.

Evans DG, Evans DJ Jr, Clegg S, Pauley JA. Purification and characterization of the CFA/I antigen of enterotoxigenic *Escherichia coli*. Infect Immun 1979; 25:738-748.

Evans DG, Graham DY, Evans DJ Jr, Opekun A. Administration of purified colonization factor antigens (CFA/I, CFA/II) of enterotoxigenic *Escherichia coli* to volunteers: response to challenge with virulent enterotoxigenic *Escherichia coli*. Gastroenterology 1984; 87:934-940.

Field M, Graf LH Jr., Laird WJ, Smith TL. Heat-stable enterotoxin effects on guanylate cyclase activity, Cyclic GMP concentration and ion transport in small intestine. Proc Natl Acad Sci USA 1978; 75:2800-2804.

Gasstra W, de Graaf FK. Host-specific fimbrial adhesins of noninvasive enterotoxigenic *Escherichia coli* strains. Microbiol Rev 1982; 46:129-161.

Gasstra W, Svennerholm A-M. Colonization factors of human enterotoxigenic *Escherichia coli* (ETEC). Trends Microbiol 1996; 4:444-452.

Giron JA, Xu J-G, Gonz□lez CR, Hone D, Kaper JB, Levine MM. Simultaneous expression of CFA/I and CS3 colonization factor antigens of enterotoxigenic *Escherichia coli* CVD908. Vaccine 1995; 13:939-946.

Gothefers L, AhrenC, Stoll B, Barner DK, Orskov F, Salek MA, Svennerholm A-M. Presence of colonization factor antigens on fresh isolates of fecal *Escherichia coli*: A prospective study. J Infect Dis 1985; 152:1128-1133.

Haberberger RL, Scott DA,Thornton SA, Hyams KC. Diarrheal disease abroad a U.S. navy ship after a brief port visit to a high-risk area. Mil Med 1994; 159:445-448.

Jung HC, Eckann L, Yang S-K, Panja A, Fierer J, MarzyckaWroblewska E,Kagnoff M. A distinct assay of proinflammatory cytokines is expressed in human colon epithelial cells in responses to bacterial invasion. .J Clin Invest 1995; 95:55-65.

Klappworth JM, Donnerberg MS, Abraham JM, Molbey HL-T, James SP. Production of enterotoxigenic *Escherichia coli* inhibit lymphcyte activation and lymphokine production. Infect Immun 1995; 63:2248-2254.

Klemm P. Fibrial adhesins of *Escherichia coli*. Rev Infect Dis 1985; 7:321-340.

Levine MM, Ferrecio C, Prado V, Cayazzo M, Abrego P, Martinez J, Maggi G, Baldini MM, Martin W, Maneval D, Bradford K, Gures L, Lior H, Wasserman S, Nataro JP. Epidemiological studies of *Escherichia coli* diarrheal infections in a low socioeconomic level peri-urban community in Santiago, Chile. Am J Epidemiol 1993; 136:849-869.

Levine MM, Ristaino PR, Marley G, Smyth C, Knutton S, Boedeker E, Black R, Young C, Clements ML, Cheney S, Pafnaik R. Coli surface antigens 1 and 3 of colonization factor antigen II-positive enterotoxigenic *Escherichia coli*: morphology, purification and immune responses in humans. Infect Immun 1984; 44:409-420.

Lopez-Vidal Y, Calva JJ, Trujillo A, Ponce DE, Leon A, Ramos A, Svennerholm A-M, Ruiz-Palacios GM. Enterotoxins and adhesins of enterotoxigenic *Escherichia coli*: are they risk factors for acute diarrhea in the community? J Infect Dis 1990; 162:442-447.

Newcombe RG. Interval estimation for the difference between independient proprotions: comparison of elefven methods. Stat Med 1998: 17: 873-90

Oyofo BA, El-Gendy A, Wasfy MO, al-Etra SH, Churilla A, Murphy J. A survey of enteropathogens among United States military personnel during operation Bright Star 1994 in Cairo, Egypt. Mil Med 1995; 160:331-334.

Savarino SJ, brown FM, Hall E, bassily S, Youssef, Wierzba T, Peruski L, El-Masry NA, Safwat M, Rao M, Jertborn M, Svennerholm A-M, Lee YJ, Clemens JD. Safety and immunogenicity of an oral, killed enterotoxigenic *Escherichia coli*- cholera toxin B subunit vaccine in Egyptian adults.
J Inf. Dis 1998,177, 796-799.

Savarino, S.J., Hall E.R, Basily S.,Brown F.W, Youssef F., Wierzba T.F.,Peruski L,El-Masry N.A, Safwat M, Rao, M., Mohamady H.E., Abu-Elyazeed R., Naficy A., Svennerholm A-M., Jertborn M.,Lee Y.J, and Clemens J.D. 1998. Oral, inactivated, whole cell enterotoxigenic *Escherichia coli* plus cholera toxin B subunit vaccine: Results of the initial evaluation in children J. Infect Dis,1999,179, 107-114

Smyth CJ. Two mannose-resistant hemagglutinins on enterotoxigenic *Escherichia coli* of serotype O6:K15:H16 or H-isolated from travelers and infantile diarrhea. J Gen Microbiol 1982; 128:2081-2096.

Thomas LV, McConnell MM, Rowe B, Field AM. The possession of the novel coli surface antigens by enterotoxigenic *Escherichia coli* strains positive for the putative colonization factor PCF8775. J Gen Microbiol 1985; 131:2319-2326.

Svennerholm A-M, Holmgren J. Identification of *Escherichia coli* heat labile enterotoxin by means of a ganglioside immunosorbent assay (GM2-ELISA) procedure. Curr Microbiol 1978; 1:9-23.

Svennerholm A-M, Holmgren J, Sack DA. Development of oral vaccines against enterotoxigenic *Escherichia coli* diarrhea. Vaccine *1989; 7:196-198.*

Svennerholm A-M, Wikstrom M, Lindblad M, Holmgren J. Monoclonal antibodies against *Escherichia coli* heat-stable toxin (ST) and their use in a diagnostic ST ganglioside GM1-enzyme-linked immunosorbent assay. J Clin Microbiol 1986; 24:585-590.

