tachment 1. (Pr · Ed)

ICE SHEET)

ETHICAL REVIEW C[illegible], ICDDR,B.

Date APRIL 21, 1998

Principal Investigator Dr. Shams El Arifeen Trainee Investigator (if any) X

Application No. 98-007 (REVISED) Supporting Agency (if Non-ICDDR,B) X

Title of Study Safety and immunogenecity of $4x10^5$ pfu tetravalent Rhesus Rotavirus vacine, with or without zinc supplementation in Matlab, Bangladesh

Project status:
(✓) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

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

1. Source of Population:
   (a) Ill subjects — Yes (No)
   (b) Non-ill subjects — (Yes) No
   (c) Minors or persons under guardianship — (Yes) No
2. Does the study involve:
   (a) Physical risks to the subjects — (Yes) No
   (b) Social Risks — Yes (No)
   (c) Psychological risks to subjects — Yes (No)
   (d) Discomfort to subjects — (Yes) No
   (e) Invasion of privacy — Yes (No)
   (f) Disclosure of information damaging to subject or others — Yes (No)
3. Does the study involve:
   (a) Use of records, (hospital, medical, death, birth or other) — (Yes) No
   (b) Use of fetal tissue or abortus — Yes (No)
   (c) Use of organs or body fluids — (Yes) No
4. Are subjects clearly informed about:
   (a) Nature and purposes of study — (Yes) No
   (b) Procedures to be followed including alternatives used — (Yes) No
   (c) Physical risks — (Yes) No
   (d) Sensitive questions — Yes (No) N/A
   (e) Benefits to be derived — (Yes) No
   (f) Right to refuse to participate or to withdraw from study — (Yes) No
   (g) Confidential handling of data — (Yes) No
   (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — (Yes) No
5. Will signed consent form be required:
   (a) From subjects — Yes (No)
   (b) From parent or guardian (if subjects are minors) — (Yes) No
6. Will precautions be taken to protect anonymity of subjects — (Yes) No
7. Check documents being submitted herewith to Committee:
   ___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   ✓ Protocol (Required)
   ✓ Abstract Summary (Required)
   ✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
   ___ Informed consent form for subjects
   ✓ Informed consent form for parent or guardian
   ✓ Procedure for maintaining confidentiality
   ✓ Questionnaire or interview schedule *Data Forms

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

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

21/4/98

Principal Investigator

Trainee

Safety and immunogenecity of 4×10^5 pfu tetravalent Rhesus Rotavirus vaccine, with or without zinc supplementation in Matlab, Bangladesh

Abstract Summary

Study Summary:

Worldwide, rotavirus is the most common cause of hospitalization due to diarrhoea. Studies in developed countries show that the current tetravalent rhesus rotavirus vaccine is immunogenic and efficacious against severe rotavirus diarrhoea. The results from three studies in Latin America has produced mixed results. While high rates of immune response and efficacy were seen in Venezuela, efficacy was lower in Peru and Brazil and appeared to be associated with lower immune response in Brazil. No vaccine trials have been conducted in Asia and Africa. Zinc deficiency is known to be associated with impaired immune response. Existing data suggests the presence of zinc deficiency among children in Bangladesh. It is therefore likely that daily supplementation of infants with adequate amounts of zinc during the age period when the three doses of the rotavirus vaccine are given will improve the immune response to the vaccine.

A study has been proposed by the International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh (ICDDR,B) with the technical assistance of the Centres for Disease Control and Prevention (CDC) to investigate the immunogenecity of the RRV-TV rotavirus vaccine in a sample of infants in Matlab, Bangladesh. This study has already received approval from the Research and Ethical Review Committees and is expected to start soon.

It is now proposed that the approved study be expanded to test the additional hypothesis that simultaneous daily zinc supplementation will significantly improve the immunogenecity of the RRV-TV rotavirus vaccine in these infants. The objective of this study is to assess the safety and IgA serological response of 3 doses of oral tetravalent rhesus rotavirus vaccine (4×10^5 pfu /dose) in Bangladeshi infants, when it is given with or without zinc supplementation.

The same randomized, double-blind placebo-controlled design will be retained with an additional cell where infants will receive both the RRV-TV vaccine and daily zinc supplements (5mg) from 4 to 18 weeks of age. Infants will thus be randomized to one of the following three groups: (I) RRV-TV and zinc supplement, (ii) RRV-TV and placebo for zinc, and (iii) placebos for vaccine and zinc. Each group will have 50 infants.

Three doses of vaccine or placebo will be given at 6-8, 10-12 and 14-16 weeks of age along with the routine immunizations. Zinc supplements or placebos will be given daily (5 mg/day) starting 2 weeks before the first dose until 4 weeks after the third dose. Four blood samples will be obtained: at the time of the 3 doses and 4 weeks after the third dose. Stool samples will be collected at 4, 7 and 10 days after each dose. Immune responses will be compared between the three groups to assess the effect of the vaccine and supplement.

Strategies to Address Specific Ethical Issues:

1. The RRV-TV rotavirus vaccine, designed for preventing human rotavirus infection, can only be evaluated in healthy humans. Very young infants will be enrolled as the risk of the disease is greatest in that age group.

2. Safety issues of the RRV-TV vaccine has been addressed in the approved study. Briefly, the RRV-TV vaccine has been associated with small but significant excess of mild to moderate fever, usually occurring 3-4 days after the first dose of the vaccine and which was only detected by measurement and not by caretakers. Some studies have also reported higher rates of reduced

appetite, irritability and decreased activity following the first vaccine dose. Zinc supplementation, in the small doses to be given in the proposed study, is not associated with any adverse effect. There is a very small risk of infection through blood collection. No sensitive information will be collected in this study.

3. The frequent observations of the subject infant and the easy availability of ICDDR,B clinical facilities will result in early identification and management of adverse effects, if any. Sterile non-touch techniques will be used to collect blood samples to reduce the risk of infection through that route.

4. Confidentiality of collected information will be maintained by keeping all data forms private and locked at the Matlab Diarrhoea Hospital and the ICDDR,B Dhaka Offices with access limited to those working in the study. Study subjects will only be identified by study numbers in the computer databases used for analysis.

5. Infants will only be enrolled after their parents have been given a full explanation of the study and they have understood the implications of participating in this study, and have agreed to participate in writing.

6. Background and other information on the study subjects will be available from the routine surveillance of this population. The CHWs will visit the enrolled infants at 4 and 7 days after vaccination to ask the caretaker about adverse events or reactions using a standard reporting form which will take about 5-10 minutes.

7. Enrolled infants in the vaccine groups are likely to benefit from the rotavirus vaccine, and those receiving the zinc may also benefit directly. ICDDR,B will ensure that any enrolled infant in need of treatment is offered treatment if it is available in the Matlab Hospital. For other conditions, the infants will be referred to the Chandpur Government Hospital and assistance provided for travel. The results of this study will help contribute to the further evaluation of the use of the RRV-TV vaccine which has the potential of significantly reducing the risk of rotavirus diarrhoea among children. These benefits considerably outweigh the minimal risks from the study.

8. Study infants will be selected from the Matlab demographic surveillance records. Four blood samples will be obtained: at the time of the 3 doses and 4 weeks after the third dose. Stool samples will be collected at 4, 7 and 10 days after each dose.

International Centre for Diarrhoeal Disease Research, Bangladesh

RESEARCH PROTOCOL

FOR OFFICE USE ONLY

Protocol No: 98-007 Date: 2/3/98

RRC Approval: Yes/No Date:

ERC Approval: Yes/No Date:

1. Title of Project (Do not exceed 60 characters including spaces and punctuations)

Safety and immunogenecity of 4×10^5 pfu tetravalent Rhesus Rotavirus vaccine, with or without zinc supplementation in Matlab, Bangladesh

2a. Name of the Principal Investigator(s) (Last, Middle, First)	2b. Position / Title	2c. Qualifications
Arifeen, Shams, El	Epidemiologist, PHSD	MBBS, MPH, DrPH

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

Child Health Programme, PHSD

4. Contact Address of the Principal Investigator

4a. Office Location:

Public Health Sciences Division, ICDDR,B
Mohakhali
Dhaka, Bangladesh

4b. Fax No: 880-2-886050

4c. E-mail: shams@icddrb.org

4d. Phone /Ext: 880-2-871751-60/ext #2233

5a. Use of Human Subjects	5b. Use of Live Animal	5c. If Yes, Specify Animal Species
Yes ☑	Yes ☐	
No ☐	No ☑	

6. Dates of Proposed Period of Support *(Day, Month, Year - DD/MM/YY)*

01/03/98-28/02/99

7. Cost Required for the Budget Period

7a. Ist Year ($): 34,481 *2nd Year ($):* *3rd Year ($):*

7b. Direct Cost ($): 34,481 Total Cost ($): 43,101

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 as well by the external reviewers. The protocol has been revised according to the reviewer's comments and is approved.

J Patrick Vaughan
Name of the Division Director

Patrick Vaughan
Signature

1.3.98
Date of Approval

9. Certification by the Principal Investigator

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.

10. Signature of PI

Date: March 1, 1998

Table of Contents

☐ Check here if appendix is included

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	Shams El Arifeen				
Project Name	Safety and immunogenecity of 4×10^5 pfu tetravalent Rhesus Rotavirus vaccine, with or without zinc supplementation in Matlab, Bangladesh				
Total Budget	$43,101	Beginning Date	01/04/98	Ending Date	31/03/99

This is an addendum to a study proposed by the International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh (ICDDR,B) with the technical assistance of the Centres for Disease Control and Prevention (CDC) to investigate the immunogenecity of the RRV-TV rotavirus vaccine in a sample of infants in Matlab, Bangladesh.

It is now proposed that this study be expanded to test the additional hypothesis that simultaneous daily zinc supplementation will significantly improve the immunogenecity of the RRV-TV rotavirus vaccine in these infants. The objective of the study is to assess the safety and IgA serological response of 3 doses of oral tetravalent rhesus rotavirus vaccine (4×10^5 pfu /dose) in Bangladeshi infants, when it is given with or without zinc supplementation.

Worldwide, rotavirus is the most common cause of hospitalization due to diarrhoea. Studies in developed countries show that the current tetravalent rhesus rotavirus vaccine is immunogenic and efficacious against severe rotavirus diarrhoea. The results from three studies in Latin America has produced mixed results. While high rates of immune response and efficacy were seen in Venezuela, efficacy was lower in Peru and Brazil and appeared to be associated with lower immune resposnse in Brazil. No vaccine trials have been conducted in Asia and Africa. Zinc deficiency is known to be associated with impaired immune response. Existing data suggests the presence of zinc deficiency among children in Bangladesh. It is therefore likely that daily supplementation of infants with adequate amounts of zinc during the age period when the three doses of the rotavirus vaccine are given will improve the immune response to the vaccine.

The same randomized, double-blind placebo-controlled design will be retained with an additional cell where infants will receive both the RRV-TV vaccine and daily zinc supplements (5mg) from 4 to 18 weeks of age. Infants will thus be randomized to one of the following three groups: (i) RRV-TV and zinc supplement, (ii) RRV-TV and placebo for zinc, and (iii) placebos for vaccine and zinc. Each group will have 50 infants. Three doses of vaccine or placebo will be given at 6-8, 10-12 and 14-16 weeks of age along with the routine immunizations. Zinc supplements or placebos will be given daily (5 mg/day) starting 2 weeks before the first dose until 4 weeks after the third dose when the last blood sample is obtained. Immune responses will be compared between the three groups to assess the effect of the vaccine and supplement.

