PHSD 2001

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

CENTRE
FOR HEALTH AND
POPULATION RESEARCH

MEMORANDUM

17 June 2001

To : Dr K. Zaman
Public Health Sciences Division

From: David A Sack, M D
Chairman, Research Review Committee (RRC)

Sub : Approval of protocol # 2001-014

Thank you for your memo of 17th June 2001 attaching a modified version of the protocol # 2001-014 entitled "A randomised, placebo-controlled study of the safety, reactogenicity and immunogenicity of an orally administered human rotavirus vaccine (RIX4414) in healthy children in Bangladesh". The modified version of the protocol is hereby approved upon your satisfactory addressing of the issued raised by the Committee in its meeting held on 11th June 2001.

Thank you.

cc: Acting Associate Director
Public Health Sciences Division

ICDDRB: Centre for Health & Population Research **RRC**

APPLICATION FORM

RESEARCH PROTOCOL

Protocol No.:

2001 - 014

FOR OFFICE USE ONLY

RRC Approval: Yes/ No Date:

ERC Approval: Yes/No Date:

AEEC Approval: Yes/No Date:

Project Title:

A randomised, placebo-controlled study of the safety, reactogenicity and immunogenicity of an orally administered human rotavirus vaccine (RIX4414) in healthy children in Bangladesh

Theme: ***(Check all that apply)***

- ☐ Nutrition
- ☐ Emerging and Re-emerging Infectious Diseases
- ☐ Population Dynamics
- ☐ Reproductive Health
- X Vaccine evaluation
- ☐ Environmental Health
- ☐ Health Services
- X Child Health
- ☐ Clinical Case Management
- ☐ Social and Behavioural Sciences

Key words: Vaccine, rotavirus, immunogenicity, reactogenicity, diarrhoea, Bangladesh

Principal Investigator: Dr. K. Zaman **Division**: Public Health Sciences Division

Phone: 8811751-60, ext. 2246; 8810115

Address: Child Health Programme, PHSD, ICDDR,, B; Dhaka **Email**: kzaman@icddrb.org

Co-Principal Investigator(s):

Co-Investigator(s): Dr. Md. Yunus, Dr. Shams El Arifeen, Dr. Tasnim Azim, Dr. ASG Faruque, Prof. Shahid Karim (Shishu Hospital, Dhaka), Dr. Robert F Breiman, Dr. David A Sack

Student Investigator/Intern:

Collaborating Institute(s): University of Cincinnati, USA

Population: Inclusion of special groups (*Check all that apply)*:

Gender

- X Male
- X Females

Age

- X 0 – 5 years
- ☐ 5 – 9 years
- ☐ 10 – 19 years
- ☐ 20 +
- ☐ > 65
- ☐ Pregnant Women
- ☐ Fetuses
- ☐ Prisoners
- ☐ Destitutes
- ☐ Service providers
- ☐ Cognitively Impaired
- ☐ CSW
- ☐ Others (specify ----------)
- ☐ Animal

Project / study Site (*Check all the apply)*:

- ☐ Dhaka Hospital
- ☐ Matlab Hospital
- ☐ Matlab DSS area
- ☐ Matlab non-DSS area
- ☐ Mirzapur
- X Dhaka Community
- ☐ Chakaria
- ☐ Abhoynagar
- ☐ Mirsarai
- ☐ Patyia
- ☐ Other areas in Bangladesh
- ☐ Outside Bangladesh
  name of country:
- ☐ Multi centre trial
  (Name other countries involved)

Type of Study *(Check all that apply)*:

- ☐ Case Control study
- ☐ Community based trial / intervention
- ☐ Program Project (Umbrella)
- ☐ Secondary Data Analysis
- ☐ Clinical Trial (Hospital/Clinic)
- ☐ Family follow-up study
- ☐ Cross sectional surve
- ☐ Longitudinal Study
- ☐ Record Review
- ☐ Prophylactic trial
- ☐ Surveillance / monitoring
- X Others- vaccine evaluation

Revised on: 17 October 2000

Targeted Population *(Check all that apply)*:

- X No ethnic selection (Bangladeshi)
- ☐ Bangalee
- ☐ Tribal groups
- ☐ Expatriates
- ☐ Immigrants
- ☐ Refugee

Consent Process *(Check all that apply)*:

- X Written
- ☐ Oral
- ☐ None
- X Bengali language
- X English language

Proposed Sample size: Total sample size: 566 _____ ☐

Sub-group 90 toddlers_____ ☐

_____ ☐

476 infants_____ ☐

_____ ☐

Determination of Risk: Does the Research Involve *(Check all that apply)*:

- ☐ Human exposure to radioactive agents?
- ☐ Fetal tissue or abortus?
- ☐ Investigational new device?
- ☐ Human exposure to infectious agents?
- X Investigational new drug
- ☐ Existing data available via public archives/source (specify_____)
- X Pathological or diagnostic clinical specimen only
- ☐ Existing data available from Co-investigator
- ☐ Observation of public behaviour
- ☐ New treatment regime

Yes/No

X ☐ Is the information recorded in such a manner that subjects can be identified from information provided directly or through identifiers linked to the subjects?

☐ X Does the research deal with sensitive aspects of the subject's behaviour; sexual behaviour, alcohol use or illegal conduct such as drug use?

Could the information recorded about the individual if it became known outside of the research:

☐ X a. place the subject at risk of criminal or civil liability?

☐ X b. damage the subject's financial standing, reputation or employability; social rejection, lead to stigma, divorce etc.

Do you consider this research *(Check one)*:

- ☐ greater than minimal risk
- ☐ no risk
- X no more than minimal risk
- ☐ only part of the diagnostic test

Minimal Risk is "a risk where the probability and magnitude of harm or discomfort anticipated in the proposed research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or tests. For example, the risk of drawing a small amount of blood from a healthy individual for research purposes is no greater than the risk of doing so as a part of routine physical examination".

Yes/No

X ☐ Is the proposal funded? Yes

If yes, sponsor Name: USAID, NVPO, WHO

Yes/No

☐ ☐ Is the proposal being submitted for funding ?

If yes, name of funding agency: (1)

(2)

Do any of the participating investigators and/or their immediate families have an equity relationship (e.g. stockholder) with the sponsor of the project or manufacturer and/or owner of the test product or device to be studied or serve as a consultant to any of the above?

IF YES, submit a written statement of disclosure to the Director.

Dates of Proposed Period of Support *(Day, Month, Year - DD/MM/YY)* *years*	**Cost Required for the Budget Period ($)** *a. Ist Year 2nd Year 3rd Year Other*
Beginning date As soon as possible	*399,838 (including 3 months from 2nd year)*
End date 15 months from the date of starting	*b. Direct Cost* :$ 319,870 *Total Cost* : $399,838

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.

PERSSON — Name of the Division Director

Signature

31/5 2001 — Date of Approval

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.

Signature of PI Kgaman

Date: 31.5.2001

Name of Contact Person (if applicable)

Table of Contents

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 K. Zaman

Project Name: A randomised, placebo-controlled study of the safety, reactogenicity and immunogenicity of an orally administered human rotavirus vaccine (RIX4414) in healthy children in Bangladesh.

Total Budget 399,838 Beginning Date As soon as possible
Ending Date 15 months from date of starting

A safe and effective vaccine is needed to reduce the enormous public health burden associated with rotavirus illness, especially in developing countries. About 40,000 children with rotavirus diarrhea are treated each year at the ICDDRB hospitals. Globally there are an estimated 600,000 to 1 million deaths annually. Unanticipated adverse events (intussusception) experienced with a rhesus rotavirus vaccine have accelerated efforts to develop and evaluate alternative vaccine candidates so that a safe and effective public health tool would become available.

We propose to conduct a randomized, placebo-controlled, self-contained study within one community in Bangladesh to assess reactogenicity and immunogenicity of a live, attenuated human-derived rotavirus vaccine (RIX4414) among young children. This is the first study of this vaccine in Bangladesh. This vaccine has however been studied in adults, toddlers and infants in the US and Europe, and studies are being planned for South Africa, Mexico, Brazil and Venezuela. An efficacy study is currently underway in infants in Finland.

Although the vaccine has already undergone safety, immunogenicity studies in other countries, the investigators plan on repeating the safety studies in an older group prior to studies in infants to insure the vaccine's safety in the less well-nourished children of Bangladesh. Thus, in the first part of this study, safety of escalating doses (viral concentrations of $10^{5.0}$ and $10^{6.0}$ ffu) will be assessed in 90 toddlers (2-4 years old). Children will be randomised such that 30 children will receive the study vaccine of 10^5 ffu, 30 children of 10^6 ffu and 30 children will receive the placebo. Reactogenicity and adverse events will be assessed after a single dose of each virus concentration or placebo through daily home visits for the first 2 weeks and then twice weekly home visits for another 4 weeks, and thereafter monthly active surveillance to complete 6 months of follow up. Stool samples will be collected from all subjects before each dose and 7 days after each dose for rotavirus antigen detection.

If the evaluation in 2 to 4 years old children reveals no unacceptable reactogenicity and no other concerns about safety, then two and three doses of the study vaccine($10^{5.0}$ ffu) and two doses of the vaccine ($10^{6.0}$ ffu), administered 1 month apart, will be evaluated in infants 6-14 weeks old. This part will be conducted provided there are no safety concerns in the first study part among toddlers. This will be a safety, and immmunogenicity study of two or three doses of the study vaccine when randomized to infants at 6 to 14 weeks of age. Since the second part will aim to identify the most suitable dose of the vaccine for

Bangladesh infants, a larger number of infants will need to be enrolled in order to determine if there is a difference in immune responses between the two doses. Thus a total of 476 infants will be randomised to receive either 2 doses of $10^{5.0}$ ffu (136 subjects), 3 doses of $10^{5.0}$ ffu (136 subjects) or 2 doses of $10^{6.0}$ ffu (136 subjects) or placebo (68 subjects). Safety and reactogenicity assessment will be done through home visits as for the first part. Serum specimens will be collected from all subjects to evaluate IgA antibodies to rotavirus (not serotype specific) by ELISA. Specimens will also be available for neutralization assay against G1, G2, G3, G4, G9, and the vaccine strain, although neutralizing antibodies will not be a primary outcome. Stool samples will be collected from the first 150 enrolled subjects on days 0, 3, 7, 14, 21 after each dose for rotavirus antigen detection to assess vaccine viral shedding.

Routine EPI vaccines should be administered according to local recommendation (at 6, 10 and 14 weeks of age) concomitantly with the study vaccine. The study will form the foundation for conducting Phase III studies of effectiveness and safety in Bangladesh, where we have shown that rotavirus is responsible for substantial public health burden. Ultimately, the goal will be to have available a practical, safe, and effective vaccine for use in prevention of rotavirus morbidity globally.