Svennerholm AM, Åhren C, Jertborn M. Vaccines against ETEC infections. Part I: Oral inactivated vaccines against ETEC, In: Levine MM, Woodrow CG, Kaper JB, Cobo GS, editors, New Generation vaccines 2nd Edition. Marcel Dekkar, Inc. New York. 1997: 865-73

Wenneras C, Svennerholm A-M, Ahren C, Czerkinsky C. Antibody-secreting cells in human peripheral blood after oral immunization with an inactivated enterotoxigenic *Escherichia coli* vaccine. Infect Immun 1992; 60:2605-2611.

Wenneras C, Svennerholm A-M, Czerkinsky C. Vaccine-specific T cells in human peripheral blood after oral immunization with an inactivated enterotoxigenic *Escherichia coli* vaccine. Infect Immun 1994; 62:874-879.

Wolf M, Taylor D, Boedeker EC, Hyams E, Maneval D, Levine M, Tamuza K, Wilson R, Eschevarria P. Characterization of enterotoxigenic *Escherichia coli* isolated from US troops deployed to the Middle East. J Clin Microbiol 1993; 31:851-856.

WHO. Geneva 1996 State of World's vaccines and immunization., Ed. Sheila Davey, Pg 116.

World Health Organisation, Geneva. 1999. New frontiers in the development of vaccines against enterotoxigenic (ETEC) and enterohaemorrhagic (EHEC) *E. coli* infections. Weekly Epidemiological Record, 13, 98-100.

Dissemination and Use of Findings

Describe explicitly the plans for disseminating the accomplished results. Describe what type of publication is anticipated: working papers, internal (institutional) publication, international publications, international conferences and agencies, workshops etc. Mention if the project is linked to the Government of Bangladesh through a training programme.

The information obtained from this study will help in the field-testing of the ETEC vaccine and future evaluation of its protective efficacy.

Principal Investigator: Last, first, middle _____

Collaborative Arrangements

Describe briefly if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization. **(DO NOT EXCEED ONE PAGE)**

This is a collaborative study between the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), the Department of Medical Microbiology and Immunology, Goteborg University, Goteborg, Sweden and the Johns Hopkins University, Baltimore, MD, USA.

TIME FRAME (two years)

a) Enrollment of children, administration of vaccine and laboratory assays (ASC response to vaccine antigens, stimulation of MNC for cytokine assays) - first 9 months.

b) Completion of laboratory assays (vaccine-specific ELISA for plasma and faecal samples and cytokine assays)- next 12 months.

c) Evaluation of results and report writing. Plan and initiate study to progress to children from 3 months to 3 years for protective efficacy studies.

TASK OF EACH INVESTIGATOR

Dr. Firdausi Qadri

- Setting up of procedures for cytokines, RT-PCR, ELISAs etc.
- Supervise work in the laboratory and coordinate specimen collection from the study subjects.
- Coordinate laboratory techniques between Sweden and Bangladesh.
- Analyze data.

Prof. Ann-Mari Svennerholm

- Coordinate study in Bangladesh with that carried out in other countries in terms of comparison of results, vaccines and reagents
- Scientific and academic feedback.

Prof. V. I. Mathan

- Scientific and academic feedback.

Dr. R. B. Sack

Scientific and academic feedback

Principal Investigator: Last, first, middle _____

Biography of the Principal Investigator

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

Name	Position	Date of Birth
Dr. Firdausi Qadri	Senior Scientist, LSD ICDDR,B,; Dhaka, Bangladesh	31th March, 1951

Academic Qualifications (Begin with baccalaureate or other initial professional education)

Institution and Location	Degree	Year	Field of Study
University of Liverpool	Ph.D	1981	Biochemistry
University of Dhaka	M.Sc	1977	Biochemistry

Research and Professional Experience

~~Concluding with the present position, list, in chronological order, previous positions held, experience, and honours. Indicate current membership on~~ any professional societies or public committees. List, in, chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. **(DO NOT EXCEED TWO PAGES, USE CONTINUATION SHEETS).**

1. Senior Scientist, Immunologist, Immunology Section, LSD, ICDDR, working from 1988 to present

2. Assistant Professor, Dept. of Biochemistry, Dhaka University. From 1981 to 1988.

3. Presently involved in studies on the immune response in patients with diarrhoea due to cholera and ETEC diarrhoea. An extensive evaluation of the immune response is being carried out involving both the systemic and mucosal components. This involves the B and T cell responses and the immunomodulators including cytokines and inflammatory markers etc.

4. Studies on the immune response to oral cholera and ETEC vaccines in Bangladeshi individuals in phase I safety and immunogenicity trials.

Relevant publications and proceedings on oral vaccines

Qadri F, Wenneras C, Bardhan PK, Hossain J, Albert MJ, Sack RB, Svennerholm A-M. B cell response to enterotoxigenic *Escherichia coli* (ETEC) in vaccinees and patients after oral immunization and infection (submitted).

Rudin A, Wiklund G, Qadri F, Wenneras C, Svennerholm A-M. Infection with colonization factor antigen I expressing enterotoxigenic *Escherichia coli* may boost immune responses against heterologous CFAs in primed subjects. Epidemiol Infect 119:391-393.

Wenneras C, Qadri F, Bardhan PK, Sack RB, Svennerholm A-M. Intestinal antibody responses to enterotoxigenic *Escherichia coli* in vaccinees and patients after oral immunization and infection (Submitted).

Qadri F, Hossain J, Begum YA, Jonson G, Azim T, Sack RB, Albert MJ, Svennerholm A-M. Immune response to the mannose sensitive hemagglutinin (MSHA) in patients with cholera caused by *Vibrio cholerae* serogroups O1 and O139. 8th International Congress of Mucosal Immunology. Clin Immunol Immpath 1994; 76:S105, P615.

Qadri F, Hossain J, Chowdhury A, Azim T, Salam MA, Khan AM, Sack RB, Albert MJ. Antigen-specific antibody secreting cells in peripheral blood due to *Vibrio cholerea* O139 (W20/34). 12th European Immunology Meetings, Barcelona, June, 1994.