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

Name	Professional Discipline / Specialty	Role in the Project
1. Shams El Arifeen	Epidemiologist (Child Health Prog., PHSD)	Principal Investigator
2. Mohammad Yunus	Scientist and Head, Matlab Health Research Prog., PHSD	Co-Principal Investigator
3. Joseph Bresee	Medical Epidemiologist (CDC)	Co-Principal Investigator
4. Abdullah Hel Baqui	Senior Epidemiologist (Head, Child Health Prog., PHSD)	Co-Investigator
5. MA Wahed	Assoc. Scientist (Head, Biochemistry and Nutrition, LSD)	Co-Investigator
6. Tasnim Azim	Immunologist (Head, Virology, LSD)	Co-Investigator
7. J Patrick Vaughan	Epidemiologist (Director, PHSD, ICDDR,B)	Co-Investigator
8. Robert E. Black	Epidemiologist (Chairman, DIH, JHUSPH)	Co-Investigator
9. Roger Glass	Chief, Viral Gastroenteritis Unit (CDC)	Co-Investigator

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be tested:

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

Daily zinc supplementation in infants starting 2 weeks before the first scheduled dose at 6 weeks of age and continuing 4 weeks beyond the third dose at 14 weeks will significantly improve the immunogenecity of the RRV-TV rotavirus vaccine in infants.

Scientific basis:

The efficacy and immunonogenecity of the RRV-TV rotavirus vaccine in developing countries have been poor, which is in sharp contrast to results in developed countries. Observational studies have demonstrated that zinc deficiency is associated with impaired immune functions while experimental studies show improvement in immune response following zinc supplementation. The prevalence of zinc deficiency among Bangladeshi children is estimated to be high (at least 30%). It is therefore plausible to hypothesize that supplementation with zinc will improve the zinc status of the infants and thus improve the immune response to the RRV-TV rotavirus vaccine as measured by seroconversion.

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).**

1. To assess the effect of zinc supplementation on the immune response to 4×10^5 pfu RRV-TV rotavirus vaccine as measured by serological tests of blood samples obtained at the time of each of the three doses of the vaccine and 4 weeks after the third dose. A four-fold increase in antibody titer over the baseline levels is usually considered a positive immune response.

2. To assess the safety of three doses of the RRV-TV rotavirus vaccine, as measured by the prevalence of adverse effects in the first 7 days after each dose based on interviews of the caretaker and physical examination.

3. To assess the shedding of vaccine virus in the stool, based on samples obtained 4, 7 and 10 days after each dose.

4. To assess the effect of zinc supplementation on the plasma zinc levels to be measured at baseline and again 4 weeks after the third vaccine dose.

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).**

Background

This is an addendum to a study proposed by the International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh (ICCDR,B) with the technical assistance of the Centres for Disease Control and Prevention (CDC) to investigate the immunogenecity of the RRV-TV rotavirus vaccine in a sample of infants in Matlab, Bangladesh. It is anticipated that this study will commence in March 1998.

It is now proposed that this study be expanded to test the additional hypothesis that daily zinc supplementation starting 2 weeks before the first scheduled dose at 6 weeks and continuing 4 weeks beyond the third dose at 14 weeks will significantly improve the immunogenecity of the RRV-TV rotavirus vaccine in these infants. The same randomized, double-blind placebo-controlled design will be retained with an additional cell where infants receiving the RRV-TV vaccine will also receive zinc supplements.

ICDDR,B operates a field research site in Matlab, a rural area of Bangladesh. About 105,000 people are provided with health services by ICDDR,B in the intervention and they are also visited once a month as part of an intensive demographic and health surveillance system, the Demographic Surveillance System (DSS).

This addendum only presents the rationale for expanding the study, and describes the changes necessary in the study design.

Review of the literature

Diarrhoea is an important cause of morbidity and mortality in the developing countries. In Bangladesh, diarrhoea was associated with about 19-26% of childhood deaths (Baqui, in press; Salway, 1994). In Bangladesh, rotavirus is associated with about 4% of all diarrhoea cases among under-5 children in the community, but it is the most common (about 40%) cause of diarrhoea with dehydration in this age group (Black, 1982). Worldwide, rotavirus is responsible for only 6% of all diarrhoeal episodes, although, about a fifth of all deaths due to diarrhoea can be attributed to this virus (de Zoysa 1985). Rotavirus is the single most important cause of dehydrating diarrhoea in infants and young children in the world. In a recent study based on a surveillance of diarrhoea cases in the Dhaka hospital of ICDDR,B, 20% of under-5 children with diarrhoea excreted rotavirus in their stools and 92% of these cases were in children <2 years (Unicomb, in press). In Bangladesh, children in the first two years of life have 0.5 episodes of rotavirus diarrhoea per year (Black, 1982). The incidence is much less in older children.

WHO and other international public health agencies have attached high priority to the development of rotavirus vaccines. The rhesus rotavirus tetravalent vaccine (RRV-TV) is the most promising candidate vaccine. This vaccine contains strains with VP7 (G Type) specificities for the 4 common human rotavirus strains (G serotypes 1, 2, 3 and 4). These strains also predominate in Bangladesh, where serotypes 2 (27%) and 1 (19%) are the most common (Bingnan, 1991). The immunogenecity and efficacy of two different doses of the vaccine have been evaluated. Tables 1 and 2 presents a summary of these trials.

The lower dose (4×10^4 pfu) vaccine elicited an IgA (to RRV) response in about 75% of cases in the US and Peru but only 58% in Brazil (Bernstein, 1995; Lanata, 1996; Linhares, 1995; Santosham, unpublished). The neutralizing antibody response to human serotypes was low in all four sites: 88% produced neutralization antibody response to RRV in the US study (not reported for the Peru and Brazilian

studies), and 16-45% induced neutralization antibody response to the 4 human serotypes. The efficacy of the lower dose RRV-TV vaccine was more than 80% against severe rotavirus diarrhea in the US, but was only 40-47% in Peru and Brazil. There are some differences in the definitions of severe rotavirus diarrhoea in these studies.

The immune response to the higher dose (4×10^5 pfu) vaccine was similar to that of the 10-fold lower dose. In one US study, 56% of the infants produced a rotavirus-specific IgA response (Rennels, 1996), while in another US trial and in Venezuela, IgA seroconversion occured in 84-93% of the infants (Santosham, unpublished; Perez-Schael, 1997). A neutralizing antibody response to RRV was observed in 77-90% of the infants while only 10-45% demonstrated neutralization antibody responses to the 4 human serotypes.

Table 1: Serconversion rates of RRV-TV vaccine by vaccine dose and location

			Seroconversion rates (%)					
				Neutralizing antibody				
Location	Citation	Vaccine dose	ELISA (RRV IgA)	RRV	G1	G2	G3	G4
USA	Bernstein, 1995	4×10^4	74	88	19	26	26	28
Peru	Lanata, 1996	4×10^4	75	-	36	16	32	28
Brazil	Linhares, 1995	4×10^4	58	-		16-45		
USA	Rennels, 1996	4×10^5	56	90	14	31	29	14
USA	Santosham, unpublished	4×10^5	93	83	24	24	26	19
Venezuela	Perez-Schael, 1997	4×10^5	84	77	45	33	28	10

Efficacy against severe rotavirus disease was about 80% in Venezuela and in one of the US trials (Perez-Schael, 1997; Rennels, 1996), while somewhat lower efficacy (69%) was observed in the study in a Native American population (Santosham, unpublished). The epidemiology of the disease in Venezuela is believed to be more like what is seen in developed countries and may explain the similarity of vaccine efficacy in the US and Venezuela.

The lower efficacy in Peru and Brazil may be a function of the lower dose used in these studies or differences in the epidemiology of the disease, specifically age at first infection relative to age at immunization. It is also believed that the lower efficacy seen in developing countries may be due to lower immune response, as was seen in Brazil where only 58% of the participants produced a IgA response (Linhares, 1995). However, a similar modest IgA response was also seen one of the US studies using the higher dose vaccine (Rennels, 1996).

No trials have yet been performed in Asia and Africa with this vaccine. The Consensus Workshop on Rotavirus Vaccines for the Immunization of Children in Developing Countries held in Geneva on 9-10 January, 1997 recommended that immunogenecity trials be conducted in poor, developing countries like Bangladesh. These studies should also address determinants of immune response and the effect of zinc or vitamin A supplmentation on vaccine response. It is important that we better understand the link between the immune response and protective effects of rotavirus vaccination and identify ways to improve the immune response and presumably the efficacy of the vaccine in developing countries.

SAFETY AND IMMUNOGENECITY OF 4×10^5 PFU TETRAVALENT RHESUS ROTAVIRUS VACCINE, WITH OR WITHOUT ZINC SUPPLEMENTATION IN MATLAB, BANGLADESH

SHAMS EL ARIFEEN

1998-007 (Rev)

Table 2: Efficacy of RRV-TV vaccine by vaccine dose, location and features of rotavirus diarrhea

Location	Citation	Vaccine dose	Duration of follow up	Features of rotavirus diarrhea	Efficacy (%)
USA	Bernstein, 1995	4×10^4	2 seasons	- Severity score 9-14	59
				- Severity score 15-20	82 (ns)
				- Duration >3 days	92
Peru	Lanata, 1996	4×10^4	2 years	- Severity score ≥14 points	30 (ns)
				- With vomiting	40
				-≥6 liq stools/day	40
Brazil	Linhares, 1995	4×10^4	2 years	- Any rotavirus diarrhea	35
				- >5 stools/day	47
USA	Rennels, 1996	4×10^5	1 season	- Severity score >8 points	68
				- Severity score >14 points	80
USA	Santosham, unpublished	4×10^5	1 year	- Severity score >14 points	69
			2 year	- Severity score >14 points	44
Venezuela	Perez-Schael, 1997	4×10^5	19-12 months	- Severity score 9-14	47
				- Severity score 15-20	88
				- Duration >4 days	71

Childhood malnutrition is extremely prevalent in Bangladesh. It is likely that children will also be deficient in micronutrients like zinc, though empirical data are limited. Early unpublished data from studies done at ICDDR,B suggest that at least a third of the children are likely to be deficient in zinc defined as serum levels below 9.2 μmol/L (M.A. Wahed, personal communication). In neighboring India, 38-50% of children have been found to be zinc deficient (Sazawal, 1995; Sachdev 1988). Recent studies in India, Vietnam and Mexico have demonstrated a reduction in diarrhoea incidence following zinc supplementation (Sazawal, 1997; Ninh Xuan, 1996; Rosaldo, 1997). Another study from Bangladesh has demonstrated a reduction in the severity and duration of diarrheal episodes when zinc is given during the episodes (Roy, 1997).