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

Name	Professional Discipline/ Specialty	Role in the Project
1. Dr. K. Zaman	Epidemiologist (Child Health Programme)	Principal Investigator
2. Dr. Md. Yunus	Senior Scientist & Head Matlab HRP	Co-investigator
3. Dr. Shams El Arifeen	Epidemiologist & Head (Child Health Programme)	Co-investigator
4. Dr. Tasnim Azim	Virologist, Lab. Sciences Division	Co-investigator
5. Dr. ASG Faruque	Scientist, Clinical Sciences Division	Co-investigator
6. Prof. Shahid Karim	Paediatric Surgeon, Shishu Hospital, Dhaka	Co-investigator
7. Dr. Robert F Breiman	Head, Progrm. Infect Dis & Vaccine Scien.	Co-investigator
8. Dr. David A Sack	Director, ICDDR,B	Co-investigator

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be tested:

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

1. Human rotavirus vaccine (RIX4414) is safe and immunogenic in infants

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).

Part 1 (Safety assessment in toddlers)

Primary

- For each vaccine concentration (10^5 and 10^6 ffu), to assess the safety and reactogenicity of a single oral dose of the rotavirus vaccine *versus* placebo in healthy children 2-4 years old.

Secondary

- To assess viral shedding in each study group.

Part 2 (Safety and immunogenicity assessment in infants)

Primary

- To assess the immunogenicity of the rotavirus vaccine after any dose in the study vaccine groups (infants aged 6-14 weeks).

Secondary

- For each viral concentration, to assess the safety and reactogenicity of two/three oral doses of the rotavirus vaccine *versus* placebo.
- To assess viral shedding in a subset of subjects.

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).**

A safe and effective rotavirus vaccine is needed to reduce the enormous public health burden associated with rotavirus illness, especially in developing countries (at least 600,000 deaths worldwide annually) (Miller & McCann, 2000). Earlier unanticipated adverse events experienced with a rhesus rotavirus vaccine have intensified efforts to develop and evaluate other vaccine candidates so that a safe and effective public health tool would become available. For these reasons, GPV/VRD has designated rotavirus vaccine development and evaluation as an urgent priority.

Rotaviruses, non-enveloped, double-stranded RNA viruses, infect both humans and animals and are distributed worldwide. In humans, rotavirus causes diarrhea of varying severity ranging from mild to severe. Deaths occur from rotavirus diarrhea when the diarrhea is sufficiently severe to cause dehydration. Most illnesses occur in infants, although adults and the elderly may also be affected. Rotavirus is the most common cause of severe diarrhea leading to hospital treatment in every country where etiologies have been monitored, including both developed and developing countries. In developing countries, the number of deaths due to rotavirus is estimated to be between 600,000 and one million, almost all under age 2 years. Almost all children have been infected by the time they are 3 years of age, and previous infection protects children from subsequent illness. Re-infections are frequent, but subsequent illnesses tend to be less severe than the first infection. Thus, it is believed that if the first rotavirus infection occurs with an attenuated vaccine strain, subsequent infections with virulent wild-type rotaviruses will not cause the severe life-threatening diarrhea that now occur. Vaccination with a safe and effective vaccine has the potential of averting the deaths of up to one million children per year, most of whom live in developing countries. In addition, such a vaccine will avert hospitalizations that occur in children in all parts of the world.

At ICDDR,B rotavirus accounts for about 40% of the diarrheal illnesses of patients under 5 years of age as determined by the surveillance carried out at both the Dhaka and Matlab hospitals. Deaths due to rotavirus in these settings are extremely rare because rehydration is provided; however, in many areas of Bangladesh, adequate treatment may not be given, resulting in an estimated 20,000 rotavirus deaths annually (Unicomb et al., 1997).

Rotavirus has several serotypes based on G and P antigens. In most industrialized countries, G1 is the most common type by far. Other serotypes also occur and seem to vary from year to year and by geographic location. Recently, G9 has emerged as being common in Bangladesh, India and parts of Africa. Infection with serotype G5 has also been recently reported from some developing countries.

Previously, it was felt necessary to have a multi-valent vaccine that included all relevant serotypes. For this reason, the tetravalent rhesus reassortant vaccine was developed. It is not clear however that a vaccine needs to be multi-valent. Based on studies of the natural history of rotavirus infections, it seems that a first infection is generally the most severe, regardless of serotype, suggesting that there is some heterotypic protection, at least against severe disease. Secondly, since rotavirus infection occurs commonly, subsequent rotavirus infections increase serotype-specific and heterotypic protection. In the US, multi-centre vaccine trials using tetravalent and monovalent rhesus vaccines, monovalent vaccine (G1) induced nearly the same protection as the tetravalent.

Since most severe infections occur between the ages of 4 months and 2 years of age, vaccination should begin early in life, and immunization should be completed prior to 6 months. From a logistical standpoint, the vaccine can best be delivered if it can be included within the EPI program where the provisions are already in place for the cold chain.

Because tetravalent rhesus rotavirus vaccine (Rotashield), licensed in 1998 in the US was associated with intussusception (IS) (Anonymymous, 1999, Murphy et al., 2001), investigations of new rotavirus vaccines must be evaluated for association with IS. The company and the investigators feel that it is unlikely that RIX4414 will lead to IS but this will need to be determined during all clinical trials. The reasons that RIX4414 is unlikely to cause IS includes the following. Wild type rotavirus does not appear to be a cause of IS since the seasonality with rotavirus is so marked in industrialized countries, yet there is no corresponding seasonality for IS. Secondly, RIX4414 is a human strain, typical of the viruses that every child will become infected with early in life, so vaccination does not induce an exposure that would not otherwise occur, unlike administration of rhesus rotavirus vaccine which represented a virus that would not normally infect children. Thirdly, the risk of IS is primarily during the age 4 to 12 months, and we propose completing the vaccination prior to this time period. Nevertheless, all subjects receiving RIX4414 during clinical trials will be informed of the risk of IS, and we will ensure that any potential case of IS is rapidly identified, transported efficiently, diagnosed using state of the art methods, treated with air insufflation when possible and other standard procedure as practised by paediatric surgeons in Dhaka.

The active ingredient in RIX4414 is based on the human rotavirus strain 89-12. The 89-12 HRV was obtained from the stool of a 15-month-old infant with mild rotavirus diarrhea in December 1988 in Cincinnati (USA). The 89-12 strain belongs to the serotype G1P1A and genotype P8. A two-year prospective study showed that natural infection with such strains circulating in this community during 1988 and 1989 provided protection against subsequent illness and against re-infection during a two year follow up, since none of the children infected with the strain developed another episode of rotavirus diarrhoea (Bernstein et al., 1991). The strain was developed as a vaccine by the Virus Research Institute (VRI), now called AVANT, a biotechnology company in the US. The initial isolate was passaged in primary African Green Monkey Kidney cells (AGMK), and then further passaged on an approved AGMK cell line to produce initial clinical lots of vaccine based on uncloned P33 89-12 HRV strain. It was passaged in tissue culture using approved cell lines and was found to be attenuated when administered to volunteers. Clinical studies in US children and infants demonstrated the safety, immunogenicity and

efficacy of the vaccine in infants (Bernstein et al., 1998; Bernstein et al., 1999). A phase 2b trial with two doses of 10^5 ffu of 89-12 in US infants demonstrated 89% protection against any rotavirus gastroenteritis and even higher protection against severe rotavirus gastroenteritis during the first year after vaccination (Bernstein et al., 1999). In the second year the efficacy was 65%. The overall efficacy for 2 years was 76% against any rotavirus infection and 84% against severe rotavirus and 100% against any very severe rotavirus (Bernstein et al, 2000). Table 1 presents summary results of the studies conducted with rotavirus 89-12 strain.

Table 1: Summary results of the studies conducted with human rotavirus 89-12 strain

Country (reference)	Sample size	Age group	Side-effects		Immunogenicity	Efficacy (95% CI)
			Vaccine	Placebo		
USA (Bernstein et al, 1999)	215	10-16 weeks	Temperature 38.1°C- 38.5 °C - 19% 38.6°C- 38.9 °C - 6% ≥ 39.0 - 4% Diarrhoea - 17% Vomiting- 15%	5% 2% 1% 9% 9%	Immune response to vaccinees 94.4%, placebo 3.8%	89% (65-96%)
USA (Bernstein et al, 1998)	34	Adults	Fever (>38°C) - 0 Diarrhoea- 1	0 0	Serum antibody rise to vaccinees 2, placebo 0	Not done
	40	2-12 years	Fever (>38°C) - 0 Diarrhoea- 1	0 0	Serum antibody rise to vaccinees 4, placebo 0	
	9	6-26 weeks	Fever (>38°C) - 1 Diarrhoea- 1	0 0	Serum antibody rise to vaccinees 2, placebo 0	
	42	6-26 weeks	Fever (>38°C) - 4 Diarrhoea- 2	5 1	Serum antibody rise to vaccinees 19, placebo 0	

SB Biologicals has acquired the strain and taken over the manufacturing process and clinical development of the 89-12 vaccine from AVANT and has implemented several process changes and prepared a lyophilized HRV vaccine based on a cloned P43 89-12 HRV strain (referred to as RIX4414).

It is known that the same vaccine can achieve different levels of immune response and efficacy in different study populations, and therefore it is important to test the study vaccine in different geographic settings. GSK Biologicals is therefore commencing trials in Asia, Latin-America and Africa to begin testing of its HRV vaccine's effectiveness in preventing gastroenteritis.

The aim of this dose-ranging study is to identify the most suitable dose and regimen of the vaccine for Bangladesh infants and to determine if there is a difference in immune responses between the two viral concentrations using two different schedules. The rationale for the use of several doses and several viral concentrations is four fold:
First, to implement rotavirus vaccination as part of the existing EPI schedule, infants should be vaccinated at a very early age (6-8 weeks old) and with a short interval between doses. At this young age, infants are expected to have considerable levels of circulating maternal antibodies. The importance of maternally acquired anti-rotavirus specific antibodies has been evaluated in several clinical trials performed in infant-mother pairs. As expected, the presence of maternally acquired antibodies was shown to interfere with live attenuated oral rotavirus vaccine in infants (Pickering et al, 1995). The administration of an additional dose might overcome the potential interference of maternal antibodies. Therefore, the immunogenicity of GSK Biologicals' HRV vaccine needs to be evaluated at two and at three doses.

Second, as mentioned earlier, GSK Biologicals has implemented several process changes to the uncloned P33 89-12 vaccine from AVANT, including 10 additional passages on Vero cells, which may have led to further attenuation of the vaccine virus strain. A higher viral concentration may be needed in Bangladesh to reach the seroconversion rates observed in the USA (study with 89-12 P33) or Finland (study 003 with GSK Biologicals' HRV vaccine, unpublished). Moreover, due to the circulation of other pathogens in Bangladesh, the immunogenicity of the vaccine could be negatively affected. Therefore, immunogenicity of GSK Biologicals' HRV vaccine needs to be evaluated at two viral concentrations.