Qadri F, Wenneras C, Mannoor K, Salam MA, Albert MJ, Svennerholm A-M. Mannose-sensitive haemagglutinin (MSHA)-specific antibody response and cytokine production in peripheral blood and the gut in cholera.9th International Congress of Muccosal Immunology, 27-31 January, 1997., Sydney, Australia. Immunol Cell Biol 1997; 75(Suppl 1):A130.

Wenneras C, Qadri F, Bardhan P, Sack RB, Czerkinsky C, Svennerholm A-M. Antibody and cytokine response determined in duodenal biopsy extracts after oral immunization of human volunteers with a prototype vaccine to enterotoxigenic *Escherichia coli.* 12th European Immunology Meeting, 14-17 June, 1994, Barcelona, Spain. p.500.

Wenneras C, Qadri F, Bardhan P, Sack RB, Svennerholm A-M. Intestinal immunity to enterotoxigenic *Escherichia coli* (ETEC) infection and vaccination. 9th International Congress of Mucosal Immunology, 27-31 January, 1997, Sydney, Australia. Immunol Cell Biol 1997; 75(Suppl 1):A64.

Wenneras C, Qadri F, Czerkinsky C, Bardhan P, Sack RB, Sevenerholm A-M. Intestinal immune responses in patients infected with enterotoxigenic *E. coli.* 8th International Congress of mucosal immunology, 17-20 July, 1995, San Diego, USA. Clin Immunol Immunopath 1995; 76:S103,601.

Qadri F, Makela H, Holmgren J, Albert MJ, Mannoor K, Kantele A, Saha D, Salam MA, Kantele JM. Enteric infections in an endemic area induce a circulating antibody-secreting cell response with homing potentials to both mucosal and systemic tissues. J Infect Dis 1998 000-000

Qadri F, Jonson G, Begum YA, Wenneras C, Albert MJ, Salam MA, and Svennerholm A-M. Immune response to the mannose-sensitive hemagglutinin in patients with cholera due to *Vibrio cholerae* O1 and O139. Clin Diagn Lab Immunol 1997; 4, 429-434

Qadri F, Wenneras C, Albert MJ, Hossain J, Mannoor K, Begum YA, Mohi, G, Salam MA, Sack RB, and Svennerholm A-M, Comparison of the immune responses in patients infected with *Vibrio cholerae* O139 and O1. Infection and Immunity, 65, 3571- 3576

Projects involved in over the last 3 years

Safety and immunogenicity study of the oral bivalent B subunit O1/O139 whole cell cholera vaccine in Bangladeshi volunteers. Funding SAREC

Further evaluation of the oral inactivated ETEC vaccine and studies on the immune responses in acute watery diarrhoea (July 1996-December 1998). Funding Sida-SAREC

Epidemiology and ecology of *Vibrio cholerae* infection in Bangladesh
(July 1996- 2001). Funding- NIH

Response to comments of reviewers

Reviewer 1

1. As suggested, children with ongoing antibiotic treatment will not be enrolled in the study. Only children who have not have any antibiotic over the last seven days will be selected
2. We have recently carried out a study to determine the prevalence of ETEC diarrhoea in the 2% surveillance of diarrhoeal patients at ICDDR,B and found that the incidence increases from 3 months onwards. It is possible that the vaccine can be given at one of the EPI schedules, probably with the dose given at 41/2 months. This has to be considered when a large protective efficacy trial is conducted.
3. Studies in Egypt and phase I studies in Bangladesh in adults and children have shown that a 3 day follow-up after vaccination is sufficient. Since the ETEC vaccine is a killed vaccine it is sufficient to observe reactogenicity for 3 days only.
4. As a benefit, all children in the study will be under supervision of a trained physician during the entire period of the study. In case of illness, medication will be given as and when necessary. Since this is a double blind study the same benefit has to be given to both the groups. All the children irrespective of their groups, will be given a month's supply of multivitamin supplement at the end of the study.

Reviewer 2

1. A primary safety endpoint has now been defined as was suggested (page 9).
2. The sample size has been increased after taking into consideration the prevalence of ETEC in children in Bangladesh (Page 9).
3. The IgA specific ASC response will only be studied to only a few of the vaccine antigen.Since a two colour ELISPOT procedure will be used this will require much less numbers of mononuclear cells. In the younger children only the serum and the fecal samples will be used for antibody assays and the cytokine assays. These have been clarified in the methods section (Page 10).
4. Data analyses section has been written in more detail now.
5. The completeness of dosing will be monitored. Those ingesting at least 90% of the vaccine will be studied further. Provision has been made in the case report (Appendix V) to monitor the intake of the dose.
6. The results from the children will be analyzed separately and together from information obtained from both groups.
7. The same buffer will be used for the two groups in the study and has been clarified.
8. Diarrhoea has been defined in the protocol (page 9) and the reaction form (appendix V) modified as well.
9. Results from children taking only one dose of the vaccine will also be used for data analysis. Those children who are not able to take a second dose of the vaccine due to fever or diarrhoea will be excluded from the study and not be given a dose later.
10. A positive response will be defined in terms of a two fold increase over the individuals baseline value. Only ASC levels > 10 ASCs per 10^7 MNCs in the post immunization specimen will be considered a responder.