Zinc deficiency is associated with impaired immune functions as evident by (i) decreased number of T-cells, (ii) altered T-helper and suppressor cell functions, (iii) depressed delayed hypersensitivity reaction, (ii) deficiency in natural killer cells and antibody dependent cytotoxicity (Cunningham-Rundler, 1979; Acar, 1983; Frost, 1977). Studies of the effect on humoral or secretory immunity are limited in number and results have been inconsistent. In some studies, depressed Ig levels and reduced or unaffected response to B-cell-specific mitogens have been seen (Cunningham-Rundler, 1979). Animal studies have also reported diminished counts of splenic plaque-forming cells, an index of humoral immune stimulation, in response to inoculation with sheep RBC (Beisel, 1982). A more recent study did not, however, detect any difference in antibody titers to a range of antigens including rotavirus between zinc deficient and sufficient children (Brussow, 1995). These findings suggest that infants in developing countries may respond poorly to diseases or to live attenuated vaccines due to impaired immune functions secondary to zinc deficiency.

The higher titer (4×10^5 pfu) formulation of RRV-TV has been shown to be safe, immunogenic and efficacious in the US and Venezuela. We hypothesize that in zinc deficient populations, zinc supplements are likely to improve the immune response to this vaccine, and hence it will be more efficacious.

Continuation Sheet (Number each sheet consecutively)

Significance and Rationale:

This study will help answer the question whether the observed poor immune response and efficacy of RRV-TV rotavirus vaccine in developing countries is due to nutritional deficiencies and whether the immune response can be enhanced by improving the zinc status of the infants. The improved immune response should translate into higher efficacy since the vaccine is very immunogenic and efficacious in developed countries. The availability of an efficacious vaccine against rotavirus infection, even if contigent on simulatanous zinc supplementation, would go a long way in reducing severe morbidity and mortality due to diarrhoea. If zinc supplmentation is found to improve the immune response to rotavirus vaccine, follow-up studies would be needed to assess correct dosage of zinc and approporiate delivery strategies. Effects on the response to other common childhood vaccines can also be investigated.

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).**

Study design

This will be a randomized 3-cell double-blind, placebo-controlled safety and immunogenecity trial of the RRV-TV vaccine. Children will be randomized to one of the following three groups: (i) RRV-TV and zinc supplement, (ii) RRV-TV and placebo for zinc, and (iii) placebos for vaccine and zinc. The placebos will look and taste exactly like the vaccine or supplement. Three doses of vaccine or placebo will be given at 6-8, 10-12 and 14-16 weeks of age along with the routine immunizations. Zinc supplements or placebos will be given daily (5 mg/day) starting 2 weeks before the first dose until 4 weeks after the third dose when the last blood sample is obtained.

Selection criteria:

Inclusion criteria:

1. Infants of either sex living in the Matlab MCH-FP intervention area who are 4-6 weeks old at the start of supplementation.
2. Voluntary written consent given by at least one parent or guardian.

Exclusion criteria:

Infants will be not be enrolled if they:

1. Die prior to enrollment or no longer live in the study area at the time of the enrollment visit.
2. Are older than 6 weeks of age at the time of assignment of identification number by ICDDR,B's DSS.
3. Have clinically significant chronic disease
4. Have a immunosuppressed household member
5. Are enrolled in any other concurrent vaccine or nutritional supplementation trial

Study outcomes:

1. The main outcome will be a four-fold rise in anti-rotavirus IgA titer against rhesus rotavirus strains. However, increase in titers of neutralization antibodies to RRV will also be measured.
2. Proportion of subjects that shed rotavirus at 4, 7 and 10 days after each dose.
3. Serum zinc levels in blood samples collected before the first vaccination (i.e., 2 weeks after start of zinc supplementation) and 4 weeks after the third dose.
4. Assessment of vaccination safety by interviewing the caretaker and measuring the rectal temperature at 4 and 7 days following each vaccination. Questions will be asked about gastrointestinal symptoms (cramps, anorexia, vomiting, stool frequency and consistency) and respiratory symptoms (cough, runny nose and wheezing) for each of the first 7 post-vaccination days after each dose.

Sample size

It is assumed that the IgA seroconversion in the placebo cell will be 20%. We make further assumptions that 50% of the vaccine only and 80% of the vaccine and zinc infants will seroconvert after 3 doses. These are revised estimates and are slightly different from the estimates used in the original study

design. A sample of 45 infants will be required in each cell to detect these differences with a significance level of 0.05 and power of 80%. Assuming 10% lost-to-follow-up, this translates into (45+5)=50×3=150 infants. This is almost double the total sample originally planned for.

In Matlab, an average of 225 births occur each month. Assuming that 20% of the infants can not be enrolled due to delayed identification (age>6 weeks), and 20% refusal, about 144 infants should be available for enrollment per month. Therefore, most of the enrollment can hypothetically be completed in 1-2 a months. However, it is anticipated that enrollment will be restricted to villages closer to the Matlab Hospital to simplify logistics and reduce travel time. This will, however, lengthen the time of enrollment.

Randomization procedure

Once enrolled infants are assigned their unique DSS identification numbers and their parents/caretakers agree to participate, they will be assigned a study ID number which will indicate whether the infant will receive vaccine+zinc, vaccine only, or only placebo. The numbers will be assigned by sequentially proceeding down a pre-printed list of numbers. These numbers will be randomized in blocks of 6 to assure an equal number of subjects in each study group at any given time. The person assigning the number will not be involved in the care of the infant or in the measurement of outcomes. The numbers on the vaccine, zinc and placebo vials will correspond to this number. All dates of zinc/placebo supplementation and vaccine/placebo administration will be documented.

Study vaccine, zinc supplements and placebos

Study vaccine and the vaccine placebo has already been described. The zinc supplement group will receive zinc acetate providing elemental zinc 5 mg/day daily for about 14 weeks. This dose is equivalent to the Recommended Daily Allowance for infants (Behrman, 1992) and was chosen to achieve maximal benefits from safe and acceptable levels of supplementation. Infants have been given up to 10 mg of zinc daily in previous studies in ICDDR,B with no reported adverse effects. The supplement or the corresponding placebo will be prepared in 40 ml bottles containing 8 doses of 5 ml (5 mg of elemental zinc) each and pre-labeled according to the randomization schedule. They will be sent to the respective community volunteers after being labeled again with the child's name, mother's name and DSS number and Study ID number.

Procedures for administering vaccine/placebo and zinc/placebo

The procedures for administering the vaccine and the corresponding placebo have been described previously. Briefly, infants will be brought to the Matlab Hospital by the Health Assistant on the day of vaccination. Blood samples will be collected prior to the administration of the vaccine or placebo. The vaccine or placebo will be given orally using a small needle-less syringe. Other EPI vaccines will also be administered to all infants.

Daily zinc/placebo supplementation will be done by community volunteers specifically selected for this study. It is estimated that one community volunteer can be given the responsibility of 2 infants who they will visit daily. Each morning, the community volunteer will visit the two infants and personally feed them the supplement or placebo from the vial labeled with the name of the child using a small syringe without a needle. The volunteers, who will need to be literate, will keep simple records of their activities, i.e., daily supplementation status of each child. One Research Officer will be responsible for supervising and resupplying the volunteers once a week and collecting the volunteer records.

Withdrawal from the study

An enrolled infant's parents may refuse to participate at any point. This will in no way affect their ability to seek care at the ICDDR,B health facilities.

Follow-up of study infants

Stool sample collection has been described previously. In addition to the previously proposed two samples at 4 and 7 days after each dose, another sample will be collected at 10 days. Four blood samples will be collected by trained nurses at the Matlab Hospital at the time of vaccinations (3 doses) and 4 weeks after the third dose. The blood samples at the second and third doses will be collected according to previously defined procedures and will be used only for serological tests.

The two blood samples at the first dose and 4 weeks after the third dose will be used for both serology and assessment of plasma zinc. These two samples will be collected by venupuncture using trace mineral free plastic syringes and stainless steel syringes and placed in heparinized (50 IU/ml) plastic tubes. The tubes and syringes will be rendered trace mineral-free by overnight soaking in 50% nitric acid, rinsed (3 times) with doubly deionized water and allowed to drain dry. Plasma will be immediately separated by centrifugation (600 × g for 5 min) and split into 2ml for serology and 200μl for assessment of zinc. Both samples will be immediately frozen to -20°C until analysis. Samples will be transported once a week to the ICDDR,B Dhaka Laboratories for testing purposes.

Information on socio-demographic characteristics, morbidity, immunization and other information on the study subjects will be available from the routine surveillance of this population.

Laboratory assessment

The laboratory investigations for assessing serological response to the vaccine has been described previously. However, assays for neutralization antibodies will only be performed for RRV.

Assessment of serum zinc

Assays of plasma zinc will be performed at the ICDDR,B Biochemistry Laboratory, where the methods are already well established. An atomic absorption spectrophotometer will be used for the estimation of plasma zinc concentration. All infants in the zinc supplemented group and 50% of the infants in the other two groups, who will all receive a placebo for zinc, will be tested twice for plasma zinc, once at the time of the first dose and again 4 weeks after the third dose.

Surveillance for adverse reactions

The CHWs will visit the enrolled infants at 4 and 7 days after vaccination to ask the caretaker about adverse events or reactions using a standard reporting form (Adverse Reaction questionnaire). Information will be collected for each of the 7 days following vaccination. A rectal temperature will also be taken and recorded in the form.

Data management

Questionnaires and data collection forms will be edited at Matlab and transported to Dhaka once a week. All data entry will be done in Dhaka. One Data Management Assistant will be assigned for data entry and cleaning. Data will be analyzed by the Principal Investigator.

Time table

Enrollment will be completed within 4 months of the start of the study. Assuming that newborns are identified within 4-6 weeks after birth, supplementation starts 2 weeks prior to the first dose at 6-8 weeks, 3 doses are given about 4 weeks apart, and the last blood sample is collected 4 weeks after the third dose, a total of about 8 months will be needed to complete follow-up of all infants. It is expected that preliminary laboratory testing can be completed in about 2 months and a preliminary report of results will be prepared in another 2 month. Thus, the entire study can be completed in about 12 months.

Safety considerations:

In previous trials, the RRV-TV vaccine has been associated with small but significant excess of mild to moderate fever, usually occuring 3-4 days after the first dose of the vaccine and which was only detected by measurement and not by caretakers. Some studies have also reported higher rates of reduced appetite, irritability and decreased activity following the first dose. There has been no evidence of higher risk of vomiting and diarrhoea among the vaccinated.

Zinc supplementation, in the small doses to be given in the proposed study, is not associated with any adverse effect. Sterile non-touch techniques will be used to collect blood samples to reduce the risk of infection through that route.

The frequent observations of the subject infant and the easy availability of ICDDR,B clinical facilities will result in early identification and management of adverse effects, if any.

Study significance and benefits:

Enrolled infants in the vaccine groups will directly benefit from the rotavirus vaccine. Treatment of the enrolled infants will also be ensured. The results of this study will help contribute to the further evaluation of the use of the RRV-TV vaccine which has the potential of significantly reducing the risk of rotavirus diarrhoea among children.

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 equipments 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).**

The Matlab field study area offers excellent facilities for this study. The Demographic Surveillance System (DSS), with regularly updated demographic information on a 210,000 population will serve as an excellent sampling frame for the study as approporiate individuals can be easily identified and selected. ICDDR,B has established means of communication within Matlab based on road and river transport. The existing field data collection staff offers a pool of higly experienced and trained individuals to choose from.