Thirdly, in Bangladesh as in other countries following the EPI schedule, OPV is a part of the routine schedule. Concomitant administration requires that rotavirus vaccine and OPV do not interfere and compromise the immune response to either of the vaccines. Previous studies with a bovine rotavirus vaccine (RIT 4237) indicated that the take of the bovine rotavirus vaccine was suppressed by co-administration with OPV while the response to OPV was not affected (Vodopija et al, 1988). Studies with the rhesus rotavirus vaccine (RRV or RRV-TV) in Georgia, USA indicated that the rhesus rotavirus vaccine was safe and immunogenic when co-administered with OPV and there was no significant interference due to concomitant administration (Ho Mei-Shang et al, 1989; Ing et al, 1991). A study in USA with 1278 infants assessing the efficacy of RRV-TV vaccine when given with or without concurrent OPV (Rennels et al, 1996), showed no significant

difference in efficacy of RRV-TV when given concomitantly with OPV. Although interference was not observed in studies conducted in USA, the results cannot be extrapolated to developing countries where the potency of OPV and vaccination schedule varies, and a high rate of poliovirus vaccine failure is reported (WHO, 1995). Indeed a study was conducted in Thailand to assess the simultaneous administration of oral rhesus-human reassortant tetravalent vaccine (RRV-TV) and OPV. Results observed in this study suggested that OPV is likely to interfere with the take of RRV-TV vaccine but the interfering effect could be compensated by administering additional doses (two versus three doses) or by using a higher concentration of the rotavirus vaccine (Migasena et al, 1995).

Fourthly, to warrant shelf life of the vaccine, the commercial lots will have a release titer at a high viral concentration, to ensure that at the expiry of the shelf life, the actual titer is at least equal to the titer for which efficacy was determined. The use of the two viral concentrations in the clinical assay will ensure that there is no safety issue for any of the viral concentration.

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).**

Experimental design and methods

Study site: The study will be conducted in a suburb of Dhaka in the area of Mirpur, where phase II ETEC vaccine and killed cholera vaccine trials have been undertaken and several other studies are ongoing. The study will be in one section (section 11) of Mirpur which has a population of about 350,000. Average monthly income per family is about US$60. The study subjects will be recruited through home visits by the locally recruited field workers.

Overview of study

Part 1 (Safety assessment in toddlers)

In study part 1, the safety in toddlers will be assessed in a small number of subjects. The purpose of this study is to assess the safety and reactogenicity of the proposed doses of the candidate vaccine when administered orally in a small number of toddlers before clinical development continues in the target population, infants. The safety data collected during the fifteen-day follow-up period (day 0 to day 14) will be reviewed by the medical monitor without breaking the study blind. If there are no safety concerns during this blinded review of the safety data collected, participants in the study part 2 will be vaccinated with the viral concentration of the proposed doses.

Toddlers (2-4 years old) Recruitment, consent, and randomization (1:1:1) with 30 subjects in $10^{5.0}$ ffu (1 dose), 30 subjects in $10^{6.0}$ ffu (1 dose) and 30 in placebo group.	One dose of vaccine or placebo, with surveillance for adverse events (by home visits) and fecal excretion of virus

Part 2 (Double-blind safety and immunogenicity assessment in infants)

In study part 2, the safety and immunogenicity of the candidate vaccine will be assessed in the target age group - infants approximately 6 weeks of age. Escalating doses (viral concentrations of 10^5 ffu and 10^6 ffu) will be evaluated. All infants will receive by co-administration the recommended EPI vaccines at 6-10-14 weeks, including OPV.

Infants (6-14 weeks old) Recruitment, consent, and randomization (2:2:2:1) with 136 subjects in $10^{5.0}$ ffu (2 doses), 136 subjects in $10^{5.0}$ ffu (3 doses), 136 subjects in $10^{6.0}$ ffu (2 doses) and 68 in placebo group.	Two/three doses of vaccine or placebo, 4 weeks apart, with surveillance for adverse events (by home visits) and blood sampling at each study visit for antibody titer changes. Fecal excretion of virus in the first 150 enrolled subjects.

The investigator and his staff will be aware of the possible increased risk of intussusception and will consider this diagnosis among children presenting symptoms of intussusception. Symptoms consistent with intussusception are severe colicky abdominal pain, persistent vomiting, bloody stools, abdominal bloating and fever up to 41°C. The investigator and his staff will take appropriate actions to evaluate and treat the condition.

A child with suspected intussusception will be referred to a local hospital in Dhaka (Children's hospital, Shishu Hospital). The hospital has the experience and capacity for diagnosis, non-surgical and surgical management of intussusception, and a pediatric surgeon at this hospital is a co-investigator on the protocol to assume responsibility for the care of children suspected of having IS. During the study course, the parents/guardians will be instructed to contact the investigator and his staff immediately should the subject manifest any signs or symptoms they perceive as serious.

In case a subject is diagnosed with intussusception this will be immediately reported to the sponsor. The diagnosis of intussusception should be documented by imaging. **Annex II** provides instruction on the evaluation of intussusception. Each case of suspected or confirmed intussusception will be reviewed by an independent DSMB (data safety monitoring board).

Outline of the study:

- Single community — Mirpur, Bangladesh (north part of Dhaka).
- Randomized, placebo-controlled, self-contained study.
- Subjects eligible for inclusion in the study will be sequentially allocated a subject number in the order in which they are enrolled and will be administered the study vaccine or placebo according to a randomization list prepared by the sponsor.
- The study will begin with a single administration of the $10^{5.0}$ ffu/$10^{6.0}$ ffu dose to 2-4 years old children. The study will proceed further provided there are no safety concerns raised after the first dose in the study group.
- Routine EPI vaccines should be administered according to local recommendation (at 6, 10 and 14 weeks of age for the study part 2 only) concomitantly with the study vaccine.
- Vaccine is given with calcium carbonate buffer at time of vaccination; total dose of vaccine and buffer is 1.5 ml.
- Study subjects will be followed at their homes daily for 2 weeks and then twice weekly for another 4 weeks after each dose of vaccination to assess reactogenicity and adverse events.
- Stool sample collection:

Study Part 1: Stool samples (fecal swabs) will be collected on the day of each dose and on day 7 after each dose from all subjects.
Study Part 2: Stool samples (fecal swabs) will be collected on the day of each dose and on days 3, 7, 14 and 21 after each dose from the first 150 enrolled subjects.

- Serum specimens will be collected at each study visit and 4 weeks after the last dose from all subjects only in part 2.
- Data collection will be by conventional paper case report forms (CRF's).

Sample size considerations

During the first phase of the study (immunization of children 2-4 years of age), the outcome of interest is reactogenicity. Even if intussusception were to occur with a relative risk similar to that observed with RRV-TV, we do not expect to observe intussusception with the small numbers included in this Phase I study. We will however use fever as a measure of reactogenicity. Based on previous studies in the USA involving active surveillance, we expect a background rate of 5% of fever (among placebo recipients). With 90 subjects (30 with $10^{5.0}$ ffu, 30 with $10^{6.0}$ ffu and 30 placebo), we will have 80% power (p=0.05) to detect a 35% incidence of fever among vaccinated subjects (assuming a background rate of 5% among controls). The same sample size assumptions hold true for the infant study.

For the second part involving both safety and immunogenicity, the primary outcome measure of interest is immunogenicity, although through this and all subsequent studies, reactogenicity will continue to be monitored. With 136 vaccinees in each vaccine group ($10^{5.0}$ 2 doses, $10^{5.0}$ 3 dose, $10^{6.0}$ ffu 2 doses) and 68 subjects receiving placebo, we will be able to detect a difference in take rates of 15% between the two dose levels (70% vs. 85%). The sample size will be sufficient to detect a difference in take rates of 12% (75% vs. 87%) between 2 doses of $10^{5.0}$ ffu and $10^{6.0}$ ffu. We are also assuming that 35% of infants will be exposed to wild type rotavirus during the 8 week study period and our numbers will be sufficient to differentiate a take rate between vaccine responses and background responses. If the proportion among controls is actually lower, and 70% of children have a four-fold rise in serum antibody titer, then the number of subjects required to achieve significance will be lower.

Study Cohort

Numbers of subjects / centres: Field workers will identify children of appropriate age, inform families of the purpose and scope of the study and offer participation. It is planned to vaccinate 10-12 children daily, and first dose of vaccines are expected to be complete within 2 weeks of the start of the study.

Inclusion criteria for enrollment: Male or female, between 24 months and 48 months of age at the time of the first dose of vaccine for participation in the study part assessing the vaccine in toddlers. Male or female, between 6 and 14 weeks of age at the time of the first dose of vaccine for participation in the study part assessing the immonogenicity and safety of the vaccine in infants. Written informed consent, free of chronic or serious medical condition as determined by history and physical exam within 3 days of the first dose of vaccine, plan to stay in community for at least 3 months.

Exclusion criteria for enrollment: Malnutrition (<-2Z score weight for age), fever (>38° C), acute or chronic illness, use of antimicrobial drug within previous 14 days, hypersensitivity to any of the vaccine components (vaccine composition in **annex III**), use of any investigational drug during previous 30 days, any uncorrected congenital malformation of the gastrointestinal tract, use of any immunosuppressing drugs during the last 14 days (likelihood is remote), any evidence by physical exam of immunosuppresing condition, administration of gamma globulin or any other blood product, previous intussusception or abdominal surgery, known pregnant person in the home.

Elimination criteria during study: If an exclusion criteria develops while participating in the study, the subject will be eliminated from the study.

Definitions:
Fever: Rectal temperature > 38°C
Diarrhoea: Three or more looser stools than normal stools in a 24 hour period.

Contraindications to vaccination: Not applicable.

Conduct of the study: The study will be conducted according to Good Clinical Practices, the Declaration of Helsinki, and local rules and regulations of Bangladesh and the ICDDR,B. It will be reviewed and approved by the Ethical Review Committee (ERC) of the ICDDR,B prior to starting the study. The ERC is a recognized committee for review of research protocols involving humans and has a Multiple Project Assurance (MPA) with a US agency (USAID).

Informed consent: The parents of the subjects will sign a consent form informing them of the nature of the study, its rationale, and its risks and benefits. The informed consent document will embody the elements of the consent as described in the Declaration of Helsinki, and the ICH Harmonized Tripartite Guidelines for Good Clinical Practices. The investigator or designate will describe the protocol to parents of potential subjects face to face. The subject information and consent form may be read to the parents of potential subjects, but in any event, the investigator will give the parents ample opportunity to inquire about the details of the study and ask questions before signing the consent form **(annex I).** The consent form will describe the possible side effects including the potential risk of IS. While the likelihood is extremely low of intussusception being temporally linked to participation in this small study, we will insure that health care workers are trained to recognize symptoms and clinical signs of intussusception—this is the principal reason for such aggressive, active surveillance for adverse events. We will have an arrangement with a local hospital (Children's hospital, Shishu Hospital in Dhaka) with experience and capacity for diagnosis, non-surgical and surgical management of intussusception. Any patient meeting a case-definition consistent with intussusception will be transported to the hospital for evaluation and treatment.