ABSTRACT SUMMARY

Acute diarrhoeal diseases are a major health problem in developing countries. Infection with ETEC is one of the most common causes of diarrhoea among children in developing countries and it also contributes to malnutrition. The prevalence of toxin types and colonization factors (CFs) of enterotoxigenic *Escherichia coli* (ETEC) was recently studied in fresh samples (n=4662) obtained from a 2% routine surveillance of diarrheal stools for two years from September 1996 to August 1998. It was observed that the prevalence of ETEC was 14% with over 70% isolated in children 0-5 years age range, of whom 93% were in the 0-3 years age range (Qadri et al. 1999). The infection could by prevented by a safe and immunogenic vaccine that is protective in children. One such vaccine undergoing evaluation is the oral inactivated ETEC vaccine consisting of recombinant cholera toxin B subunit (BS) and a mixture of formalin- inactivated whole cell ETEC bacteria expressing colonization factors (BS-CFA ETEC vaccine). The vaccine has been tested in a number of countries in phase-I safety and immunogenicity trails in adults (Svennerholm et al., 1997). These initial vaccine trials have been extended in Egypt to larger field based phase II and phase III studies. It has been tested in adults as well as in children in the 2-12 years old age group (Savarino et al. 1998, 1999) and is now being tested in 6-18 months old children (WHO, 1999). A phase I study carried out in Bangladeshi children in the 3-10 year range has shown that the vaccine is safe and immunogenic (Qadri et al. , manuscript submitted). A phase-II safety and immunogenicity study now needs to be carried out in Bangladesh in children who are most at risk to the consequences of ETEC diarrhea i.e. children from 6 months to 3 years of age. Based on the results of this study, the vaccine can subsequently be extended to a large phase III, protective efficacy trial.

Clarification of points as required in the attached list are as follows:

1. Only children in the 6 month to 3 year age range will be included since ETEC diarrhoea is a major cause of morbidity and mortality in this age and a vaccine is needed urgently for them. Only children whose guardians or parents have given voluntary consent to participate in this study will be enrolled.
2. Methods used have very little chance of physical risk.
3. Since only blood will be collected there is little risk other than a minimum discomfort at puncture site. After feeding the oral ETEC vaccine the child will be kept under observation for 30 minutes and will also be monitored for side-effects for 3 consecutive days.
4. The interview records will be kept in a locker under supervision. Only the investigators will have access to it.
5. Informed consent will be obtained from the guardian or parent of the child. The potential risks and the procedures to be carried out are mentioned in the consent form.
6. The interview will be conducted in the homes of the children for obtaining history prior to enrollment. Monitoring the side-effects will also be carried out in subsequent home visits. Approximately 30 minutes to 1 hour will be required for the interview.
7. The vaccine trial will benefit children in any ETEC endemic country if the trial shows that the vaccine is safe and immunogenic. This will lead to future studies for effectiveness.

Principal Investigator: Last, first, middle _____

8. The activity will require collection of stool and small amount of blood from about a third of the total children that will be enrolled in the study.

Appendix: Ia

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
IMMUNE RESPONSE STUDIES TO ORAL ETEC VACCINE
CONSENT FORM
(children, 6 months to less than 18 months)
Immunization and Safety studies of the ETEC vaccine

Diarrhoeal diseases are major health problem, and a germ called enterotoxigenic *Escherichia coli* (ETEC) is responsible for many cases of diarrhoea among children and adults in Bangladesh. Like some other diseases, it may be possible to prevent some of the diarrhoeal diseases including ETEC diarrhoea using effective vaccines.

We are conducting a study at this hospital to observe how healthy children and adults respond to an oral killed ETEC vaccine. The vaccine to be used in this study has been tested in adults and children, and has been found to be safe. Studies carried out in Sweden and Egypt have found the vaccine to be safe, adverse events noted in about 2-6% of recipients which include nausea, mild abdominal cramps, 1-2 loose stools per day during the 3 day observation period.

We request for your permission to enroll your child in this study. For the purpose of this study, your child will be asked to drink 19 ml of this vaccine (about 4 teaspoon) twice, fourteen days apart. We will collect stool samples (5 gm) from your child 4 times during the course of the study.
There will be no direct benefit to your child as a result of his/her participation in this study, however, results of this study will improve our knowledge about this vaccine and thus benefit the society. We will be happy to answer to your questions related to the vaccine and the study, and also to provide you with the results as and when they become available.
You are free to accept or reject our proposal to enroll your child in this study even after enrollment you will be able to withdraw your child from the study at any time. If you agree to our proposal of enrollment of your child in the study, please indicate that by putting your signature or the impression of your left thumb at the specified space below.

Thank you for your cooperation.

_____
Signature/LTI of the guardian

Date: _____

_____
Signature of the investigator

Date: _____

_____
Signature of witness

Date: _____

Principal Investigator: Last, first, middle _____

Appendix: Ib

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
IMMUNE RESPONSE STUDIES TO ORAL ETEC VACCINE
CONSENT FORM
(CHILDREN, 6 months to less than 18 months)
Immunization and collection of blood and stool

Diarrhoeal diseases are major health problem, and a germ called enterotoxigenic *Escherichia coli* (ETEC) is responsible for many cases of diarrhoea among children and adults in Bangladesh. Like some other diseases, it may be possible to prevent some of the diarrhoeal diseases including ETEC diarrhoea using effective vaccines.

We are conducting a study at this hospital to observe how healthy children and adults respond to an oral killed ETEC vaccine. The vaccine to be used in this study has been tested in adults and children, and has been found to be safe. Studies carried out in Sweden and Egypt have found the vaccine to be safe, adverse events noted in about 2-6% of recipients which include nausea, mild abdominal cramps, 1-2 loose stools per day during the 3 day observation period.

We request for your permission to enroll your child in this study. For the purpose of this study, your child will be asked to drink 19 ml of this vaccine (about 4 teaspoon) twice, fourteen days apart. To determine his/her response to the vaccine, we will collect venous blood (about 1.5 ml, about 1/4th teaspoon) three times during the course of this study (at the beginning before immunization, and 7 and 21 days later). Other than momentary pain and a very small chance of bruising at the site of the prick of the needle, drawing of a few drops of blood will not cause any harm to your child. To minimize the chance of infection, we will take aseptic precautions and use disposable, sterile needles for drawing blood. We will also collect his/her stool samples (5 gm) four times during the course of this study.
There will be no direct benefit to your child as a result of his/her participation in this study, however, results of this study will improve our knowledge about this vaccine and thus benefit the society. We will be happy to answer to your questions related to the vaccine and the study, and also to provide you with the results as and when they become available.
You are free to accept or reject our proposal to enroll your child in this study even after enrollment you will be able to withdraw your child from the study at any time. If you agree to our proposal of enrollment of your child in the study, please indicate that by putting your signature or the impression of your left thumb at the specified space below.

Thank you for your cooperation.