The Matlab hospital has the facilities and staff to collect and store blood and stool samples. Study subjects in need of treatment can be treated there or referred to other facilities. The twice a day shuttle between Matlab and Dhaka will ensure that the samples and data forms reach Dhaka on time. The ICDDR,B laboratories in Dhaka are equipped and have the experience with most of the laboratary tests. Facilities for neutralization assays will be set-up there with technical assistance from CDC. The Dhaka-based data processing unit of the Public Health Sciences Division will be expanded, if necessary, to meet the needs of this study.

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).**

Baseline features will be examined to assess the randomization. The proportion of infants with reported adverse reaction will be compared for each dose for each of the first 7 days after vaccination. Two serologic measures (proportion with $\geq$4-fold increase in titer and geometric mean titers) will be compared between the three groups. The mean plasma zinc levels will be compared between groups at baseline and after the last dose of vaccine.

The proportions will be compared using χ^2-tests and rate ratios (95% confidence intervals). The geometric mean titers (GMT) and plasma zinc levels will be compared using non-parametric tests. Multiple regression analysis will be done to simultaneously adjust for different factors.

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.

The RRV-TV rotavirus vaccine, designed for preventing human rotavirus infection, can only be evaluated in healthy humans. Very young infants will be enrolled as the risk of the disease is greatest in that age group (<2 years).

Infants will only be enrolled after their parents have been given a full explanation of the study and they have understood the implications of participating in this study, and have agreed to participate in writing. ICDDR,B will ensure that any enrolled infant in need of treatment is offered treatment if it is available in the Matlab Hospital. For other conditions, the infants will be referred to the Chandpur Government Hospital and assistance provided for travel.

Confidentiality of collected information will be maintained by keeping all data forms private and locked at the Matlab Diarrhoea Hospital and the ICDDR,B Dhaka Offices with access limited to those working in the study. Study subjects will only be identified by study numbers in the computer databases used for analysis.

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.

No animal will be used in this 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.

Acar S, Ersey F, Sanil O, et al (1983) Cell-mediated immunity, serum and lymphocyte zinc levels in Hodgkin's disease, In: Prasad AS eds. Zinc deficiency in human subjects. New York: Alan R. Liss. 255-60.

Baqui AH, Black RE, Arifeen SE, Hill, K, Mitra SN and Sabir AA. Causes of childhood deaths in Bangladesh: results of a nation-wide verbal autopsy study. Bull WHO (in press).

Beisel WR (1982). Single nutrients and immunity. Am J Clin Nutr. 35 suppl:417-468.

Bernstein DI, Glass RI, Rodgers G, et al (1995). Evaluation of rhesus rotavirus monovalent and tetravalent reassortant vaccines in US children. JAMA 273:1191-6.

Black RE, Brown KH, Becker S, et al (1982). Longitudinal studies of infectious diseases and physical growth of children in rural Bangladesh. Am J Epidemiol. 115:315-24.

Bingnan F, Unicomb L, Rahim Z, Banu NN, Podder G, Clemens J, Van Loon FPL, Rao MR, Malek A, Tzipori S (1991). Rotavirus-associated diarrhea in rural Bangladesh: two-year study of incidence and serotype distribution. J Clin Microbiol. 29:1359-1363.

Brussow H, Sidoti J, Dirren H, Freire WB (1995). Effect of malnutrition in Ecuadorian children on titers of serum antibodies to various microbial antigens. Clin Diagnostic Lab Immun. 2:62-68.

Cunningham-Rundler C, Cunningham-Rundler S, Garafolo J (1979). Increased T-lymphocyte function and thymopoietin following zinc repletion in man. Fed Proc. 38:1222.

de Zoysa I, Feacham RV (1985). Interventions for the control of diarrhoeal diseases among youngf children: rotavirus and cholera immunization. Bull WHO.63:569-83.

Frost P, Chen JC, Rabbani I, et al (1977). The effect of immune deficiency on the immune response. In: Brewer GJ, Prasad AS, eds. Zinc metabolism: current aspects in health and disease. New York: Alan R. Liss. 143-50.

Lanata CF, Midthun K, Black RE, et al (1996). Safety, immunogenecity, and protective efficacy of one and three doses of the tetravalent rhesus rotavirus vaccine in infants in Lima, Peru. J Infect Dis. 174:268-75.

Linhares AC, Gabbay YB, De Freitas RB, et al (1995) Immunogenecity, safety and efficacy of rhesus-human reassortant rotavirus (RRV-tetravalent) vaccine in Belem, Brazil. Presented at the Fifth Rotavirus Vaccine Workshop, Atlanta, Georgia, U.S.A., October 16-17.

Ninh Xuan N, Thissen J, Cillette L, et al (1996) Zinc supplementation increases growth and circulating insulin-like growth factor I (IGF-I) in growth retarded Vietnamese children. Am J Clin Nutr. 63:514-9.

Perez-Schael I, et al. (1996). RRV-TV efficacy in Venezuela, World Pediatric Congress, Mexico City, Mexico, December 1996.

Rennels MB, Glass RI, Dennehy PH, et al (1996). Safety and efficacy of high-dose rhesus-human reassortant rotavirus vaccines- report of the national multicenter trial. Pediatr. 97:7-13.

Rosaldo JL, Lopez P, Munoz, E, et al (1997). Zinc supplementation reduces morbidity, but neither zinc nor irion supplementation affected growth or body composition of Mexican preschoolers. Am J Clin Nutr. 65:13-19.

Roy SK, Tomkins AM, Akramuzzaman SM, Behrens RH, Haider R, Mahalanabis D, Fuchs G (1997). Randomised controlled trial of zinc supplementation in malnourished Bangladeshi children with acute diarrhoea. Arch Dis Child. 77:196-200.

Sachdev HPS, Mittal NK, Mittal SK, et al (1988). A controlled trial on utility of oral zinc supplementation in acute dehydrating diarrhoea in infants. J Pediatr Gastr Nutr. 7:877-81.

Salway SM, Nasim SM (1994). Levels, trends and causes of mortality in children below 5 years of age in Bangladesh: findings from a national survey. J Diarrhoeal Dis Res. 3:187-93.

Sazawal S, Black RE, Bhan MK, et al (1995). Effect of zinc supplementation during acute diarrhoea on duration and severity of the episode - a community based, double-blinded controlled trial. N Eng J Med. 333:839-44.

Sazawal S, Black RE, Bhan MK, et al. (1997). Efficacy of zinc supplementation in reducing the incidence and prevalence of acute diarrhoea - a community based, double-blinded controlled trial. Am J Clin Nutr. 66:413-8.

Unicomb LE, Kilgore PE, Faruque ASG, Hamadani JD, Fuchs GJ, Albert J, Glass RI (1997). Anticipating rotavirus vaccines: hospital based surveillance for rotavirus diarrhoea and estimates of disease burden in Bangladesh. Pediatr Inf Dis J. 16:947-51.

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 findings from this study will be published in internal (institutional) publications and peer-reviewed journals, and disseminated in in-country and international conferences. The study is not linked to any Government of Bangladesh activity at this time.

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)**

ICDDR,B will collaborate with the Johns Hopkins University, USA for technical assistance and funding support (from USAID funding to JHU). An agreement will be signed when the funding arrangements have been finalized. Technical assistance will also be available from the Centers for Disease Control and Prevention (CDC), USA as part of the agreement for the originally proposed safety and immunogenecity trial.

Biography of the Investigators

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
Shams El Arifeen	Epidemiologist Child Health Programme, PHSD, ICDDR,B	November 29, 1959

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

Institution and Location	Degree	Year	Field of Study
Dhaka Medical College, University of Dhaka, Dhaka Bangladesh	M.B.B.S.	1983	Medicine
Johns Hopkins University School of Hygiene and Public Health, Baltimore, USA	M.P.H.	1991	Epidemiology
Johns Hopkins University School of Hygiene and Public Health, Baltimore, USA	Dr.P.H.	1997	International Health

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).**

Nov'1983 - Nov'1984: **In-service Trainee:** Dhaka Medical College Hospital, Dhaka, Ministry of Health & Family Welfare, Government of Bangladesh;

Nov'1984 - Jul'1986: **Medical Officer:** Dhaka Medical College Hospital; Tongi Thana Health Complex; and Tangail Sadar Upazila, Ministry of Health & Family Welfare, Government of Bangladesh

Jul'1986 - Jul'1992: **Medical Officer (*In-charge, Urban Section*):** Expanded Programme on Immunization (E.P.I.) Project, Dhaka, Ministry of Health & Family Welfare, Government of Bangladesh

Aug'1992 - Sep'1992: **Consultant:** Community Health Division, International Centre for Diarrhoeal Disease Research, Bangladesh

Oct'1992 - Sep'1994: **Research Investigator/Senior Research Investigator:** Urban Health Extension Project/MCH-FP Extension Project (Urban), International Centre for Diarrhoeal Disease Research, Bangladesh;

Sep'1994 - Oct'1997: **MCH-FP Program Specialist:** MCH-FP Extension Project (Urban)/Operations Research Project, International Centre for Diarrhoeal Disease Research, Bangladesh

Oct'1997 - current: **Epidemiologist:** Child Health Programme, Public Health Sciences Division, International Centre for Diarrhoeal Disease Research, Bangladesh;

Professional Organizations:

Bangladesh Medical and Dental Council 1984-

Delta Omega Honorary Public Health Society Alpha Chapter 1991-

Continuation Sheet (Number each sheet consecutively)

Research and Other Experience:

- Experience in epidemiologic, operations, and qualitative research methods - design, implementation, and analysis:
  - Cross-sectional study assessing the prevalence and risk factors of household injury among Baltimore City residents (1990)
  - Longitudinal study on the effects of birth weight, intrauterine growth retardation and prematurity on infant growth and survival in the slums of Dhaka, Bangladesh (1993-1997)
  - The delivery of a basic service package of primary health care services from urban clinics - evaluation of quality and access (1996-1997)
  - Safety of vitamin A supplementation in infancy using immunization contacts
  - Validation of the clinical case definition of measles and evaluation of the efficacy of different Measles vaccines
  - Immunization coverage evaluation surveys in urban and rural areas of Bangladesh (1987-1990)
  - Formative evaluation using qualitative research methods for a proposed health and sanitation project in Greater Chittagong district (1991)
- Teaching Assistant, Course titled **"Management of Health Systems in Developing Countries"**, 1991-92 academic year. School of Hygiene & Public Health, Johns Hopkins University, Baltimore, Maryland, USA.

Publications:

- Baqui AH, Black RE, Arifeen SE, Hill, K, Mitra SN and Sabir AA. **Causes of childhood deaths in Bangladesh: results of a nation-wide verbal autopsy study**. Bull WHO (in press).
- Baqui AH, Arifeen SE, Amin S & Black RE. **Levels and Correlates of Maternal Nutritional Status in Urban Bangladesh** European Journal of Clinical Nutrition, 1994;48:349-57
- Baqui AH, de Francisco A, Arifeen SE, Siddique AK, Sack RB. **Bulging fontanelle after supplementation with 25,000 IU of vitamin A in infancy using immunization contacts**. Acta Paediatrica. 1995;85:863-6
- Arifeen SE, Mahbub AQM, (editors). **A Survey of Slums in Dhaka Metropolitan Area - 1991**. October 1993. (ICDRR,B Working Paper No. 39) (Urban MCH-FP Working Paper No. 11).