Total duration of follow-up after vaccination: According to recommendations of the European Committee for Proprietary Products (CPMP) all vaccinated children will be followed up for at least 6 months after the last immunization considering the safety issue (CPMP, 1999). Since the long-term follow up does not require any active surveillance for

the subjects, and since no efficacy end point is part of this protocol, active surveillance of diarrheal events will not be done.

The protocol will be reviewed for scientific quality by the Research Review Committee (RRC) of the ICDDR,B. The Research Review Committee (RRC, with 13 members), composed of clinicians, epidemiologists, social scientists, laboratory scientists, and demographers/population scientists from both within and outside the center, reviews all scientific research proposals of the center, evaluates their scientific merit, competence of Principal Investigators, and relevance to the Centre's objectives and priorities.
The protocol will be reviewed by the Immunization Research Technical Advisory Group of the World Health Organization. Further, a data safety monitoring board (DSMB) including a safety monitor will be constituted who will be responsible for the monitoring and review of the safety and conduct of the trial. One GCP monitor will be appointed who will check Case Report Forms (CRFs) for completeness and correspondence with source documents, monitor regulatory files and validate data files in computer.

Approval by National Drug Administration. Since the protocol involves the use of an experimental vaccine, the protocol will be reviewed and approved by the National Drug Administration of Bangladesh prior to initiation of the study.

General Study Aspects
Routine EPI vaccines should be administered according to local recommendation (at 6, 10 and 14 weeks of age for the study part 2) concomitantly with study vaccine.

Outline of study procedures
For all study parts:
Prior to vaccination (up to day before vaccination)
- Inform community leaders about study and answer questions.
- Approach families with children in appropriate age group living near Mirpur clinic to determine their interest in the study.
- Inform families about study and answer their questions.

For toddlers (part 1):
Day 0 (Visit 1)
- Conduct history and physical exam, including measurements of height and weight and rectal temperature.
- Insure that the subject meets all inclusion and exclusion criteria.
- Obtain signed informed consent.
- Determine randomization code
- Prepare study vaccine and administer dose 1.
- Collection of stool sample from all subjects for rotavirus antigen detection by ELISA.

Day 1 to day 14
- Carry out active surveillance with all subjects by visiting house daily for 2 weeks. Measure rectal temperature daily. Use daily form to record symptoms or lack of symptoms. Have physician available at clinic and inform family of subjects that the physician is available should there be any illness during the time of the study. If subjects are brought to the clinic, record all symptoms in patient medical records. These medical records will be the source documents for the passive surveillance.

Day 7

- Collection of stool sample from all subjects for rotavirus antigen by ELISA.

Day 15 – day 42

- Twice weekly active surveillance for 4 weeks following each dose and inquiring about symptoms or illnesses among subjects.
- On day 42, insure that all concomitant medication taken during the study have been recorded.

Day 43 to 6 months.

- Monthly active surveillance to complete 6 months of followup. With each visit, inquire about symptoms or illnesses among subjects.
- At end of 6 months, terminate subject from the study

For infants(Part 2)

Day 0 (Visit 1)

- Same procedures as performed for toddlers in part 1.
- Obtain a blood sample for serology
- Prepare study vaccine and administer dose 1
- Collection of stool sample for rotavirus antigen by ELISA

Day 3, 7, 14, and 21 (first 150 subjects only)

- Collection of stool sample for rotavirus antigen by ELISA

Day 1-day 27

- Carry out active surveillance with all subjects by visiting house daily for 2 weeks and thereafter twice weekly. Measure rectal temperature daily. Use daily form to record symptoms or lack of symptoms. Have physician available at clinic and inform family of subjects that the physician is available should there be any illness during the time of the study. If subjects are brought to the clinic, record all symptoms in patient medical records. These medical records will be the source documents for the passive surveillance.

Day 28

- Insure that subject is eligible for dose 2 according to inclusion and exclusion criteria
- Obtain a serum blood sample for serology
- Prepare study vaccine and administer dose 2
- Collection of stool sample from subjects (first 150 only) for rotavirus antigen by ELISA

Day 29 – day 42

- Carry out active surveillance with all subjects by visiting house daily for 2 weeks. Measure rectal temperature daily. Use daily form to record symptoms or lack of symptoms. Have physician available at clinic and inform family of subjects that the physician is available should there be any illness during the time of the study. If subjects are brought to the clinic, record all symptoms in patient medical records. These medical records will be the source documents for the passive surveillance.

Day 31, 35, 42, 49 (first 150 subjects only)

- Collection of stool sample for rotavirus antigen by ELISA

Day 43-day 55

- Twice weekly active surveillance inquiring about symptoms or illnesses among subjects.

Day 56

- Insure that subject is eligible for dose 3 according to inclusion and exclusion criteria

- Obtain a serum blood sample for serology
- Prepare study vaccine and administer dose 3
- Collection of stool sample from subjects (first 150) for rotavirus antigen by ELISA

Day 57-day 70

- Carry out active surveillance with all subjects by visiting house daily for 2 weeks. Measure rectal temperature daily. Use daily form to record symptoms or lack of symptoms. Have physician available at clinic and inform family of subjects that the physician is available should there be any illness during the time of the study. If subjects are brought to the clinic, record all symptoms in patient medical records. These medical records will be the source documents for the passive surveillance.

Day 59, 63, 70, 77 (first 150 subjects only)

- Collection of stool sample for rotavirus antigen by ELISA

Day 71- 83

- Twice weekly active surveillance inquiring about symptoms or illnesses among subjects.

Day 84

- Obtain a serum blood sample for serology.
- Insure that all concomitant medication taken during the study have been recorded.

Day 85 to 6 months after third dose

- Monthly active surveillance to complete 6 months of follow up. With each visit, inquire about symptoms or illnesses among subjects.
- At end of 6 months, terminate subject from the study

Laboratory testing

Fecal samples will be collected as whole stool samples by the parent of the subject or the field worker using a stool cup and taken to the field clinic within 8 hours of passing of the sample. At the clinic, it will be placed in a glass vial with saline (about 10% feces to saline ratio), labelled and placed into a refrigerator (+4°C). Specimens will be transported to the ICDDR,B virology lab within 48 hours and maintained at +4° C until tested. Blood specimens will be obtained by venipuncture (about 3 ml) in clinic using sterile technique. Blood will be placed into a vacutainer without anticoagulants. After blood has clotted, it will be centrifuged and serum transferred to three labelled cryovials (3 aliquots) and frozen at –20° C.

Stool samples, diluted in PBS will be tested for the presence of rotavirus antigen using ELISA (Unicomb et al, 1993; Azim et al., 1999). Stools found to be positive by ELISA will be further characterized by polyacrylamide gel electrophoresis and by reverse-transcriptase-polymerase chain reaction to determine G and P genotype (Unicomb et al., 1999, Gouvea et al., 1990; Gentsch et al., 1992). A sample of the presumptive vaccine strains will be confirmed as vaccine strains at Smith Kline Biologicals using sequencing. Strains of rotavirus will be typed following a scheme described previously (Unicomb et al., 1999). Anti-rotavirus IgA antibody titers will be determined by ELISA using standard methods (Ward, 1989). Serum samples will be available for neutralization assays, though this is not a stated outcome of the study.

Since the ELISA IgA titers are a crucial outcome of the study and since we want to insure that there is agreement between different studies with the vaccine from different countries, but also want to develop our capacity for carrying out a validated assays in Dhaka, we will split the serum samples and have them tested in the laboratory of either Dr. Ward or Smith Kline Biologicals where the assay has been validated. Dr. Ward has agreed to be a consultant to assist with the standardization of the assay.

Data Safety Monitoring Committee (DSMB)

A committee will be formed including pediatricians to evaluate the reactogenicity data before moving from the older age to the younger age group. The committee will prepare a letter with its recommendations for the file at each stages in the study.

Expected outcomes and timeframe

1. We expect the vaccine to be safe as determined by our adverse event surveillance when given to toddlers and to infants. We expect to detect no cases of intussusception in either group. The vaccine administration may be associated with mild elevations of temperature with a rate significantly higher than control levels, but this mild reactogenicity will not be perceived as clinically important.
2. We expect that most (>70% of infants) will develop a significant increase in serum IgA antirotavirus antibody titer as determined by ELISA and this take rate will be significantly higher than in those receiving the placebo.
3. We expect that the vaccine strain will be detected in a small proportion of fecal specimens of toddlers, but a high proportion of infants when tested 7 days following vaccination. It may still be detected from a few of the infants, when tested 28 days following vaccination.

Following approvals from the appropriate committees and regulatory bodies, we expect Part 1 (the safety studies in toddlers) can be carried out during a 5 month time frame (including information to the community about the study, training of workers, identification of eligible children, setting of a clinic etc). Following collection and analysis of safety data, the Data Safety Monitoring Board will meet to review the safety data. If found sufficiently safe to proceed, the infants in Part 2 can be recruited and vaccinated within about 6 months. Testing the serum specimens for IgA anti-rotavirus antibody will require an additional month. Thus we expect a report summarizing safety and IgA serology can be available within 12-15 months of starting the study, thus preparing the way for a follow-up efficacy study if appropriate. The infants will however be followed for a total of six months after the last dose of vaccine to detect any late adverse events if they were to occur.

Serology will be presented both as the number of infants with 4 fold rises in antibody titer as well as geometric mean titers in the vaccine and placebo groups

Previous experience of the laboratory in the proposed field of research

ICDDR,B scientists have conducted a variety of studies to define the epidemiology and microbiology of rotavirus infections. The Centre also conducted phase II safety and immunogenicity studies with rhesus rotavirus tetravalent vaccines and was prepared to conduct a phase III study with that vaccine when efforts to further evaluate that vaccine were discontinued. The Centre has a long history of studies of prevention of diarrheal diseases including rotavirus as well as cholera, typhoid, shigella, and ETEC. Dr. Sack has studied the vaccine based on the HRV strain 89-12 in safety, immunogenicity and efficacy studies in the US.

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 International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) has large multi-disciplinary international and national scientific research staff. Existing field, hospital, laboratory and office facilities will be used for this study. ICDDR,B scientists have conducted a variety of vaccine studies including rotavirus.

Field site
The field component of the study will be carried out in urban slums in Mirpur in Dhaka. This site is being used currently for a phase II study of the ETEC vaccine.

Clinical facilities
Sishu hospital is the largest paediatric hospital in Bangladesh. The hospital has the experience and capacity for diagnosis, non-surgical and surgical management of intussusception, and a pediatric surgeon at this hospital is a co-investigator on the protocol to assume responsibility for the care of children suspected of having IS.