_____
Signature/LTI of the guardian

Date: _____

_____
Signature of the investigator

Date: _____

_____
Signature of witness

Date: _____

Principal Investigator: Last, first, middle _____

Appendix IIa

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
IMMUNE RESPONSE STUDIES TO ORAL ETEC VACCINE
CONSENT FORM (CHILDREN 18 months to 3 years)
Immunization and Safety studies of the ETEC vaccine

Diarrhoeal diseases are major health problem, and a germ called enterotoxigenic *Escherichia coli* (ETEC) is responsible for many cases of diarrhoea among children and adults in Bangladesh. Like some other diseases, it may be possible to prevent some of the diarrhoeal diseases including ETEC diarrhoea using effective vaccines.

We are conducting a study at this hospital to observe how healthy children and adults respond to an oral killed ETEC vaccine. The vaccine to be used in this study has been tested in adults and children, and has been found to be safe. Studies carried out in Sweden and Egypt have found the vaccine to be safe, adverse events noted in about 2-6% of recipients which include nausea, mild abdominal cramps, 1-2 loose stools per day during the 3 day observation period.

We request for your permission to enroll your child in this study. For the purpose of this study, your child will be asked to drink 34 of this vaccine (about 7 teaspoon) twice, fourteen days apart. We will also collect stool (5 gm) from your child four times during the study. There will be no direct benefit to your child as a result of his/her participation in this study, however, results of this study will improve our knowledge about this vaccine and thus benefit the society. We will be happy to answer to your questions related to the vaccine and the study, and also to provide you with the results as and when they become available.
You are free to accept or reject our proposal to enroll your child in this study even after enrollment you will be able to withdraw your child from the study at any time. If you agree to our proposal of enrollment of your child in the study, please indicate that by putting your signature or the impression of your left thumb at the specified space below.

Thank you for your cooperation.

_____
Signature/LTI of the guardian

Date: _____

_____
Signature of the investigator

Date: _____

_____
Signature of witness

Date: _____

Principal Investigator: Last, first, middle _____

Appendix IIb

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
IMMUNE RESPONSE STUDIES TO ORAL ETEC VACCINE
CONSENT FORM (CHILDREN 18 months to 3 years)
Immunization and collection of blood and stool

Diarrhoeal diseases are major health problem, and a germ called enterotoxigenic *Escherichia coli* (ETEC) is responsible for many cases of diarrhoea among children and adults in Bangladesh. Like some other diseases, it may be possible to prevent some of the diarrhoeal diseases including ETEC diarrhoea using effective vaccines.

We are conducting a study at this hospital to observe how healthy children and adults respond to an oral killed ETEC vaccine. The vaccine to be used in this study has been tested in adults and children, and has been found to be safe. Studies carried out in Sweden and Egypt have found the vaccine to be safe, adverse events noted in about 2-6% of recipients which include nausea, mild abdominal cramps, 1-2 loose stools per day during the 3 day observation period.

We request for your permission to enroll your child in this study. For the purpose of this study, your child will be asked to drink 34 ml of this vaccine (about 7 teaspoon) twice, fourteen days apart. To determine his/her response to the vaccine, we will draw 3 ml (about half a teaspoonful) of blood from a vein on his/her forearm, 3 times during the course of this study (at the beginning of the study and 7 and 21 days later). We will also collect his/her stool samples (5 gm) 4 times during the study. Other than momentary pain and a very small chance of bruising at the site of insertion of the needles, drawing of 3 ml of blood or finger prick will not cause any harm to your child. To minimize the chance of infection, we will take aseptic precautions and use disposable, sterile syringe and needles for drawing blood. There will be no direct benefit to your child as a result of his/her participation in this study, however, results of this study will improve our knowledge about this vaccine and thus benefit the society. We will be happy to answer to your questions related to the vaccine and the study, and also to provide you with the results as and when they become available.
You are free to accept or reject our proposal to enroll your child in this study even after enrollment you will be able to withdraw your child from the study at any time. If you agree to our proposal of enrollment of your child in the study, please indicate that by putting your signature or the impression of your left thumb at the specified space below.

Thank you for your cooperation.

_____
Signature/LTI of the guardian

Date: _____

_____
Signature of the investigator

Date: _____

_____
Signature of witness

Date: _____

Principal Investigator: Last, first, middle _____

Appendix III

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH BANGLADESH, (ICDDR,B)

Study Subject Selection Form

Safety and immunogenicity study of the oral BS-CFA ETEC vaccination in Bangladeshi children

Name of the examiner _____

Date of interview ☐☐ ☐☐ ☐☐
DD MM YY

1. Basic data

1.1. Family No. |_|_|_|_|

1.2 Screening No. |_|_|_|_|

1.3 Study child's name |_____|

1.4 Study child's ID |_|_|_|_|

1.5 Respondent

1= Mother 2= Father 3= Other

1.6 Sex 1 Male 2 Female

1.7 Age in months |_|_|

1.8 Date of birth ☐☐ ☐☐ ☐☐
(English calendar) DD MM YY

1.9 Date of birth ☐☐ ☐☐ ☐☐
(Bengali calendar) DD MM YY

If day is unknown write 15. Month and year must be written. You can write according to Bengali calender at the time of interview, and then translate into English later.]