Papers under Preparation

- Arifeen SE, Black RE, Antelman G, Baqui AH. **Infant Growth Patterns in the Slums of Dhaka in Relation to Birth Weight, Intrauterine Growth Retardation and Prematurity**
- Arifeen SE, Black RE, Antelman G, Nahar Q, Alamgir S, Mahmud H, Baqui AH. **Determinants of Infant Growth in the Slums of Dhaka: Size and Maturity at Birth and Breastfeeding**
- Arifeen SE, Black RE, Antelman G, Nahar Q, Mahmud H, Alamgir S, Baqui AH. **Effect of Birth Weight, Intrauterine Growth Retardation and Prematurity on Infant Survival: a Prospective Study in the Slums of Dhaka, Bangladesh**
- Arifeen SE, Black RE, Antelman G, Baqui AH. **Exclusive Breastfeeding Reduces ARI and Diarrhoea Deaths Among Infants in Dhaka Slums**
- Arifeen SE, Kane T, Amin S, Baqui AH. **Situation Analysis of Clinic-based FP and MCH Services in Dhaka City: Service Availability, Functioning and Quality**
- Arifeen SE, Baqui AH. **Vitamin A Supplementation in the First 6 Months of Life: Does it Reduce Diarrhoea and ARI Morbidity?**

Biography of the Investigators

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
Mohammad Yunus	Scientist and Head, Matlab Health Research Programme, PHSD, ICDDR,B	January 5, 1945

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

Institution and Location	Degree	Year	Field of Study
Dhaka Medical College, University of Dhaka, Dhaka Bangladesh	M.B.B.S.	1968	Medicine
London School of Hygiene and Tropical Medicine, London, UK	M.Sc.	1982	Community Health in Developing Countries

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).**

Nov'1968 - Sep'1976: **Physician:** Cholera Research Laboratory, Bangladesh

Oct'1976 - Apr'1978: **Deputy Chief Physician:** Cholera Research Laboratory, Bangladesh

Apr'1978 - Mar'1980: **Physician-in-charge:** Cholera Research Laboratory and International Centre for Diarrhoeal Disease Research, Bangladesh

Mar'1980 - Oct'1983: **Head:** Matlab Station, International Centre for Diarrhoeal Disease Research, Bangladesh

Oct'1983 - Sep'1985: **Coordinator:** Matlab Station and MCH-FP Extension Project, International Centre for Diarrhoeal Disease Research, Bangladesh

Sep'1985 - Dec'1996: **Coordinator:** Matlab Health and Research Centre, International Centre for Diarrhoeal Disease Research, Bangladesh

Dec'1996 - present: **Head:** Matlab Health (Services) Research Programme, International Centre for Diarrhoeal Disease Research, Bangladesh

Professional Organizations:

Bangladesh Medical Association	1972-
Bangladesh Association for the Advancement of Science	1979-
National Anti-Tuberculosis Association	1979-
Public Health Association of Bangladesh	1980-
Nutrition Society of Bangladesh	1991-
Bangladesh Population Association	1993-
Bangladesh Environmental Society	1994-

Continuation Sheet (Number each sheet consecutively)

Selected Recent Publications:

Islam MS, Hasan MK, Miah MA, Yunus M, Zaman K, Albert MJ. **Isolation of Vibrio cholerae O139 synonym Bengal from the aquatic environment in Bangladesh: implications for disease transmission.** Appl Environ Microbiol 1994 May;60(5):1684-1686.

Clemens J, Rao M, Ahmed F, Ward R, Huda S, Chakraborty J, Yunus M, Khan MR, Ali M, Kay B, et al. **Breast-feeding and the risk of life-threatening rotavirus diarrhea: prevention or postponement?** Pediatrics 1993;92:680-685.

Baqui AH, Sack RB, Black RE, Chowdhury HR, Yunus M, Siddique AK. **Cell-mediated immune deficiency and malnutrition are independent risk factors for persistent diarrhea in Bangladeshi children.** Am J Clin Nutr 1993;58:543-548.

de Francisco A, Chakraborty J, Chowdhury HR, Yunus M, Baqui AH, Siddique AK, Sack RB. **Acute toxicity of vitamin A given with vaccines in infancy.** Lancet 1993;342:526-527.

Islam MS, Hasan MK, Miah MA, Qadri F, Yunus M, Sack RB, Albert MJ. **Isolation of Vibrio cholerae O139 Bengal from water in Bangladesh.** Lancet 1993;342:430.

Henry FJ, Briend A, Fauveau V, Huttly SA, Yunus M, Chakraborty J. **Gender and age differentials in risk factors for childhood malnutrition in Bangladesh.** Ann Epidemiol 1993;3:382-386.

Henry FJ, Briend A, Fauveau V, Huttly SR, Yunus M, Chakraborty J. **Risk factors for clinical marasmus: a case-control study of Bangladeshi children.** Int J Epidemiol 1993;22:278-283

Yunus M, Aziz KMA, Islam MS. **Perceptions on health and disease in the Matlab Community. In: Fauveau V, ed. Matlab: Women, Children & Health.** ICDDR,B Special Publication No. 35. July 1994; 257-274.

Huq A, Colwell RR, Chowdhury AMR, XU B, Moniruzzaman M, Islam MS, Yunus M, Albert MJ. **Coexistence of Vibrio cholerae 01 and 0139 Bengal in plankton in Bangladesh (letter).** Lancet 1995; 345:1249.

Clemens J, Rao M, Sack D, Ahmed F, Khan MR, Chakraborty J, Kay B, Huda S, Yunus M, Van Loon F, Svennerholm A-M, Homgren J. **Impaired immune response to natural infection as a correlate of vaccine failure in a field trial of killed oral cholera vaccine.** Am J Epidemiol 1995; 142: 759-64.

Zaman K, Baqui AH, Yunus M, Sack RB, Bateman OM, Chowdhury HR, Black RE. **Acute respiratory infections in children: a community based longitudinal study in rural Bangladesh.** J Trop Pediatric 1997; 43:133-7.

Zaman K, Baqui AH, Yunus M, Sack RB, Bateman OM, Chowdhury HR, Black RE. **Association between nutritional status, cell-mediated immune status and acute lower respiratory infections in Bangladeshi children.** Eur J Clin Nutr 1996; 50:309-314.

Yunus M, Aziz KMA, Zaman K. **Message for parents: Diarrhoea.** Child Health Dialogue 4th Quarter, 1996;5:5.

Sack RB, Rahman M, Yunus M, Khan EH. **Antimicrobial resistance in organisms causing diarrhoeal diseases.** Clin Infect Dis 1997; 24(1 suppl): S102-5.

Biography of the Investigators

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

1. Surname: BRESEE — Date of birth:

   First name(s): JOSEPH — Nationality: USA

2. Degree(s) (subjects, university or school, year)

1986-1990 M.D., Baylor College of Medicine, Houston, TX
1982-1986 B.A., Philosophy, University of Texas, Austin, TX

3. Most recent posts held (type of post, institution/authority, dates)

1995 present

Medical Epidemiologist, Respiratory and Enteric Virus Branch
Division of Viral and Rickettsial Diseases
National Center for Infectious Diseases
Centers for Disease Control and Prevention, Atlanta, GA

4. Recent publications: list only the five most important and relevant publications over the last five years (papers in press or submitted for publication are also acceptable).
   Please give full bibliographic references (author(s), title, journal, volume, page numbers, year)

Bresee JS, Mast EE, Coleman PJ, et al. Hepatitis C virus infection associated with intravenous immune globulin: a cohort study. JAMA 1996; 276:1[illegible]3-7.

Bresee JS, Fischer MA, Dowell SF, et al. Vitamin A therapy for respiratory syncytial virus infection-a randomized, placebo-controlled trial in the United States. Pediatr Infect Dis J 1996;15:777-81.

Dowell SF, Papic L, **Bresee, JS**, et al. Treatment of respiratory syncytial virus infection with vitamin A: a randomized, placebo-controlled trial in Santiago, Chile. Pediatr Infect Dis J 1996;15:782-6.

Bresee JS, Glass RI, Gentsch JL, Ivanoff B. Current status and future priorities for rotavirus vaccine development, evaluation, and implementation in developing countries. Report for the Consensus Workshop: Rotavirus Vaccines for Immunization of Children in Developing Countries, World Health Organization, Geneva, 9-10 January, 1997

Bresee JS, Glass RI. Astroviruses, enteric adenoviruses, and other gastroenteritis viruses. Tropical Infectious Diseases: Principles, Pathogens, and Practice. 1997 Guerrant RL, Krogstad, DJ, Maguire, JH, Walker, DH, Weller, PF eds., Churchill Livingstone, Geneva, Switzerland (in press)

Biography of the Investigators

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
Abdullah H Baqui	Senior Epidemiologist & Head Child Health Programme, PHSD, ICDDR,B	March 31, 1953

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

Institution and Location	Degree	Year	Field of Study
Dhaka Medical College, University of Dhaka, Dhaka Bangladesh	M.B.B.S.	1976	Medicine
Johns Hopkins University School of Hygiene and Public Health, Baltimore, USA	M.P.H.	1985	International Health
Johns Hopkins University School of Hygiene and Public Health, Baltimore, USA	Dr.P.H.	1990	Public Health/Epidemiology

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).**

1977-1978 Medical Intern, Dhaka Medical College, Dhaka, Bangladesh
1978-1981 Medical Officer, Matlab Health Research Station, ICDDR,B, Bangladesh
1981-1987 Physician-in-Charge, Clinical Services, Matlab Health Research Station, ICDDR,B
1987-1990 Senior Medical Officer/Assistant Scientist, Department of Epidemiology, ICDDR,B
1990-1994 Head, Research and Evaluation, Urban Health Extension Project, ICDDR,B
1994-1994 Associate Project Director, Urban Health Extension Project, ICDDR,B
1990-1998 Research Associate/Assistant Scientist, Dept. of International Health, Johns Hopkins University
1994-1997 Project Director, MCH-FP Extension Project (Urban)/Operations Research Project, ICDDR,B
1994-present Principal Investigator, RISC Project, ICDDR,B
1998-present Sr. Epidemiologist and Head, Child Health Programme, PHSD, ICDDR,B and Adjunct Assistant Professor, Dept. of International Health, Johns Hopkins University

Selected Publications:

Baqui AH, Black RE, Sack RB, Yunus M, Siddique AK and Chowdhury HR. "Epidemiologic and clinical characteristics of Acute and Persistent Diarrhoea in Rural Bangladeshi Children." Acta Pediat Scand Suppl 381:15-21, 1992

Baqui AH, Sack RB, Black RE, Yunus M, Haider K, Alim ARM, Siddique AK. "Enteropathogens associated with Acute and Persistent Diarrhoea in Rural Bangladeshi Children". The Journal of Infectious Disease 1992; 166:792-6

Continuation Sheet (Number each sheet consecutively)

Baqui AH, Black RE, Sack RB, Chowdhury HR, Yunus M, Siddique AK. Malnutrition, Cell-Mediated immune deficiency and diarrhoea: A community-based longitudinal study in rural Bangladeshi children. Am J Epidemiol 1993; 137(3):355-65.