Laboratory facilities
Existing laboratory facilities in ICDDR,B will be used.

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 characteristics of the subjects receiving vaccine and placebo will be examined for group comparability. Any significant baseline differences will be controlled for during data analysis. The frequency distribution will be examined to assess the distribution of data. Proportion of infants with adverse events will be compared between the different groups (vaccine, placebo, different doses and viral concentrations). Proportion of subjects that shed rotavirus at different time periods will be compared. Serological measures (proportion with $\geq$ 4-fold increase in titer and geometric mean titer) will be compared between different viral concentrations/ doses and placebo recipients.

The proportions will be compared using χ^2-tests and rate ratios (95% confidence intervals). The geometric mean titers (GMT) will be compared using non-parametric tests. Multivariate 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.

Human rotavirus vaccine (RIX4414), designed for preventing rotavirus will be evaluated among young children since the risk of the disease is greatest in that age group. Infants will 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. The study will be conducted according to Good Clinical Practices, the Declaration of Helsinki, and local rules and regulations of Bangladesh and the ICDDR,B.

The investigator and his staff will be aware of the possible increased risk of intussusception and will consider this diagnosis among children presenting symptoms of intussusception. The investigator and his staff will take appropriate actions to evaluate and treat the condition. A child with suspected intussusception will be referred to a local hospital in Dhaka (Children's hospital, Shishu Hospital). The hospital has the experience and capacity for diagnosis, non-surgical and surgical management of intussusception, and a pediatric surgeon at this hospital is a co-investigator on the protocol to assume

responsibility for the care of children suspected of having IS. During the study course, the parents/guardians will be instructed to contact the investigator and his staff immediately should the subject manifest any signs or symptoms they perceive as serious. No subjects will be deprived of existing care facilities.

Confidentiality of collected information will be maintained by keeping all data forms private and locked at the ICDDR,B Dhaka office with access limited to those working in the study. Study subjects will only be identified by study numbers.

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.

References

Anonymous. Withdrawl of rotavirus vaccine recommendation. JAMA 1999; 282: 2113-2114.

Azim T et al. Immune response of children who develop persistent diarrhoea following rotavirus infection. Clin Diag Lab Immunolo 1999; 6: 690-695.

Bernstein DI, Sander DS, Smith VE, Sciff GM, Ward RL. Protection from rotavirus reinfection: 2-year prospective study. J Infect Dis 1991: 164: 277-83.

Bernstein DI, Sack DA, Rotstein E et al. Efficacy of live, attenuated, human rotavirus vaccine 89-12 in infants: a randomized placebo-controlled trial. Lancet 1999; 354: 287-290.

Bernstein DI et al. Safety and immunogenicity of live, attenuated human rotavirus vaccine 89-12. Vaccine 1998; 16: 381-387.

Bernstein DI, Sack DA et al. Paper presented in Infectious Disease Society American, 2000. Abstract # 200225.

Committee for Proprietary Medicinal Products (CPMP). Note for guidance on clinical evaluation of new vaccines. The European Agency for the Evaluation of Medicinal Products, Human Medicines Evaluation Unit. London, May 1999.

Gentsch et al. Identification of group A rotavirus gene 4 types by polymerase chain reaction. J Clin Microbiol 1992; 30: 1365-73.

Gouvea et al. Polymerase chain reaction of amplification and typing of rotavirus nucleic acid from stool specimens. J Clin Microbiol 1990; 28: 276-282.

Ho Mei-Shang et al. Simultaneous administration of rhesus rotavirus vaccine and oral poliovirus vaccine:immunogenicity and reactogenicity. Pediatr Infect Dis J 1989;8:692-6.

Ing DJ, Glass RI et al. Immunogenicity of tetravalent rhesus rotavirus vaccine administered with buffer and oral polio vaccine. AJDC 1991;145:892-7.

Migasena S et al. Simultaneous administration of oral rhesus-human reassortant tetravalent (RRV-TV) rotavirus vaccine and oral poliovirus vaccine (OPV) in Thai infants. Vaccine 1995; 13(2):168-74.

Miller MA, McCann L. Policy analysis of the use of hepatitis B, Haemophilus influenzae type B, Streptococcus pneumonia-conjugate and rotavirus vaccines in National immunization schedule. Health Economics 2000; 9: 19-35.

Murphy TV, Garguiullo PM, Masssoudi MS et al. Intussusception among infants given an oral rotavirus vaccine. N Engl J Med 2001; 344: 564-72

Pickering LK, Morrow AL, Herrera I et al. Effect of maternal rotavirus immunization on milk and serum antibody titers. J Infect Dis 1995;172(3):723-8.

Rennels M, Ward RL et al. Concurrent oral poliovirus vaccine and rhesus-human reassortant rotavirus vaccination effects on immune responses to both vaccines and on efficacy of rotavirus vaccines. The US Rotavirus Vaccine Efficacy Group. J Infect Dis J 1996;173:306-13.

Unicomb et al. Anticipating rotavirus vaccines: hospital-based surveillance for rotavirus diarrhoea and estimates of disease burden in Bangladesh. Peditr Infect Dis J 1997; 16: 947-51.

Unicomb et al. A one year survey of rotavirus strains from three locations in Bangladesh. Arch Virol 1993; 132: 201-208.

Unicomb et al. Evidence of high-frequency genomic reassortment of group A rotavirus strains in Bangladesh: emergence of type G9 in 1995. J Clin Microl 1999; 37: 1885-91.

Vodopija I et al. Combined vaccination with live oral polio vaccine and the bovine rotavirus RIT 4237 strain. Vaccine 1988;4:233-6.

Ward R et al. Antibody to rotavirus on protection of adults challenged with a Human Rotavirus. J Infect Dis 1989; 159: 79-88.

World Health Organisation collaborative study group on oral poliovirus vaccine. Factors affecting the immunogenicity of oral poliovirus vaccine: a prospective evaluation in Brazil and Gambia. J Infect Dis 1995;171:1097-1106.

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 publications and peer reviewed journals, and disseminated in national and international conferences.

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)**

3.1 Collaboration with other scientists and research institutions

Dr. Richard Ward (University of Cincinnati, USA) will be a consultant on the project to assist with the validated serology IgA anti-RV. We currently are carrying out ELISA IgA serology at ICDDR,B; however, Dr. Ward's lab is the one which is generally regarded as having the reference lab for such serology and it will be helpful to have the methods used in Dhaka correspond with those of Dr. Ward for future studies.

The protocol is being planned to complement similar studies in South Africa under the direction of Dr. Duncan Steele. During the course of the studies, we plan to have joint investigator meetings and generally coordinate our work.

CV of Dr. K. Zaman

1. **(I) Name :** K. Zaman

   (ii) Designation : Epidemiologist

   (iii) Address : Child Health Programme, Public Health Sciences Division, ICDDR,B,Dhaka, Bangladesh, Tel: 8811751-60 ext. 2246, Fax: 880 2 8826050 Email: kzaman@icddrb.org

2. **Academic background:**

Degree	University	Field	Year
PhD	Johns Hopkins University USA	International Health	1999
MPH	Johns Hopkins University USA	International Health	1992
MBBS	Rajshahi University Bangladesh	Medicine, Paediatrics	1978

3. **Field of speciality:** Epidemiology, Infectious diseases, International Health, Paediatrics

4. **(a) Research experience:** Experienced in the design, implementation, and analysis of data from clinical and community-based epidemiological studies for 20 years
   (b) Other experience: Patient care: Clinical care of the patients with diarrhoeal and respiratory diseases
   Teaching: Served as a faculty member in different courses on 'Epidemiological methods in Public Health' organized by the ICDDR,B
   Teaching Assistant: Department of International Health, Johns Hopkins University, USA
   Administration: Overall supervision and management of ICDDRB Matlab Diarrhea Treatment Centre, MCH-FP clinic and Staff clinic

Publications of Dr. K. Zaman

1. Zaman K, Yunus M, Rahman A, Chowdhury HR, Sack DA. Efficacy of a packaged rice ORS among children with cholera and cholera like illness. Acta Paediatrica 2001 (in press).

2. Zaman K. Children's fluid intake during diarrhea: a comparison of questionnaire responses with data from observations. Doctor of Philosophy dissertation. **Johns Hopkins University School of Hygiene and Public Health**, Baltimore, Maryland, USA, 1999.

3. **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 Pediatrics** 1997;43:133-137.

4. **Zaman K**, Baqui AH, Yunus M, Sack RB, Chowdhury HR, Black RE. Malnutrition, cell-mediated immune deficiency and acute upper respiratory infections in rural Bangladeshi children. **Acta Paediatrica** 1997; 86: 923-927.

5. **Zaman K**, Zeitlyn S, Chakraborty J, Francisco A de, Yunus M. Acute lower respiratory infections in rural Bangladeshi children: patterns of treatment and identification of barriers. **Southeast Asian J Trop Med Pub Hlth** 1997;28:99-106.

6. **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.

7. **Zaman K**, Yunus M, Baqui AH, Hossain KMB. Surveillance of Shigellosis in rural Banghladesh : A 10-year review. **J Pak Med Asso** 1991; 41: 75 - 78.

8. **Zaman K**, Yunus M, Hossain KMB. Changing pattern of bio and serotypes of Vibrio cholerae 01 in rural Bangladesh. **J Pak Med Asso** 1987; 37: 57.

9. **Zaman K**, Baqui AH, Yunus M. Hypokalaemia and urinary anomalies in children with diarrhoea in rural Bangladesh. **J Pak Med Asso** 1986; 36: 20 - 21.

10. **Zaman K**, Islam MR, Baqui AH, Yunus M. Hypokalaemia in children with diarrhoea in rural Bangladesh. **Indian J Med Res** 1985; 81: 169 - 174.

11. **Zaman K**. Islam MR, Baqui AH, Yunus M. Nutritional status and electrolyte anomalies in children with diarrhoea in rural Bangladesh. **Nutr Rep Int** 1984; 30: 865 - 871.

12. **Zaman K**, Yunus M, Baqui AH. 100 years of cholera. **The Pulse** 1984; 53: 11 - 12.

13. **Zaman K**, Yunus M, Baqui AH, Hossain KMB, Khan MU. Cotrimoxazole resistant Shigella dysenteriae type 1 outbreak in a family in rural Bangladesh. **Lancet** 1983; ii: 796 - 797.

14. Henning B, Stewart K, **Zaman K**, Alam AN, Brown KH, Black RE. Lack of therapeutic effect of Vitamin A for non-cholera, watery diarrhoea in Bangladeshi children. **Eur J Clin Nutn** 1992; 46 : 437- 443.

15. The cholera working group, ICDDR,B: Albert MJ, Ansaruzzaman M, Bardhan PK, Faruque ASG, Islam MS, Mahalanabis D, Sack RB, Salam MA, Siddique AK, Yunus M, **Zaman K** (in alphabetical order).A large epidemic of cholera like disease in Bangladesh caused by Vibrio cholerae non 01. **Lancet** 1993;342:387-390.