Principal Investigator: Last, first, middle _____

1.10 Height (cm) □□□.□

1.11 Weight (kg) □□.□

1.12 % of NCHS (W/A) □□□.□

1.13 % of NCHS (W/H) □□□.□

1.14 Oedema (Present) 1 = Yes 2=No

1.15 Presence of illness 1=No 2=Fever 3= Diarrhoea 4=others

1.16 Previous immunization

1.16.1 DPT 1=No 2= 1 dose 3=2 dose 4= 3 dose

1.16.2 OPV 1=No 2= 1 dose 3=2 dose 4= 3 dose

1.16.3 Measles 1=No 2= Yes

1.16.4 BCG 1=No 2= Yes

Principal Investigator: Last, first, middle _____

Appendix IV

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH BANGLADESH, (ICDDR,B)

Safety and immunogenicity study of the oral BS-CFA ETEC vaccination in Bangladeshi children

History form of enrolled study subjects

Day 0 / 7 / 14 / 21

Date □□ □□ □□

DD MM YY

Vaccine lot No. ---------- Name of the examiner ----------

1. Basic data

1.1. Family No. |_|_|_|_|

1.2 Screening No. |_|_|_|_|

1.3 Study child's name |_____|

1.4 Study child's ID |_|_|_|_|

1.5 Date of vaccination □□ □□ □□
DD MM YY

1.6 Vaccination Primary=1 Secondary=2

2.0 Presence of illness
1=Yes 2=No

If yes

2.1 Chief Complain:

2.2 History of present illness:

2.3 Physical Examination

2.3.1 General Examination

Pulse (rate/min) □□□

Heart rate/min □□□

Respiratory rate/min □□□

Temperature (°C) □□. □

Anaemia: 1=No 2=mild 3=moderate 4=severe

Clubbing: 1= No 2=Yes

Jaundice: 1= No 2=Yes

Cyanosis: 1= No 2=Yes

Dehydration: 1= No 2=Some 3= Severe

Skin Condition: 1= Scabies 2=Eczema 3= Others

2.3.2 Systemic Examination

2.3.2.1 Alimentary System

Abdominal distension: 1=No 2=Moderate 3=Severe

Abdominal tenderness: 1=No 2=Yes if tender 3= localized 4= generalized

Liver palpable: 1=No 2=Yes

Spleen palpable: 1=No 2=Yes

2.3.2.2 Respiratory System:

Lungs(auscultation): 1=Clear 2=Added sound; if added sound 3=Ronchi 4=Rales

2.3.2.3 Cardiovascular System:

Heart(auscultation): 1=Normal 2=Added sound

2.3.2.4 Nervous System:

Appearance: 1=Normal 2=Irritable 3=Lethargic

2.3.2.5 Locomotor System:

Movement 1=Normal 2=Restricted

Principal Investigator: Last, first, middle _____

Appendix V

Adverse event form

Surveillance after Primary vaccination

Day 1

1.0 Completeness of dosing. Amount ingested: 1=100% 2=≥ 90% 3=<90%

2.0 LOCAL REACTION

Parameters	
Abdominal pain	1= No 2=Mild 3= moderate 4=severe
Cramp	1= No 2= Yes
Distension	1=No 2= moderate 3= severe
Vomiting If yes, number/day	1=No 2= Yes
Other symptoms *Diarrhoea [watery stool or loose stool]	1=No 2= Yes
If yes above number/day (> 3)	

*Diarrhoea defined as passing of ≥ 3 loose or liquid stools per day

3.0 SYSTEMIC REACTION

Parameters	
Fever (oral or rectal temp>37.8°C If yes, temperature (°C)	1= No 2= Yes
Loss of appetite/ Poor feeding	1= No 2= Yes
Irritability	1= No 2= Yes
Nausea	1= No 2= Yes
Dizziness	1= No 2= Yes
Exanthema	1= No 2= Yes
Other	1= No 2= Yes

4.0 Relation to vaccine

1= None 2= Possible 3= Probable 4= Highly probable

5.0 Therapy required

1= No 2= Yes

6.0 Outcome

1= resolved 2= improved 3= unchanged 4= worse

Principal Investigator: Last, first, middle _____

Appendix V

Adverse event form

Day 2

Surveillance after Primary vaccination

1.0 Completeness of dosing. Amount ingested: 1=100% 2=≥ 90% 3=<90%

2.0 LOCAL REACTION

Parameters	
Abdominal pain	1= No 2=Mild 3= moderate 4=severe
Cramp	1= No 2= Yes
Distension	1=No 2= moderate 3= severe
Vomiting If yes, number/day	1=No 2= Yes
Other symptoms *Diarrhoea [watery stool or loose stool]	1=No 2= Yes
If yes above number/day (> 3)	

*Diarrhoea defined as passing of ≥ 3 loose or liquid stools per day

3.0 SYSTEMIC REACTION

Parameters	
Fever (oral or rectal temp>37.8°C If yes, temperature (°C)	1= No 2= Yes
Loss of appetite/ Poor feeding	1= No 2= Yes
Irritability	1= No 2= Yes
Nausea	1= No 2= Yes
Dizziness	1= No 2= Yes
Exanthema	1= No 2= Yes
Other	1= No 2= Yes

4.0 Relation to vaccine
1= None 2= Possible 3= Probable 4= Highly probable

5.0 Therapy required
1= No 2= Yes

6.0 Outcome
1= resolved 2= improved 3= unchanged 4= worse

Principal Investigator: Last, first, middle _____

Appendix V

Adverse event form

Day 3

Surveillance after Primary vaccination

1.0 Completeness of dosing. Amount ingested: 1=100% 2=≥ 90% 3=<90%

2.0 LOCAL REACTION

Parameters	
Abdominal pain	1= No 2=Mild 3= moderate 4=severe
Cramp	1= No 2= Yes
Distension	1=No 2= moderate 3= severe
Vomiting If yes, number/day	1=No 2= Yes
Other symptoms *Diarrhoea [watery stool or loose stool]	1=No 2= Yes
If yes above number/day (> 3)	

*Diarrhoea defined as passing of ≥ 3 loose or liquid stools per day

3.0 SYSTEMIC REACTION

Parameters	
Fever (oral or rectal temp>37.8°C If yes, temperature (°C)	1= No 2= Yes
Loss of appetite/ Poor feeding	1= No 2= Yes
Irritability	1= No 2= Yes
Nausea	1= No 2= Yes
Dizziness	1= No 2= Yes
Exanthema	1= No 2= Yes
Other	1= No 2= Yes