Baqui AH, Black RE, Yunus M, Haque ARMA, Chowdhury HR, and Sack RB. "Methodologic Issues in Diarrhoeal Diseases Epidemiology : Definition of Diarrhoeal Episodes." International Journal of Epidemiology 1991;20(4).

Baqui AH, Sack RB, Black RE et al. Malnutrition and cell-mediate immune deficiency are independent risk factors for persistent diarrhoea in Bangladeshi children. Am J Clin Nutr 1993; 58:453-8.

Baqui AH, Black RE, Mitra AK, Chowdhury HR, Zaman K, Fauveau V, Sack RB. Diarrhoeal diseases: The Matlab experience. In: Fauveau V. ed. Mother and child health in Bangladesh: What has been learned in Matlab. Dhaka: ICDDR,B 1993

Baqui AH, Yunus M, and Zaman K. "Community-Operated Treatment Centers Prevented Many Cholera Deaths". J Diar Dis Research 1984; 2(2): 92-98.

Baqui AH, Yunus M, Zaman K. "Surveillance of Patients attending a rural diarrhea hospital in Bangladesh". Tropical and Geographical Medicine 1991; 43(1-2):17-22.

Baqui AH, Zaman K, Yunus M, Mitra AK, Hossain KMB and Banu H."Epidemiological and Clinical Characteristics of Shigellosis in Rural Bangladesh". J Diar Dis Research 1988; 6(1):21-28.

Baqui AH, Arifeen SA, Amin S, Black RE. Levels and Correlates of Maternal Nutritional Status and Consequences for Child Survival in Urban Bangladesh. Eur J Clin Nutr 1994, 48,349-357

Baqui AH, Francisco A de, Arifeen SE, Siddique AK and Sack RB. Bulging fontanelle after supplementation with 25,000 IU vitamin A in infancy using EPI contacts. Acta Paed Scand 1995, 84:863-6

Detailed Budget for New Proposal

Project Title: Safety and immunogenecity of 4×10^5 pfu tetravalent Rhesus Rotavirus vaccine, with or without zinc supplementation in Matlab, Bangladesh

Name of PI:	Shams El Arifeen			
Protocol Number:	Name of Division: PHSD			
Funding Source: USAID/WHO	Amount Funded (direct): $34,481	Total: $43,101		Overhead (%): 25%
Starting Date:	March 1, 1998	Closing Date:		February 28, 1999
Strategic Plan Priority Code(s):	14 and 11			

Sl. No.	Account Description						US $ Amount Requested		
							1st Yr	2nd Yr	3rd Yr
	Personnel	Salary Support							
		Position	Effort%	#	Months	Salary			
	M.A. Wahed, Assoc. Scientist	NOC/11	5	1	12	1500.00	900		
	Research Officer	GS5/7	100	1	9	464.00	4,176		
	Research Officer-Lab	GS5/7	100	1	4	464.00	1,856		
	Nurse-midwife	GS4/7	100	1	2	356.00	712		
	Health Assistant	GS3/7	100	5	2	297.00	2,970		
	Sub Total						10,614		
	Local Travel						7,580		
	International Travel						0		
	Sub Total						7,580		
	Supplies and Materials (Description of items)			Subjects	# per Subject	Rate			
	Laboratory supplies						500		
	Office supplies						250		
	Zinc supplements			150	14	0.50	1,050		
	Sub Total						1,800		
	Interdepartmental Services								
	Plasma zinc assessment			100	2	3.50	700		
	Serum IgA ELISA			70	4	5.00	1,400		
	Neutralization Antibody Assay			70	4	10.00	2,800		
	Stool EIA/ELISA			70	4	5.00	1,400		
	Photocopy			150	20	0.05	150		
	Sub Total						6,450		
	Other Contractual Services						0		
	Female Attendent			1	2	85.00	170		
	Community Volunteer			75	8	11.11	6,667		
	Meals for study subjects			150	4	2.00	1,200		
	Sub Total						8,037		
	Other Operating Costs						0		
	Capital Expenditure						0		
	TOTAL DIRECT COST						34,481		

Note 1: The salary of the Principal Investigator (Shams El Arifeen) and 2-months salary of a Co-Investigator (Abdullah Hel Baqui) are paid for by JHU

Note 2: WHO has committed $18,000 for this study. The remainder is expected from USAID/Washington's CHR funds with JHU

C.R. 25.3.98
Dr. Budget Office

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 proposed additional budget for the activities outlined in this addendum is US$35,679. The costs of the investigators will be covered seperately. Overhead will be charged at the standard centre rate of 25%.

Personnel: One Research Officer (GS5) will be hired for 8 months to supervise and support the community volunteers. The additional time for staff hired for the original study has also been budgeted.

Travel: This includes the travel costs for the investigators and research officer, plus the additional travel costs of the health assistants who will be bringing the study subjects for vaccinations and blood sampling and will visit them for stool sample collection.

Supplies: This includes the cost of zinc supplements, and miscellaneous laboratory and other costs.

Interdepartmental: Costs of laboratory tests and photocopying of questionaires have been budgeted.

Other direct costs: For the implementation of the daily zinc supplementation, 75 community volunteers will be needed. They will be paid at Taka 500 (US$11.11) per month for 4 months each. The additional time of the female attendents have been budgted. The cost of meals to be provided to the caretakers of the study subject when they come to Matlab hospital has also been included.

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).**

None required

APPENDIX

International Centre for Diarrhoeal Disease Research, Bangladesh

Voluntary Consent Form (ENGLISH)

Title of the Research Project: Safety and immunogenecity of 4×10^5 pfu tetravalent Rhesus Rotavirus vaccine, with or without zinc supplementation in Matlab, Bangladesh

Principal Investigator: Shams El Arifeen

Before recruiting into the study, the study subject must be informed about the objectives, procedures, and potential benefits and risks involved in the study. Details of all procedures must be provided including their risks, utility, duration, frequencies, and severity. All questions of the subject must be answered to his/ her satisfaction, indicating that the participation is purely voluntary. For children, consents must be obtained from their parents or legal guardians. The subject must indicate his/ her acceptance of participation by signing or thumb printing on this form.

Investigators should seek consent from the mother, father or appropriate guardian using the following information, given as an explanation in Bangla, together with a copy of the printed Bangla version.

"Diarrhoea is a very serious disease in Matlab, which causes many deaths in young children. These children usually have loose, watery stools and vomiting which may last for several days. As you probably already know, most of these ill children can be treated by the community health workers but some children may die, particularly if they do not receive treatment early with Oral Rehydration Solution (ORS). We are investigating a new vaccine that has a very good chance of preventing some of these illnesses.

ICDDR,B is working with the World Health Organisation and some scientists from the Centers for Disease Control and Prevention (CDC) in the USA, with the permission of the Government of Bangladesh, to try and find out if this new vaccine will work here in Matlab. We are also trying to find out if the action of the vaccine can be improved by also giving small quantities of an esssential food component called zinc. To help us do this we are asking if you will allow your child to join this trial. If you agree, your baby will receive a small amount of solution with either with zinc or without zinc. A person assigned from your neighbourhood will come and visit you everyday to feed this solution to your child. This will continue from when the child is 1 month old till it is a about 4½ months. Your baby will receive the normal 3 injections with vaccines against diphtheria, pertussis and tetanus given by the EPI programme. Your baby will also be given 3 doses of either rotavirus vaccine or solution without vaccine (placebo) by mouth as a small amount of liquid. Neither you, your home healthcare worker, nor your study assistant will know if your child has received the rotavirus vaccine, zinc or the placebo, since they will have the same color and taste. You will have no choice of which you will receive (vaccine, zinc or placebo); this will be decided by chance, like the flip of a coin.

We will also need to follow your child until it reaches about 18 weeks old and we will need to take blood 4 times to see if the new vaccines are working well. We will collect 4ml (less than 1 teaspoonful) of blood frown the arm of your child before each vaccine dose and 4 weeks after the last dose. In addition, we will need to get stool samples 3 times after each dose of vaccine (we can get this from a dirty diaper).

If you allow your child to take part in this study, he or she may be protected from diarrhoea caused by rotavirus. Also, your family will receive health advice and lunch on days you come to the clinic, and free transport to and from the clinic.

The vaccine has been given to more than 7,000 infants all over the world, and we are asking about 150 children to take part in this study. In about 1 in every 5 children, the rotavirus vaccine causes a mild fever, which often happens 3-4 days after your child takes the vaccine and lasts less than one day. Some children also become fussy and don't eat well after getting the vaccine (less than one in 10). All of these side effects appear to occur only after the first of the three doses, and the vaccine should not cause any other serious problems. Drawing blood from your baby may cause some soreness and bruising over the area where the blood was drawn. If your child does get ill at any time, we will look

after him or her in your village or at the Matlab hospital. Since the vaccine contains a live virus, if someone in the household has severe health problems (like people being treated for cancer or people with AIDS), children in the same household should not be in the study. Also, if your child has severe health problems, they should not take part in the study.

Once you have agreed to have your child take part in the study, you can contact your home healthcare worker, , if you have any problems or questions. Also you may contact Dr. Muhammad Yunus or Dr. Joseph Bresee or Dr. Shams El Arifeen at the address and number below at any time:

ICDDR,B Matlab Hospital
Phone: 807485

You do not have to let your child take part in the study. It is completely voluntary, and if you decide not to take part in the study, it will not change the care your child or family receives in any way. Also, once you have agreed to take part in the study, you may remove your child from the study at any time without any penalty or change in the routine care your child receives. All records from this study kept at Matlab Diarrhoea Hospital in the Dhaka offices of ICDDR,B will be kept private and in a locked location. Only people doing the study will be able to look at them. Any study records that are taken from ICDDR,B will not have any of the names of families or children who took part in the study on them.

Do you have any questions? Yes No

Can your baby join in this trial? Yes No

We would like to be able to store any leftover blood and stool samples at ICDDR,B after the study is complete, and maybe test them as part of future research studies. If any of this future testing finds something important for your infant's health, we will try to let you know. At some future time we might ask to test your child's stored samples for a health problem that may run in your family. If so, we will tell you what that could mean and we would only test if you agree to it. Even if you agree to have your child's samples stored, you may ask to withdraw from this testing at any time in the future. You would do this by asking Dr Yunus at the Matlab Hospital to discard the blood or stool samples.

Do you agree to let ICDDR,B store and use your infant's samples in the event of future research?