16. Baqui Abdullah H, Yunus M, **Zaman K**. Community-operated treatment centres prevented many cholera deaths. **J Diar Dis Res** 1984; 2: 92 - 98.

17. Faruque ASG, Eusof A, Rahman ASMM, **Zaman K**. Study of makeshift hospital during cholera outbreak. **Bang Med Res Coun Bull** 1984; 10: 45 - 52.

18. Faruque ASG, Rahman ASMM, **Zaman K**. Young childhood diarrhoea management by mothers and village practitioners in rural Bangladesh. **Trop Geo Med** 1985; 37: 223 - 226.

19. Faruque ASG, Rahman ASMM, **Zaman K**. Young childhood diarrhoeal morbidity patterns in rural Bangladesh. **Bang Med J** 1985; 14: 66 - 69.

20. Baqui Abdullah H, **Zaman K**, Yunus M, Mitra AK, Hossain KMB, Banu H. Epidemiological and clinical characteristics of Shigellosis in rural Bangladesh. **J Diar Dis Res** 1988; 6: 21 - 28.

21. Baqui AH, Yunus M, **Zaman K**, Mitra AK, Hossain KMB. Surveillance of patients attending a rural diarrhoea treatment centre in Bangladesh. **Trop Geo Med** 1991; 43: 17 - 22.

22. Fauveau V, Yunus M, **Zaman K**, Chakraborty J, Sarder AM. Diarrhoea mortality in rural Bangladeshi children. **J Trop Pediatr** 1991; 37: 31 - 36.

23. Chowdhury HR, Fauveau V, Yunus M, **Zaman K**, Briend A. Is acute watery diarrhoea an important cause of morbidity and mortality among rural Bangladeshi children. **Trans R Soc Trop Med Hyg** 1991; 85: 128 - 130.

24. Baqui AH, Black RE, Mitra AK, Chowdhury HR, **Zaman K**, Fauveau V, Sack RB. Diarrhoeal diseases: The Matlab experience. In: Fauveau V. ed. **Matlab: Women, children and health**. Dhaka: ICDDR,B 1994: 161-186.

25, Chowdhury HR, Yunus M, Khan EH, **Zaman K**, Rahman R. Pivmecillinam resistant Shigella infections in rural Bangladesh. **Trop Doctor** 1995; 25:141-142.

26. Islam MS, Hasan MK, Miah MA, Yunus M, **Zaman K**, Sack RB, Albert MJ. Isolation of Vibrio cholerae 0139 synonym Bengal from the aquatic environment in Bangladesh: Implications for disease transmission. **Appl & Env Microbiol** 1994; 60: 1684-1686.

27. de Francisco A, **Zaman K**, Chowdhury HR, Wahed MA, Chakraborty, Yunus M. Vitamin A toxicity - a case of accidental ingestion. **Trop Doct** 1995; 25: 187.

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

29. **Zaman K.** Sack DA, Chakraborty J, Yunus M, Baqui AH, Black RE. Children's fluid intake during diarrhea: a comparison of questionnaire responses with data from observations. (submitted).

ANNEXE B. *CURRICULUM VITAE* OF other scinetist (*1 page maximum*)

Surname. **Yunus** **Date of birth: January 05, 1945**

First names(s): **Muhammad** **Nationality: Bangladeshi**

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

Degree	University/ Department	Year
M.Sc.	London School of Hygiene and Tropical Medicine, London, UK	1982
M.B.B.S.	Dhaka Medical College University of Dhaka Dhaka, Bangladesh	1968

Present and most recent posts held (type of post,institution/authority, dates)

Senior Scientist (May 2000-Current) ICDDR,B

Scientist (Jan'1993-Apr' 2000) **ICDDR,B**

Head, Matlab Health Research Programme,(Dec'1996-Current) Public Health Sciences Division, ICDDR,B

Coordinartor (Sep'1985-Nov'1996) Matlab Health and Research Centre, ICDDR,B

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 numberss, year

Yunus M, Zaman K, Khan EH,Chowdhury HR, Rahman Rahman A, Alam DS,Hoque E. *Surveillance of Vibrio Cholerae* 0139 patients atte a rural diarrhoea treatment centre [abstract]. *J Diarrhoeal Dis Res* 1995 Mar;13(1):54.

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

Sack RB, Rahman M, Yunus M, Khan EH. Antimicrobial resiatance in organisms causing diarrhoael disaeses. Clin Infect Dis 1997; 24(suppl):5102-5.

Francisco A de, Hall AJ, Unicomb L, Chakraborty J. Yunus M., Sack RB. Maternal measles antibody decay in rural Bangladeshi infants-implications for vaccianation schedules. Vaccine 1998; 16: 554-56.

Alam DS, Marks GC, Baqui AH, Yunus M, Fuchs GJ. Association between clinical type of dioarrhoea and growth of children under 5 years rural Bangladesh. Int J Epidemiol 200: 29.

ANNEX B. ***CURRICULA VITAE* OF OTHER SCIENTISTS INVOLVED**
*(1 page maximum per individual *)*

1. Surname: ARIFEEN — Date of birth: November 29, 1959

   First name(s): SHAMS EL — Nationality: Bangladeshi

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

Medicine, Surgery, Obs. & Gynae.	M.B.B.S.	Dhaka Medical College, Bangladesh	1983
Epidemiology	M.P.H.	Johns Hopkins University, USA	1991
Epidemiology and Int. Health	Dr.P.H.	Johns Hopkins University, USA	1997

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

Title:	Epidemiologist and Programme Head, Child Health Programme, PHSD, ICDDR,B
Department:	Child Health Programme, Public Health Sciences Division, ICDDR,B
Date of appointment:	January 1, 1998
Expected termination:	Not applicable

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

1. 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, Am J Clin Nutr 2000:72:1010-7.

2. Perry H, Weierbach R, Arifeen SE, Hossain I. A comprehensive assessment of the quality of immunization services in one major area of Dhaka City, Bangladesh. Trop Med Int Health 1998;3:981-92.

3. Baqui AH, Black RE, Arifeen SE, Hill, K, Mitra SN and Sabir AA. Causes of childhood deaths in Bangladesh: results nation-wide verbal autopsy study. Bull WHO 1998;76:161-171.

4. Baqui AH, de Francisco A, Arifeen SE, Siddique AK, Sack RB. Bulging fontanelle after supplementation with 25,000 of vitamin A in infancy using immunization contacts. Acta Paediatrica. 1995;85:863-6.

5. Baqui AH, Arifeen SE, Amin S & Black RE. Levels and Correlates of Maternal Nutritional Status in Urban Bangla European Journal of Clinical Nutrition, 1994;48:349-57.

CURRICULUM VITAE

Name: Tasnim Azim

Date of Birth: September 22, 1956

Nationality: Bangladeshi

Present Position: Associate Scientist and Head, Virology Laboratory, Laboratory Sciences Division, ICDDR,B.

Academic Qualifications:

Ph.D., 1989, Immunology, University of London, UK

MBBS, 1983, Medicine, University of Dhaka, Bangladesh

Publications (last three years):

1. Unicomb L, Banu NH, **Azim T**, Islam A, Bardhan PK, Faruque ASG, Hall A, Moe CL, Noel JS, Albert MJ and Glass RI. Astrovirus infection in association with acute, persistent and nosocomial diarrhea in Bangladesh. Ped. Infect. Dis. J. 1998; 17:611-614.

2. **Azim T**, Rashid A, Qadri F, Sarker MS, Hamadani J, Salam MA, Wahed MA and Albert MJ. Antibodies to shiga toxin in the serum of children with *Shigella*-associated haemolytic uraemic syndrome. J. Med. Microbiol. 1999; 48:11-16.

3. Francisco A de, Hall AJ, Alam N, **Azim T**. Hepatitis B infection in Bangladeshi mothers and infants. Southeast Asian J Trop Med Public Health. 1999; 30:296-298.

4. **Azim T**, Ahmad SM, Khuda S, Sarker MS, Unicomb LE, De S, Hamadani JD, Salam MA, Wahed M A and Albert MJ. Immune response of children who develop persistent diarrhea following rotavirus infection. Clin Diagn Lab Immunol 1999; 6: 690-695.

5. **Azim T**, Islam MN, Bogaerts J, Mian MAH, Sarker MS, Fattah KR, Simmonds P, Jenkins C, Choudhury MR and Mathan VI. Prevalence of HIV and syphilis among high risk groups in Bangladesh. AIDS 2000; 14: 210-211.

6. Hawkes S, **Azim T**. Health care systems in transition III. Bangladesh, Part II. Bangladesh's response to HIV-AIDS. J Public Health Med. 2000; 1: 10-3.

7. **Azim T**, Islam LN, Sarker MS, Ahmad SM, Hamadani JD, Faruque SM, Salam MA. Immune response of Bangladeshi children with acute diarrhoea who subsequently develop persistent diarrhoea. J Pediatr Gastroenterol Nutr 2000: 31: 528-35.

8. Schnorr Jens-Jörg, Cutts FT, Wheeler JG, Akramuzzaman SM, Alam MS, **Azim T**, Schneider-Schaulies Sibylle, ter-Meulen V. Immune modulation after measles vaccination of 6-9 months old Bangladeshi infants Vaccine (in press)

Curricula Vitae of Other Scientists Involved

1. Surname: FARUQUE Date of Birth:1.1.1951

First Name(s): ABU Nationality: Bangladeshi

2. Degree(s): MBBS, MPH

3. Most recent posts held: Scientist, Clinical Sciences Division, ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh

4. Recent Publications (no more than 5)

1. Qadri F, Das SK, Faruque ASG et al. Prevalence of toxin types and colonization factors in enterotoxigenic Escherichia coli isolated during a 2-year period from diarrhoeal patients in Bangladesh. J Clin Microbiol 2000; 38: 27-31.
2. Albert MJ, Faruque ASG et al. Case-control study of enteropathogens associated with childhood diarrhoea in Dhaka, Bangladesh. J Clin Microbiol 1999; 37: 3458-64.
3. Faruque ASG et al. Aetiological, clinical, and epidemiological characteristics of a seasonal peak of a diarrhoea in Dhaka, Bangladesh. Scand J Infect Dis 1998; 30: 393-6.
4. Faruque ASG et al. Shigellosis in children: a clinico-epidemiological comparison between *Shigella dysenteriae* type I and *Shigella flexneri*. Ann Trop Pediatr 1998; 18: 197-201.
5. Unicomb LE, Kilgore PE, Faruque ASG et al. Anticipating rotavirus vaccines: hospital-based surveillance for rotavirus diarrhoea and estimates of disease burden in Bangladesh. Pediatr Infect Dis J 1997; 16: 947-51.