4.0 Relation to vaccine

1= None 2= Possible 3= Probable 4= Highly probable

5.0 Therapy required

1= No 2= Yes

6.0 Outcome

1= resolved 2= improved 3= unchanged 4= worse

Principal Investigator: Last, first, middle _____

AppendixV

Adverse event form

Surveillance after Secondary vaccination

Day 1

1.0 Completeness of dosing. Amount ingested: 1=100% 2=≥ 90% 3=<90%

2.0 LOCAL REACTION

Parameters	
Abdominal pain	1= No 2=Mild 3= moderate 4=severe
Cramp	1= No 2= Yes
Distension	1=No 2= moderate 3= severe
Vomiting If yes, number/day	1=No 2= Yes
Other symptoms *Diarrhoea [watery stool or loose stool]	1=No 2= Yes
If yes above number/day (> 3)	

*Diarrhoea defined as passing of ≥ 3 loose or liquid stools per day

3.0 SYSTEMIC REACTION

Parameters	
Fever (oral or rectal temp>37.8°C If yes, temperature (°C)	1= No 2= Yes
Loss of appetite/ Poor feeding	1= No 2= Yes
Irritability	1= No 2= Yes
Nausea	1= No 2= Yes
Dizziness	1= No 2= Yes
Exanthema	1= No 2= Yes
Other	1= No 2= Yes

4.0 Relation to vaccine
1= None 2= Possible 3= Probable 4= Highly probable

5.0 Therapy required
1= No 2= Yes

6.0 Outcome
1= resolved 2= improved 3= unchanged 4= worse

Principal Investigator: Last, first, middle _____

AppendixV

Adverse event form

Surveillance after Secondary vaccination
Day 2

1.0 Completeness of dosing. Amount ingested: 1=100% 2=≥ 90% 3=<90%

2.0 LOCAL REACTION

Parameters	
Abdominal pain	1= No 2=Mild 3= moderate 4=severe
Cramp	1= No 2= Yes
Distension	1=No 2= moderate 3= severe
Vomiting If yes, number/day	1=No 2= Yes
Other symptoms *Diarrhoea [watery stool or loose stool]	1=No 2= Yes
If yes above number/day (> 3)	

*Diarrhoea defined as passing of ≥ 3 loose or liquid stools per day

3.0 SYSTEMIC REACTION

Parameters	
Fever (oral or rectal temp>37.8°C If yes, temperature (°C)	1= No 2= Yes
Loss of appetite/ Poor feeding	1= No 2= Yes
Irritability	1= No 2= Yes
Nausea	1= No 2= Yes
Dizziness	1= No 2= Yes
Exanthema	1= No 2= Yes
Other	1= No 2= Yes

4.0 Relation to vaccine
1= None 2= Possible 3= Probable 4= Highly probable

5.0 Therapy required
1= No 2= Yes

6.0 Outcome
1= resolved 2= improved 3= unchanged 4= worse

Principal Investigator: Last, first, middle _____

Appendix V

Adverse event form

Surveillance after Secondary vaccination

Day 3

1.0 Completeness of dosing. Amount ingested: 1=100% 2=≥ 90% 3=<90%

2.0 LOCAL REACTION

Parameters	
Abdominal pain	1= No 2=Mild 3= moderate 4=severe
Cramp	1= No 2= Yes
Distension	1=No 2= moderate 3= severe
Vomiting If yes, number/day	1=No 2= Yes
Other symptoms *Diarrhoea [watery stool or loose stool]	1=No 2= Yes
If yes above number/day (> 3)	

*Diarrhoea defined as passing of ≥ 3 loose or liquid stools per day

3.0 SYSTEMIC REACTION

Parameters	
Fever (oral or rectal temp>37.8°C If yes, temperature (°C)	1= No 2= Yes
Loss of appetite/ Poor feeding	1= No 2= Yes
Irritability	1= No 2= Yes
Nausea	1= No 2= Yes
Dizziness	1= No 2= Yes
Exanthema	1= No 2= Yes
Other	1= No 2= Yes

4.0 Relation to vaccine

1= None 2= Possible 3= Probable 4= Highly probable

5.0 Therapy required

1= No 2= Yes

6.0 Outcome

1= resolved 2= improved 3= unchanged 4= worse

Principal Investigator: Last, first, middle _____

Detailed Budget for New Proposal

Project Title: Phase II Safety and immunogenicity studies of the enterotoxigenic *Escherichia coli* (ETEC) vaccine

Name of PI: Firdausi Qadri, ICDDR,B[a]

Protocol Number: Name of Division: Laboratory Sciences Division

Funding Source: USAID Amount Funded (direct): US$ 96,957 Total:US$121,196
Overhead (25%) 24,239

Starting Date ASAP: Closing Date: Two years from starting date

Strategic Plan Priority Code(s):Diarrhoeal disease/ Child Health Research

Sl. No	Account Description	Salary Support			US $ Amount Requested		
	Personnel	**Position**	**Effort%**	**Salary**	**1st Yr**	**2nd Yr**	**3rd Yr**
	F. Qadri	Senior Scientist	25		6,150	7,380	
		Medical Officer	100		5,640		
		Medical officer	5		605		
		Research Officers (2)	100		6168	6476	
		Laboratory Attendant (1)	100		1,985	2,095	
		Health workers(3)	100		2,808	0	
		Sub Total			**23,356**	**15,951**	
	Consultants						
	Local Travel				2000	500	
	International Travel						
		Sub total			**2,000**	**500**	
	Supplies and Materials (Description of Items)						
	Immunoreagents, antibodies, cytokine kits, tissue culture plastics, ELISA plates, pipettes, ELISPOT and other supplies, etc.				15,000	15,000	
	Sub Totals				**12,000**	**15,000**	

	Other Contractual Services			
	Repair and Maintenance	1000	2000	
	Rent, Communications, Utilities	850	500	
	Training Workshop, Seminars	500	100	
	Printing and Publication	1,000	1,000	
	Sub Total	**3350**	**3600**	

	Interdepartmental Services	**1st Yr**	**2nd Yr**	**3rd Yr**
	Computer Charges	150	150	
	Pathological Tests	1500	100	
	Microbiological tests	1,500	500	
	Biochemistry Tests			
	Hospitalization charges	1500		
	Biochemistry and Nutrition	500		
	Sub Totals	**5150**	**750**	
	Computer for laboratory **Capital Expenditure** ELISA READER and computer programme for first year, replacement of micropipettes etc. for second year	**3500** **10,000**	**1800**	

DIRECT COST		**59,356**	**37,601**
INDIRECT COST		**14,839**	**9,400**
TOTAL COST	**121,196**	**74,195**	**47,001**

Budget Justifications

Please provide one page statement justifying the budgeted amount for each major item. Justify use of man power, major equipment, and laboratory services.