Yes No

Signature of Interviewer/Investigator

Date:_____

Signature/Thumb Impression of Parents/Guardian

Date:_____

রোটাভাইরাস টিকা ও দস্তা গবেষণা সম্মতিপ্র

শিশুর নাম :

জন্ম তারিখ :

ডি, এস, এস নম্বর :

বাড়ী ও গ্রাম :

গবেষণায় শিশুদের অর্ন্তভুক্তির সময় গবেষকগনদের অবশ্যই বাংলায় লেখা নিম্নলিখিত তথ্যসম্বলিত সম্মতিপত্রটি শিশুদের মাতা, পিতা অথবা উপযুক্ত অভিভাবকদের পড়ে শোনাতে এবং বুঝিয়ে বলতে হবে।

"মতলবে ডায়ারিয়া খুবই মারাত্মক একটি রোগ যা অনেক ছোট শিশুদের মৃত্যুর কারণ। এই সকল শিশুদের সাধারণত: পাতলা পানির মত পায়খানা এবং বমি দেখা দেয় যা কয়েকদিন স্থায়ী হতে পারে। আপনি সম্ভবত জানেন, এই সকল রোগাক্রান্ত শিশুদের অধিকাংশই গ্রাম্য স্বাস্থ্য কর্মীদের দিয়ে চিকিৎসা করানো যায়। কিন্তু কিছু কিছু শিশু মারা যেতে পারে যদি তাহাদেরকে সময়মত বা শুরুতে খাবার স্যালাইন দিয়ে চিকিৎসা না করানো যায়। আমরা এই সকল রোগসমূহ প্রতিরোধ করতে পারে এমন একটি সম্ভাবনাময় নতুন টিকার উপর গবেষণার কাজ পরিচালনা করছে।

আই, সি, ডি, ডি, আর, বি বাংলাদেশ সরকারের অনুমোদন নিয়া বিশ্ব স্বাস্থ্য সংস্থ্যা ও যুক্তরাষ্ট্রের সি,ডি,সি-র কিছু সংখ্যক বিজ্ঞানী মতলবে এই টিকার কার্যকারীতা পরীক্ষার জন্য কাজ করছেন। দস্তা নামক খাদ্য উপাদান অল্প পরিমানে খাওয়ালে এই টিকার কার্যকারীতার উন্নতি হবে কিনা তাও পরীক্ষা করা হচ্ছে। এই কাজে সাহায্যের জন্য আপনার শিশুকে উক্ত পরীক্ষামূলক কার্যক্রমে অর্ন্তভূক্ত হতে দিবেন কিনা তা জানতে চাচ্ছি। আপনি রাজি হলে আপনার শিশুকে অল্প পরিমানে তরল পদার্থ খায়ানো হবে যাতে দস্তা থাকতে বা নাও থাকতে পারে। আপনার এলাকার একজন নির্ধারিত ব্যাক্তি প্রতিদিন এসে আপনার শিশুকে ইহা খাওয়াবেন। ইহা আপনার শিশুর ১ মাস বয়সে শুরু হয়ে ৪.৫ (সাড়ে চার) মাস বয়স পর্যন্ত চলবে । আপনার শিশুকে নিয়মিত ই, পি, আই কর্মসূচীর ডিপথেরিয়া, পারটোসিস ও টিটেনাস এর ৩ ডোজ প্রতিষেধক টিকা ইনজেকশন প্রদান করা হইবে । সেই সংগে আপনার শিশুকে ৩ ডোজ রোটাভাইরাসযুক্ত টিকা অথবা অল্প মাত্রার টিকাযুক্ত তরল জাতীয় পদার্থ খাওয়ানো হবে। আপনি, আপনার স্বাস্থ্য কর্মী বা এই গবেশণার কর্মী কেউই জানবেন না আপনার শিশু কি রোটাভাইরাসের টিকা, দস্তা বা টিকা/দস্তা বিহীন তরল পদার্থ পাবে কারণ এগুলো সব দেখতে ও খেতে একই রকম। আপনার শিশু এগুলির মধ্যে কি পাবে তাতে আপনার কোন হাত থাকবে না -- টস করার মত করে সিদ্ধান্ত নেয়া হবে।

আমরা আপনার শিশুকে ১৮ সপ্তাহ বয়স পর্য্যন্ত অনুসরণ এবং দেখা শোনা করব। এই নতুন টিকা সঠিকভাবে কাজ করছে কিনা তা দেখবার জন্য ৪ বার রক্ত নিয়ে পরীক্ষার প্রয়োজন হইবে। প্রতিবার টিকা দেয়ার পূর্বে শিশুর হাতের শিরা হতে ৪ মি: লি: (এক চা চামচের কম) রক্ত নেয়া হবে এবং শেষ টিকা দেয়ার ৪ সপ্তাহ পর আর একবার সমপরিমান রক্ত নেয়া হবে। সেই সংগে প্রতিবার টিকার পরে ৩ বার পায়খানার নমুনা পরীক্ষার জন্য নেয়া হবে (ময়লা কাপড় হইতেও পায়খানার নমুনা নেয়া যাবে)।

আপনার শিশুকে এই গবেষণায় অংশগ্রহণ করতে অনুমতি দিলে রোটাভাইরাস জড়িত ডায়রিয়া থেকে সে হয়ত নিরাপদে থাকবে । এছাড়া আপনি যে কদিন ক্লিনিকে আসা যাওয়া করবেন সে কদিন স্বাস্থ্য উপদেশ পাবেন, এবং বিনা খরচে ক্লিনিকে আসা যাওয়া ও দুপুরের খাওয়া পাবেন ।

এ যাবত এই টিকাটি পৃথিবী জুড়ে ৭,০০০ শিশুকে দেয়া হয়েছে এবং আমরা প্রায় ১৫০ শিশুকে এই গবেষণায় অংশগ্রহণ করাতে চাচ্ছি। এই রোটাভাইরাস টিকা দেয়ার ৩-৪ দিন পর প্রতি ৫ শিশুর মধ্যে ১ জনের অল্প জ্বর হতে পারে তবে তা ১ দিনের মধ্যে ভাল হয়ে যায় । টিকা নেয়ার পর কিছু শিশু খিটখিটে হতে পারে এবং খাওয়া নিয়ে বিরক্ত করতে পারে (১০ জনের মধ্যে ১ থেকে কম জনের এরকম হয়) । এই সমস্ত পার্শ্বপ্রতিক্রিয়া ৩টি টিকার শুধুমাত্র প্রথম টিকার পর হয়, এছাড়া এই টিকার অন্য কোন সমস্যা করার কথা নয় । রক্ত সংগ্রহ করার সময় ঐ স্থানে শিশুর সামান্য জ্বালা এবং ক্ষত হতে পারে । কোন সময় আপনার শিশু অসুস্থ হয়ে পড়লে আমরা আপনার শিশুকে আপনার বাড়ীতে অথবা মতলব হাসপাতালে তার চিকিৎসা করব। টিকাটিতে জীবিত ভাইরাস থাকার কারণে যদি বাসায় কারও মারাত্মক অসুস্থতা, যেমন ক্যান্সার চিকিৎসাধীন অথবা

এইডস রোগী, থাকে তবে তা সেই বাসার শিশুকে এই গবেষণার অর্ন্তভুক্ত করা হবে না । এছাড়া আপনার শিশুরও যদি কোনও মারাত্মক অসুস্থতা থাকে তবে তারও এই গবেষণায় অংশগ্রহণ করা উচিৎ হবে না ।

আপনি এই গবেষণায় আপনার শিশুকে অংশগ্রহণ করাতে সম্মত হওয়ার পর কোন অসুবিধা হলে বা প্রশ্ন থাকলে আপনি আপনার এলাকার স্বাস্থ্যকর্মীর সাথে যোগাযোগ করতে পারবেন । এছাড়া ডাঃ মোঃ ইউনুস, অথবা ডাঃ জোসেফ ব্রেসি অথবা ডাঃ সাম্‌স এল আরেফিনের সাথে নীচের ঠিকানায় যোগাযোগ করতে পারবেন -

আই সি ডি ডি আর, বি মতলব হাসপাতাল
ফোন নং ৮০৭৪৮৫

এই গবেষণায় আপনার শিশুর অংশগ্রহণ বাধ্যতামূলক নয় । এটা সম্পূর্ণ আপনার ইচ্ছার উপর নির্ভরশীল এবং আপনি অংশগ্রহণ না করার সিদ্ধান্ত নিলেও আপনার শিশু ও পরিবার যে সেবা পেয়ে এসেছে তার কোন পরিবর্তন হবে না । অংশগ্রহণে সম্মত হওয়ার পরও আপনি যে কোন সময় আপনি আপনার শিশুকে এই গবেষণা থেকে বের করে নিতে পারেন । এর ফলে আপনার কোন ক্ষতি বা আপনার শিশুর নিয়মিত সেবায় কোন পরিবর্তন হবে না । এই গবেষণার সকল কাগজপত্র মতলব ডায়রিয়া হাসপাতাল ও আই সি ডি ডি আর, বি ঢাকা অফিসে তালাবদ্ধ ও গোপন রাখা হবে । এই গবেষণায় কর্মরত লোকজন ছাড়া অন্য কারও এগুলি দেখার অধিকার থাকবে না। আই সি ডি ডি আর বি থেকে অন্য কোথাও এই গবেষণার কাগজপত্র ও তথ্য পাঠানো হলে তাতে আপনার পরিবারের ও শিশুর নাম থাকবে না।

আপনার কি কোন প্রশ্ন আছে? হ্যাঁ না।

আপনার শিশু এই গবেষণায় অংশগ্রহণ করতে পারবে? হ্যাঁ না।

এই গবেষণার কাজ শেষ হলে যদি রক্ত বা পায়খানা অবশিষ্ট থাকে তাহলে আমরা তা আই সি ডি ডি আর,বি -তে জমা রাখতে চাইব এবং ভবিষ্যতে অন্য গবেষণার কাজে সেগুলো হয়ত পরীক্ষা করব। ভবিষ্যতে এই পরীক্ষায় আপনার শিশুর স্বাস্থ্য সম্পর্কে গুরুত্বপূর্ন কিছু পাওয়া গেলে আমরা আপনাকে তা জানাবার চেষ্টা করব। ভবিষ্যতে আপনার শিশুর জমাকৃত রক্ত বা পায়খানায় এমন কোন স্বাস্থ্য সমস্যা নিয়ে আমরা পরীক্ষা করতে চাইতে পারি যা আপনার পরিবারে থাকতে পারে। এমন হলে আমরা সে সম্পর্কে আপনাকে বিস্তারিত বোঝাব এবং আপনার সম্মতি নিয়েই পরীক্ষা করব। আপনি এখন আপনার শিশুর রক্ত ও পায়খানা জমা রাখতে সম্মত থাকলেও ভবিষ্যতে যে কোন সময় এগুলোর উপর আরও পরীক্ষা থেকে আমাদেরকে বিরত রাখতে পারবেন। মতলব হাসপাতালের ডা: ইউনুসকে এই ব্যাপারে জানালেই জমাকৃত রক্ত ও পায়খানা ফেলে দেয়া হবে।

ভবিষ্যতে গবেষণার কাজে ব্যবহারের জন্য আপনার শিশুর রক্ত ও পায়খানা
জমা রাখতে আপনি কি আই সি ডি ডি আর,বি -কে অনুমতি দিচ্ছেন? হ্যাঁ না।

সাক্ষাৎকার গ্রহণকারীর/গবেষকের স্বাক্ষর
তারিখ :_____

পিতা/মাতা অথবা অভিভাবকের স্বাক্ষর/টিপসই
তারিখ :_____

Project Title: **Safety and immunogenecity of 4×10^5 pfu tetravalent Rhesus Rotavirus vaccine, with or without zinc supplementation in Matlab, Bangladesh**

Enrollment Log

Study ID#	Group	DSS ID#	Name	Father's Name	Village	Date of Birth	Date of Enrollment	Informed Consent (✓)
IMM-001						___/___/___	___/___/___	
IMM-002						___/___/___	___/___/___	
IMM-003						___/___/___	___/___/___	
IMM-004						___/___/___	___/___/___	
IMM-005						___/___/___	___/___/___	
IMM-006						___/___/___	___/___/___	
IMM-007						___/___/___	___/___/___	
IMM-008						___/___/___	___/___/___	
IMM-009						___/___/___	___/___/___	
IMM-010						___/___/___	___/___/___	
IMM-011						___/___/___	___/___/___	
IMM-012						___/___/___	___/___/___	
IMM-013						___/___/___	___/___/___	
IMM-014						___/___/___	___/___/___	
IMM-015						___/___/___	___/___/___	
IMM-016						___/___/___	___/___/___	