1. Surname: Breiman Date of Birth: November 17, 1953

First Name(s): Robert Nationality: USA

5. Degree(s):

M.D. University of Arizona 1979
B.A University of Arizona 1975

Infectious Disease Fellowship—UCLA: 1984-1987
Internal Medicine Residency--- UCLA Program: 1979-1982

6. Most recent posts held:

Director, National Vaccine Program Office: 1995-2000

Chief, Respiratory Diseases Branch Epidemiology Section: 1989-1997

7. Recent Publications

Breiman RF, Keller DW, Phelan MA, Sniadack DH, Stephens DS, Rimland D, Farley MM, Schuchat A, Reingold AL Evaluation of effectiveness of the 23-valent pneumococcal capsular polysaccharide vaccine for HIV-infected patients. *Arch Intern Med.* 2000;160:2633-8.

Kombo L, Gerber M, Pickering L, Atreya CD, Breiman RF. Setting a research agenda on the association of rotavirus vaccines with intussusception: Summary of an NIH/NVPO workshop. Submitted for publication.

Nuorti JP, Butler JC, Farley MM, Harrison LH, McGeer A, Kolczak MS, Breiman RF. Cigarette smoking and invasive pneumococcal disease. *N Engl J Med* 2000 Mar 9;342:681-689.

Lieu TA, Ray GT, Black SB, Butler JC, Klein JO, **Breiman RF**, Miller MA, Shinefield HR. Projected cost-effectiveness of pneumococcal conjugate vaccination of healthy infants and young children. *JAMA* 2000;283(11):1460-8.

Hofmann J, Cetron MS, Farley MM, Baughman WS, Facklam RR, Elliott JA, Deaver KA, Breiman RF. The prevalence of drug-resistant *Streptococcus pneumoniae* in Atlanta. N Eng J Med 1995;333:481-486.

NAME:	POSITION TITLE:
David A. Sack	Director, ICDDRB,B, Centre for Health and Population Research

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.*)

INSTITUTION AND LOCATION	DEGREE (*if applicable*)	YEAR(s)	FIELD OF STUDY
Lewis and Clark College, Portland Oregon	BS	1961-65	Natural Science
University of Oregon Medical School, Portland, Oregon	MD	1964-68	Medicine
University of Iowa School of Medicine, Iowa City, Iowa		1968-70 & 1971-73	Internship & Residenc Internal Medicine
Johns Hopkins University School of Medicine, Baltimore		1974-75	Fellowship, Infectious Diseases

1. Sack DA, Merson MH, Wells JG, Sack RB, Morris GK. Diarrhea associated with heat-stable enterotoxin-producing strains of *Escherichia coli.* Lancet. ii:239-241, 1975.
2. Sack DA, Kaminsky DC, Sack RB, Itotia JN, Arthur RR, Kapikian AZ, Orskov F, Orskov I. Prophylactic doxycycline for travellers' diarrhea: Results of a prospective double-blind study of Peace Corps Volunteers in Kenya. N Engl J Med. 298:758-763, 1978.
3. Sack DA, Chowdhury AMAK, Eusof A, Ali MA, Merson MH, Islam S, Black RE, Brown KH. Oral hydration in rotavirus diarrhea. A double blind comparison of sucrose with glucose electrolyte solutions. Lancet. ii:280-283, 1978.
4. Sack DA, Islam S, Brown KH, Islam A, Kabir AKMI, Chowdhury AMAK, Ali MA. Oral therapy in children with cholera: A comparison of sucrose and glucose electrolyte solution. J Pediat. 96:20-25, 1980.
5. Sack DA, Stephensen CB. Liberation of hydrogen from gastric acid following administration of oral magnesium. Dig Dis Sci. 30: 1127-1133, 1985.
6. Clemens JD, Sack DA, Harris JR, Chakraborty J, Khan MR, Stanton BF, Kay BA, Khan MU, Yunus M, Atkinson W, Svennerholm A-M, Holmgren J. Field trial of oral cholera vaccines in Bangladesh. Lancet. ii: 124-127, 1986.
7. Sack DA, Freij L, Holmgren J. Prospects for public health benefits in developing countries from new vaccines against enteric infections. J Infect Dis. 163:503-6, 1991.
8. Sack DA, Hoque ATMS, Huq A, Etheridge M. Hypothesis: Natural infection with *P. shigelloides* protects developing-country residents against *S. sonnei* infection. Lancet 343:1413-1415, 1994.
9. Bernstein DI, Davidson B, Glass RI, Rodgers G, Sack DA for the U.S. Rotavirus vaccine efficacy group. Evaluation of rhesus rotavirus monovalent and tetravalent reassortant vaccines in U.S. children. JAMA 273:1191-6, 1995.
10. Sack DA, Shimko J, Sack RB, Gomes G, MacLeod K, O'Sullivan, D, Spriggs D. Comparison of Alternative Buffers With Peru-15, a New Live, Oral Cholera Vaccine in Outpatient Volunteers. Infect Immun 65:2107-2111, 1997.
11. Sack DA, Sack RB, Shimko J, Gomes G, O'Sullivan D, Metcalfe K, Spriggs D. Evaluation of Peru-15, a new live oral vaccine for cholera, in volunteers. J. Infect Dis 176:201-5, 1997.
12. Sack DA, Tacket CO, Cohen MB, Sack RB, Losonsky GA, Shimko J, Nataro JP, Edelman R, Levin MM, Gianella RA, Schiff G, Lang D. Validation of a Volunteer Model of Cholera Using Frozen Bacteria as the Challenge. Infect Immun 66:1968-72, 1998.
13. Wagatsuma Y. Aryeetey ME. Sack DA. Morrow RH. Hatz C, Kojima S. Resolution and resurgence of schistosoma haematobium-induced pathology after community-based chemotherapy in Ghana, as detected by ultrasound. J Infect Dis. 179:1515-22, 1999.
14. Bernstein DI, Sack DA, Rothstein E, Reisinger K, Smith VE, O'Sullivan D, Spriggs DR, Ward RL. Efficacy of live, attenuated, human rotavirus vaccine 89-12 in infants: a randomised placebo-controlled trial. Lancet. 354:287-90, 1999.
15. Faruque SM, Siddique AK, Saha MN, Asadulghani Rahman MM, Zaman K, Albert MJ, Sack DA, Sack RB. Molecular characterization of a new ribotype of Vibrio cholerae O139 Bengal associated with an outbreak of cholera in Bangladesh. J Clin Microbiol. 37:1313-8, 1999.

PROJECT TITLE: Study of safety and immunogenicity of human rotavirus vaccine (RIX4414)

PI: Dr. K. Zaman

BUDGET DETAILS

Total study period: 15 months

SALARY Position	Pay Level	# of Staff	Month	% of Effort	Monthly Rate	Sub-total	Total (US $)
Dr. K. Zaman	NOC	1	15	25%	1,260	4,725	
Dr. Md. Yunus	NOE	1	15	20%	2100	6,300	
Dr. Shams El Arifeen	P3	1	15	15%	7090	15,953	
Dr. Tasnim Azim	NOC	1	15	20%	1083	3,249	
Dr. Goutam Podder	NOA	1	15	50%	828	6,210	
Dr. ASG Faruque	NOD	1	15	10%	1811	2,717	
Medical Officer	NOA	1	15	100%	587	8,805	
Reseach Investigator	NOA	1	15	100%	587	8,805	
Data Manager	NOA	1	15	20%	587	1,761	
Project Office Manager	NOA	1	15	20%	587	1,761	
Research Officer	GS 5	1	15	100%	325	4,875	
Senior Staff Nurse	GS5	1	15	100%	325	4,875	
Admin Asst	GS4	1	15	100%	267	4,005	
Field Res Atten	spl.lev	10	15	100%	60	9,000	
Field Res Asst	GS3	10	15	100%	210	31,500	
Data entry Tech	GS3	1	15	100%	210	3,150	
Programmer	GS6	1	15	25%	422	1,583	
Sr. Lab Attendant	GS-2	1	15	100%	176	2,640	
Driver	GS-2	1	15	100%	176	2,640	
Subtotal of local salaries						124,553	
Expected salary rise						12,455	
Dr. Rob Breiman	-	1	15	10%		-	
Prof. David Sack	-	1	15	5%	13,370	10,028	
Consultant - monitoring						25,000	
Consultant - virology						10,000	
	SUB-TOTAL PERSONNEL:						182,035
TRAVEL COSTS							
International travel						20,000	
Investigator Meeting						5,000	
Local transportation cost						5,000	
	SUB-TOTAL:						30,000
SUPPLIES & OTHER COSTS							
Office rent, communications and utilities						10,000	
Mobile phone						1,500	
Drugs						1,000	
Office & field supplies						1,500	
Printing & Publications of forms						10,000	
Spoon, cups, cylinder etc.						750	
Needles, Syringes, thermometers, etc.						8,000	
Furniture						1,500	
Cold pack						100	
Lab supplies cost						4,000	
Shipping cost for sending specimen						10,000	
Cost for safety monitoring Comm						1,000	
Cold box (4)						250	
Publications						500	
Hospitalization/Attend cost (Shishu Hospital)						2,050	
Service charges (Shishu Hospital)							
- Prof. S. Karim			15	5%	100	1,500	
- Medical Officer			15	15%	30	450	
	SUB-TOTAL:						54,100
INTER-DEPARTMENTAL SERVICES							
Repair & maintenance						500	
Transport (land)						4,000	
Specimen handling fees						5,000	
IgA ELISA@4X3X525	4	4	576			9,216	
RV ELISA@ 3.3X2400	3.3	1	2430			8,019	
Culture RV						5,000	
Typing RV (G/P/Electropherotyping)						6,000	
Medical Illustration						500	
Mimeography, Library charge etc.						500	
	SUB-TOTAL:						38,735
CAPITAL ITEMS							
Computers (2)/printer/UPS/accessories etc.						5,000	
Gel electrophoresis apparatus						3,000	
Refrigerator (1)						1,000	
Freezer						6,000	
	SUB- TOTAL						15,000
TOTAL DIRECT COSTS:							319,870
OVERHEAD @25%							79,968
TOTAL PROJECT COSTS:							399,838

31.5.24

M. Rahman Chowdhury
Senior Budget & Cost Officer
ICDDR, B, Mohakhali
Dhaka-1212, Bangladesh.

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.

Budget justification

Briefly relate each item in the budget (personnel, supplies, equipment, animals, patient costs, travel, etc.) to the activities outlined in the research proposal

The total duration of the proposed study will be of 15 months. The study involves recruitment of study subjects, either one or two or three doses of vaccination to about 566 healthy children (toddlers and infants), follow up at their homes and collection of blood and stool specimens.