The budget is sufficient for a two year period for the phase II vaccine study
For this study, funds for salaries of one physician, two research officers and three field workers are essential. These have been budgeted in the protocol.
. A replacement of the ELISA Reader presently in use will be needed. The study is based mainly on ELISA and an equipment that can be connected to a computer for carrying out end point titrations will be necessary. Similarly the computer presently in use in the laboratory needs to be upgraded.

The amount budgeted in the project is sufficient to carry out the safety and immunogenicity studies on the chosen sample of study subjects. This phase II study will serve to predict the feasibility of studying the ETEC vaccine in the field.

Other Support

Describe sources, amount, duration, and grant number of all other research funding currently granted to PI or under consideration. (DO NOT EXCEED ONE PAGE FOR EACH INVESTIGATOR)

1. Study of specific and innate mechanisms of the immune response in acute watery diarrhoea due to *Vibrio cholerae* and enterotoxigenic *Escherichia coli*: Studies in patients and vaccinees (January1999-2001). Funding Sida-SAREC

2.Epidemiology and ecology of *Vibrio cholerae* infection in Bangladesh
(July 1996 -2002). Funding- NIH

Principal Investigator: Last, first, middle _____

Check List

After completing the protocol, please check that the following selected items have been included.

1. Face Sheet Included [X]
2. Approval of the Division Director on Face Sheet [X]
3. Certification and Signature of PI on Face Sheet, #9 and #10 [X]
4. Table on Contents [X]
5. Project Summary [X]
6. Literature Cited [X]
7. Biography of Investigators [X]
8. Ethical Assurance [X]
9. Consent Forms [X]
10. Detailed Budget [X]
11. External reviewers comments X
12. Response to reviewers comment X

Principal Investigator: Last, first, middle _____

Principal Investigator: Last, first, middle _____

CENTRE
FOR HEALTH AND
POPULATION RESEARCH

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
Mail : ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh
Phone : 871751-60, Telex : 675612 ICDD BJ
Fax : 880-2-883116, 886050, 871568, 871686, Cable : Cholera Dhaka

Memorandum

27 September 1999

To : Dr. Firdausi Qadri
Laboratory Sciences Division

From : Professor V. I. Mathan
Chairman, Review Review Committee

From : Protocol # 99-033

This has reference to your memo of 27th September 1999 attaching a modified copy of your protocol # 99-033 entitled "Phase II and immunogenicity studies of the enterotoxinogenic *Escherichia coli* (ETEC) vaccine in Bangadeshi children". The protocol is hereby **approved** upon your appropriate addressing of the observations made by the Research Review Committee in its meeting held on 20th September 1999.

Thanking you and wishing your success in running the protocol.

copy:- Division Director
Laboratory Sciences Division

গণপ্রজাতন্ত্রী বাংলাদেশ সরকার
ঔষধ প্রশাসন পরিদপ্তর
১০৫-১০৬, মতিঝিল বা/এ,
ঢাকা- ১০০০, বাংলাদেশ।

নং- ডিএ/১-১৫/৯২/৯৭ /৫৪৮-৯

তারিখ ঃ ২৬/৯/৯৯

বরাবর,

ডঃ ফেরদৌসী কাদরী
সিনিয়ার সায়েন্টিস্ট
ইমিউনোলোজী ল্যাবরেটোরী
ল্যাবরেটোরী সায়েন্স ডিভিশন
আই. সি. ডি. ডি. আর. বি
মাহাখালী, ঢাকা-১২১২।

বিষয় ঃ **Permission to use on oral vaccine entitled "phase II safety and immunogenicity studies of the enterotoxigenic *Escherichia coli* (ETEC) vaccine in Bangladeshi children" in a proposed study at ICDDR'B.**

সূত্র ঃ তাঁহাদের সূত্র নং নাই , তারিখ ঃ ২৩-০৯-৯৯ইং।

উপরিউক্ত বিষয় ও সূত্রের বরাতে প্রেরিত প্রোটকল অনুযায়ী WHO Guide line on Good Clinical Practice ..... অবলম্বনে বিষয়োল্লিখিত ভেকসিন (Vaccine) আই. সি. ডি. ডি. আর. বি. তে বিশেষজ্ঞের তত্তাবধানে নির্দিষ্ট শিশুদের মধ্যে ট্রায়াল (Trial) পরিচালনার জন্য এতদ্বারা অনুমতি প্রদান করা হইল।

তবে ,উল্লেখ থাকে এই যে, ভেকসিন (Vaccine)টির ব্যবহারে শিশুদের মধ্যে কোন প্রকার পার্শ্ব প্রতিক্রিয়া, বিরূপ প্রতিক্রিয়া বা ক্ষতি হইলে উহার সকল দায় দায়িত্ব ট্রায়াল (Trial) পরিচালনাকারীদের উপরই বর্তাইবে। অত্র পরিদপ্তর কোন অবস্থাতেই কোন প্রকার দায়িত্ব বহন করিবে না।

২৬/৯/৯৯

(মোঃ আবদুল মালেক)
পরিচালক (ভারপ্রাপ্ত)
ঔষধ প্রশাসন পরিদপ্তর

নং- ডিএ/১-১৫/৯২/৯৭

তারিখ ঃ .....

অনুলিপি অবগতি ও প্রয়োজনীয় ব্যবস্থার জন্য প্রেরন করা হইল ঃ

১। জনাবা নায়ার রহমান, ঔষধ তত্তাবধায়ক, ঔষধ প্রশাসন পরিদপ্তর, ঢাকা।
অনাপত্তি সূচক সার্টিফিকেট (NOC) ইস্যু করার ব্যবস্থা গ্রহনের জন্য তাঁকে অনুরোধ করা হইল।

পরিচালক (ভারপ্রাপ্ত)
ঔষধ প্রশাসন পরিদপ্তর