Project Title: **Safety and immunogenecity of 4×10^5 pfu tetravalent Rhesus Rotavirus vaccine, with or without zinc supplementation in Matlab, Bangladesh**

Study Record

Study ID# :

Group :

DSS ID# :

Subject Name :

Father's Name :

Village :

Birth Date :

Enrollment Date :

Sample Collection Checklist:

Sample	Done? (✓)	Date Collected
Consent Form		
1st Serum Sample		
1st Dose Vaccine		
Day 4 Stool after 1st dose		
Day 7 Stool after 1st dose		
Day 10 Stool after 1st dose		
2nd Serum Sample		
2nd Dose Vaccine		
Day 4 Stool after 2nd dose		
Day 7 Stool after 2nd dose		
Day 10 Stool after 2nd dose		
3rd Serum Sample		
3rd Dose Vaccine		
Day 4 Stool after 3rd dose		
Day 7 Stool after 3rd dose		
Day 10 Stool after 3rd dose		

Project Title: **Safety and immunogenecity of 4×10^5 pfu tetravalent Rhesus Rotavirus vaccine, with or without zinc supplementation in Matlab, Bangladesh**

Zinc Supplementation Record

Study ID# : DSS ID# : Subject Name : Birth Date :

Group : Village : Father's Name : Enrollment Date :

Zinc Supplementation Checklist:

Week		Day							Previous bottle checked and new bottle supplied
		1	2	3	4	5	6	7	
1	Done? (✓)								
	Date								
2	Done? (✓)								
	Date								
3	Done? (✓)								
	Date								
4	Done? (✓)								
	Date								
5	Done? (✓)								
	Date								
6	Done? (✓)								
	Date								
7	Done? (✓)								
	Date								

Project Title: **Safety and immunogenecity of 4×10^5 pfu tetravalent Rhesus Rotavirus vaccine, with or without zinc supplementation in Matlab, Bangladesh**

Adverse Reaction Questionnaire

Study ID# : DSS ID# : Subject Name : Birth Date :

Group : Village : Father's Name : Enrollment Date :

Community Health Worker:_____

Date of vaccination: 1st ____/____/____ 2nd ____/____/____ 3rd ____/____/____

Sign/Symptom (✓ if sign/symptom present)	Day after 1st vaccination							Day after 2nd vaccination							Day after 3rd vaccination						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Loose/watery stools (# of stools)																					
Vomiting (# of episodes)																					
Irritable																					
Poor appetite																					
Low activity level																					
Fever (by parent's report)																					
Temperature (taken by CHW)*																					
Other concerns**																					

- Open boxes represent days of CHW home visits

** CHW will ask "Has your child had any other symptoms or do you have any other concerns after he/she was given the vaccine?". If yes, check the appropriate box to indicate day of occurrence, and explain in the space below.

Other symptoms or concerns:

Project Title: **Safety and immunogenecity of 4×10^5 pfu tetravalent Rhesus Rotavirus vaccine, with or without zinc supplementation in Matlab, Bangladesh**

Supplemental Adverse Reaction Form

Study ID# : _____

Group : _____

DSS ID# : _____

Subject Name : _____

Father's Name : _____

Village : _____

Birth Date : _____

Community Health Worker: _____

Adverse Reaction Report:

Date of Report:

Date of most recent Rotavirus Vaccine Study vaccination:

Date of onset of suspected adverse reaction:

Description of suspected adverse reaction:

Description of treatment (if any):

Description of outcome (home treatment or referral to health care facility):

Review 1

EVALUATION OF CHILD HEALTH RESEARCH PROPOSAL

Reviewer's name:

Name of proposal: Safety and Immunogenicity of T-RRV Vaccine with or without Zinc Supplementation in Matlab

Name of proposed investigator: Shams El Arifeen

Date of review 2/11/98

The USAID Child Health Research Project requires external reviews of all proposals considered for funding. The review serves to ensure high quality of the research supported by USAID, as well as to provide specific suggestions to investigators to assist in revising research protocols. Reviewers comments will be used by USAID personnel in determination of project funding recommendations, as well as by the investigators themselves, who will receive them anonymously.

Summary: The Proposal entitled "Tetravalent Rhesus Rotavirus Vaccine with or without Zinc Supplementation..." is a well designed study of the T-RRV vaccines immunogenicity in a new population of children at high risk for rotavirus infection and disease. In the revised proposal additional statistical handling is described and criteria are established for evaluating the vaccine efficacy and further large scale relevance. In addition the effect of daily zinc supplementation on vaccine efficacy and immune response will be addressed as an additional part of the protocol. The study presented has carefully considered the circulating rotaviruses in the area of study, findings from other studies with RRV-TV, and incorporated these findings, and the existing infrastructure in Bangladesh into an effective means for immunizing and monitoring vaccine take and vaccinee responses in the Asian population. The role of zinc in increased vaccine efficacy by permitting enhanced immune responses to the vaccine are important for this and other vaccine studies and adequately handled in the proposal. As described below goals of the project are clear, well designed, appropriate and will significantly contribute to our knowledge of the RRV-TV vaccines and zinc effects on susceptible populations. This study is likely to establish both the take of the vaccine and its potential efficacy in a population with a high incidence of rotavirus disease and mortality. The study further addresses the enhanced immunogenicity in response to supplemental zinc added to the diets of vaccinees. The population chosen is well suited for addressing WHO priorities for vaccine efficacy in developing countries. The investigators are highly regarded rotavirus researchers and epidemiologists who are well suited to this study.

1) Goal: The main goal of the study is clearly defined to evaluate the immunogenicity of and side effects of the RRV-TV vaccine in infants during co-administration with other childhood vaccines including polio. Rationales for testing the added efficacy of the vaccine following zinc supplementation are compelling and provide additional interesting data which are potentially applicable to a broad range of vaccines in developing countries.

2) Design:
a) Proposed research approaches and methods are adequate to administer, evaluate inclusion criterion and test the immunogenicity of the RRV-TV vaccine and the effect of dietary zinc supplements on vaccine efficacy and the enhancement of immune responses in vaccinees.
b)The author's have clearly stated the rationales for the test groups, researched rotavirus prevalence rates and circulating rotavirus types in the demographic area. The rationales for testing the effect of zinc on the RRV vaccine are provided and quite interesting. Sample size, sampling strategy and follow up surveillance during the study are well defined and appropriate. Statistical analyses are presented, adequate and based on previous data for rotaviruses at the study site. Dropout rates and enrollments listed in the T-RRV vaccine part of the proposal are assumed for the zinc portion of the proposal and are appropriate, well defined and statistically justified. Criteria are established in the revision for evaluating vaccine efficacy for further large scale testing. In addition the effect of daily zinc supplementation on the T-RRV vaccine will be addressed as part of the vaccination study.
c) The proposed study is clearly described and logical. Immunogenicity and efficacy requirements for large scale testing of the vaccine are provided.
d) All of the proposed methods are feasible and easily handled by the author's.
e) The interview questionnaire is appropriate for the study. Laboratory methods are adequate for the proposed work.

3) Appropriateness: a) The research presented is very important to improving the health and well-being of children in Bangladesh and establishing the potential utility of RRV-TV vaccines in Asia and the nutritional effects of zinc supplements on vaccinee immune responses. As a result, this is a very important study which could have a global impact on child health and further our understanding of nutritional requirements for effective vaccination programs. This study has the potential to directly impact government policy in the prevention of RV disease and improve child health care outcomes. b) This study would scientifically establish the utility of RRV-TV vaccines in developing countries and extends previous findings to Asian populations most at risk of RV morbidity and mortality. The study should provide an understanding of immune response and dietary zinc in vaccinees.

4) Timing and Budget The project will clearly be completed within the allotted time and the overall budget seems appropriate for the study.

5) Ethics: There are no ethical considerations to be considered in this proposal. The vaccine has been successfully administered to over 7000 children in developed and developing countries. The vaccine is not in lieu of other vaccines or additional care and should in fact increase the medical care of those enrolled.

6) Background: The researchers on this proposal are experts in the field of rotavirus research and epidemiology which promise a high likelihood of success for the proposal. The design of the proposal is well suited to attain the goals of the author's.

7) Other: This proposal is straight forward and is likely to address the utility of the RRV-TV vaccine in Asian populations and the role of zinc in immune responses to vaccination. As such it is a strategically important proposal for the worldwide acceptance and administration of childhood rotavirus vaccines.

Review 2

GUIDE FOR EVALUATING USAID/CHR PROPOSALS

Name of proposal: Safety & Immunogenicity of 4×10^5 pfu TTRSV w/ w/o Zinc

Name of proposed investigator: Arifeen

Below are outlined areas for reviewers to address in making their comments on proposals. In addition to these questions, reviewers are asked to address any additional issues or concerns which they consider important.

1) *Goals:* Does the research have a clear primary goal?

yes, clear & well-stated; reasonable for trial of this size.

2) *Design:* Is the research well-designed and are methods appropriate? Please assess and suggest specific improvements to each of the following components:

good a) definitions of key concepts and variables

better b) sample size and sampling strategy than previous application

well defined c) clarity of analysis plans

d) feasibility of proposed methods

e) adequacy of any interview or record abstract forms/ of laboratory methods.

The randomization procedure on page 8 seems to suggest that the person handing out the vials will determine who gets what. If true, this should be modified so that individual is also "blinded".

3) *Appropriateness:*

a) Does the research have a potential for improving government policy for child health care, either through prevention or management of illness?

b) Does the proposed research ask any new questions or make unique contributions to practice or knowledge? Please comment briefly as to the scientific significance of the proposed research.

see next page

4) *Timing and budget:* Is the project likely to be completed in the allotted time, and is the proposed budget adequate to achieve its aims? Is the budget appropriate for the study? Please comment. Should allow a full year in addition to recruitment + follow-up phase.

5) *Ethics:* Are there any ethical objections or concerns regarding the proposed project, with regard to animal, and particularly, to the use of human subjects? no major

6) *Background:* Do the researchers appear to know about other scientific research related to their work? What additional sources should they consider, either with respect to research design or specific research findings?

literature & background, appears complete & current well-documented

7) *Other:* Address any other concerns you consider appropriate in evaluating this research proposal.

Thank you for your assistance to the USAID/CHR Project.

Appropriate. The inclusion of this supplement adds considerably to the trial of this vaccine candidate, which will also demonstrate marginal immunogenicity in this age

Principal Investigator (last, first, middle): Arifeen, Shams, El

Check List

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

1. Face Sheet Included ☑
2. Approval of the Division Director on Face Sheet ☑
3. Certification and Signature of PI on Face Sheet, ~~#9 and #10~~ ☑
4. Table on Contents ☑
5. Project Summary ☑
6. Literature Cited ☑
7. Biography of Investigators ☑
8. Ethical Assurance ☑
9. Consent Forms (only English) ☑
10. Detailed Budget ☑