Investigators: The amount budgeted for the investigators reflects a reasonable estimate of the minimum time required to implement the study. Dr. K. Zaman, PI of the study will be responsible for overall implementation of the study. Drs. Md Yunus and Shams El Arifeen will provide advice on epidemiological aspects, Dr. ASG Faruque on clinical aspects while Drs. Tasnim Azim and Goutam Podder will be responsible for laboratory methods. Prof. S. Karim (Shishu hospital) will examine suspected cases of intussusception and will take appropriate measures. Medical Officer (Shishu) will assist the Paediatric surgeon for the management of referred cases. Prof. David Sack and Dr. Rob Breiman will provide overall guidance of the study.

Research Investigator will coordinate all clinical and administrative activities.

Medical officer will be based in the clinic and be responsible for examination of the subjects, recruitment and vaccination. S/he will provide necessary treatment.

Senior staff nurse: will assist medical officer in examining patients and collection of specimens. Responsible for dispensing medicines (if required).

Field Research Assistants/attendants: Inform families of the purpose and scope of the study, offer participation and bring subjects to their clinic.

Data Manager/ Programmer: Overall data management and designing systems for analysis of data.

Data entry technician: entry of data

Senior Lab attendant: Collection and recording of samples.

Office manager: ensure overall logistics.

Consultant: Advise and guide for smooth running of the project.

GCP monitor: will check Case Report Forms (CRFs) for completeness and correspondence with source documents, monitor regulatory files and validate data files in computer.

Supplies

Computers: Two computers (one for data entry and analysis and other to be used by project office manager/Admin Asst) will be required.

Mobile phone: To communicate with the field staff for any adverse events and referral of patients.

Gel Electrophoresis apparatus: For typing of rotavirus.

Refrigerator/cold box: Keeping and transportation of specimens.

Laboratory tests: ELISA, Culture and typing for identification of rotavirus.

Transport costs: To visit families, bringing of subjects and transport cost of the families.

Hospitalization cost: Treatment cost for any hospitalized cases.

Office rent: Cost for renting the clinic

International travel: Consultation meeting with other RV partners

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)

Principal Investigator of the study " Epidemiology and surveillance of multidrug resistant Mycobacterium tuberculosis and assessment of directly observed therapy short course (DOTS) programme in selected areas of Bangladesh"
Funded by USAID, Amount: US$ 577, 604

APPENDIX Annex 1

International Centre for Diarrhoeal Disease Research, Bangladesh

Voluntary Consent Form

Title of the Research Project: A randomised, placebo-controlled study of the safety, reactogenicity and immunogenicity of an orally administered human rotavirus vaccine (RIX4414) in healthy children in Bangladesh

Principal Investigator: Dr. K. Zaman

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.

Voluntary Consent Form

Rotavirus is the most important cause of acute gastroenteritis requiring hospitalisation of young children in developed countries. Symptoms of rotavirus disease mainly include diarrhea and vomiting. Deaths occur from rotavirus diarrhea when the diarrhea is sufficiently severe to cause dehydration. In developing countries, rotavirus gastroenteritis is an enormous problem. World-wide, 60-80 young children with rotavirus gastroenteritis die every hour. At the Dhaka and Matlab hospitals, rotavirus accounts for about 40% of the diarrheal illnesses of children under 5 years of age. Deaths due to rotavirus in these settings are extremely rare because rehydration is provided; however, in many areas of Bangladesh, adequate treatment may not be given, resulting in an estimated 20,000 deaths due to rotavirus in year. The only practical solution to control this global health problem is a rotavirus vaccine for infants. Yet today, there is no rotavirus vaccine available anywhere.

Several rotavirus vaccines have been tested for infants. The previous vaccine (RotaShield™) prevented 90% of severe rotavirus gastroenteritis and worked so well that few vaccinated children had to stay in the hospital for treatment of rotavirus dehydration. But the use of the vaccine was stopped because it appeared to increase the rate of intussusception. GlaxoSmithKline (GSK) Biologicals has now developed a new rotavirus vaccine (HRV vaccine) based on a human rotavirus. Clinical studies in US children and infants demonstrated the safety, immunogenicity and efficacy of the vaccine in infants.

The study will be conducted to test how infants (aged 6-14 weeks) in Bangladesh respond to the human rotavirus vaccine. The main purpose of this study is to determine the dose regimen that is appropriate for infants in Bangladesh. We will also look for any side effects (like fever, fussiness, vomiting, diarrhea and runny nose/cough). To help us do this we are asking if you will allow your child to join this trial.

If you join, you will be required to bring your child to our clinic 3 times each 4 weeks apart. Your child will be given a physical examination and his/her height and weight will be measured. You will be asked questions about your child's medical history and the medicines that your child may be taking. Your child will be randomly assigned (like flipping a coin) to one of four groups, with a 6 out of 7 chance of receiving the investigational human rotavirus vaccine (either the low or higher vaccine dose) and a 1 out of 7 chance of receiving a placebo. Among the vaccines, each child will receive two or three doses of vaccine orally given one month apart. All study subjects will be observed closely for at least 30 minutes following the administration of vaccines/placebo. During the study, you should contact the investigator and his staff (field worker) immediately should your child manifest any signs or symptoms that you perceive as serious. The study physician and the study nurses will be available for your consultation for the entire study period. Your child will receive routine vaccinations recommend by the Expanded Program on Immunizations (EPI) at the time of the administration of the study vaccine/placebo. We will collect 3 ml blood from the arm of your child before each vaccine/placebo doses and 4 weeks after the last dose. Stool samples will be collected from a sub group of children on day 0,3, 7, 14, 21 after each dose of vaccine/placebo. To assess any adverse events a field worker will visit to your child daily for the first 2 weeks after vaccination and then twice weekly for another 4 weeks, and thereafter monthly active surveillance to complete 6 months of follow up.

There may be risks if your child enters this study. The risks may be from the use of a new human rotavirus vaccine and from collection of blood samples. Although it has been given safely to a small number of adults, toddlers, and infants, it may have unknown side-effects. The human rotavirus vaccine may cause allergic reactions. Your child must stay in the clinic for 30 minutes after each vaccine dose so that any reaction can be treated. Nevertheless, there is the possibility that any rotavirus vaccine may cause intussusception (blockage in the small intestine).Your doctor and the study staff know of the possible risk of intussusception. They have arranged for prompt examination of any child in this study with suspected intussusception. They will take appropriate action to evaluate and treat the condition. Child with suspected intussusception will be referred to a local hospital in Dhaka (Children's hospital, Shishu Hospital). The hospital has the experience and capacity for diagnosis, non-surgical and surgical management of intussusception. All these will be free of costs. Symptoms of intussusception are severe abdominal pain (colic), persistent vomiting, bloody stools, abdominal bloating and high fever (even up to 41°C). There may be momentary, mild discomfort and bruising of the skin where the needle is inserted to draw blood.

Your child's participation is completely voluntary. Refusal to take part or continue with the study will involve no penalty or loss of benefits or attention to which your child are otherwise entitled. Also you may withdraw your child from the study at any time without any penalty or change in the routine care your child receives. You may wish to receive a signed copy of this form.

Your child's participation in the study will be treated as confidential. It will be necessary for representatives of GSK or possibly health authorities / drug regulatory agencies to access your child's medical records. Your child will not be referred to by name in any report of the study. Your child's identity will not be disclosed to any person, except for the purposes described above and in the event of a medical emergency or if required by law.
Your child's data will be processed electronically to determine the outcome of this study, and to provide it to health authorities/drug regulatory agencies. Your child's data may be transferred to other countries (such as USA) for these purposes GSK complies with internal procedures to

protect personal information. The data may also be used for other medical research purposes. You may be entitled under law to access your child's personal data and to have any justifiable corrections made. If you wish to do so, you should request this from the doctor conducting the study. Right to ask questions and/or withdraw from the study

If you have any questions, please contact:

Name of investigator: Dr. K. Zaman

Address of investigator: Child Health Programme, PHSD, ICDDR,B, Dhaka

Telephone number of investigator: 8811751-60 ext. 2246

Fax number of investigator: 8826050

If the vaccine is efficacious, your child may have the benefit of being protected against rotavirus diseases. If it proves to be useful, also other children may benefit from it in the future. There will be no charge for study-related doctor visits, examinations, and laboratory tests. All vaccines will be provided free of charge.

If your child becomes ill or injured as a result of taking part in this clinical study, medical treatment will be provided according to good clinical practice and costs of such treatment will be paid for by GSK Biologicals. All participants in the study are covered by global insurance policy contracted by GSK Biologicals. If you have any questions concerning the availability of medical care or if you think you have experienced a research-related illness or injury, please contact:

Name of investigator: Dr. K. Zaman

Address of investigator: Child Health Programme, PHSD, ICDDR,B, Dhaka

Telephone number of investigator: 8811751-60 ext. 2246

Fax number of investigator: 8826050

Informed Consent Signature Form

The clinical study has been clearly explained to me and I have read and understood the information provided. I agree that my [son/daughter/ward] be enrolled in the study. I understand that my [son/daughter/ward] has the right to decline to enter the study and to withdraw from it at any time for any reasons, without consequence to his/her present or future health care and attention which my child/ward receives from his/her healthcare provider. I have been made aware of my right to access and request correction of my child's/ward's personal data.

I, _____ ,

(subject's parent or legal guardian's first name and family name)

hereby freely give my consent for my child/ward to take part in this [clinical/vaccine] study.

Participant's Name: _____

(First Name, Family Name)

Participant's signature (where applicable): _____

Parent/Guardian's name: _____

(First Name, Family Name)

Parent/Guardian's signature: _____

Relationship to participant: _____

Participant's main address: _____

Participant's phone number: _____

Date: _____ Time: _____

(DD-MM-YY)

Witness: _____

Statement by Doctor, Nurse or Project Assistant who conducted the informed consent discussion:

I have carefully explained the nature, demands and foreseeable risks and benefits of the vaccination study to the person named above and witnessed the completion of the written consent form.

Name: _____

Signature: _____

Designation: _____

Date: _____ Time: _____

(DD-MM-YY)

ANNEX II

Follow-up of intussusception cases

In light of the possible increased risk of intussusception following administration of a previously licensed rotavirus vaccine, the safety of the candidate HRV vaccine will be monitored vigilantly during the clinical studies.

The investigator will be asked to inform the parents/guardians of the signs and symptoms of intussusception. Parents/guardians/caretakers of study subjects will be asked to contact the investigator if they notice any signs or symptoms indicative of intussusception. Symptoms consistent with intussusception are severe colicky abdominal pain, persistent vomiting, bloody stools, abdominal bloating and fever up to 41°C. The investigator will be aware of the possible increased risk of intussusception and will consider this diagnosis among children presenting these symptoms. The investigator and his staff will take appropriate actions to treat the condition. Child with suspected intussusception will be referred to a local hospital in Dhaka (Children's hospital, Shishu Hospital). The hospital has the experience and capacity for diagnosis, non-surgical and surgical management of intussusception.

If any case of intussusception should occur during this clinical study, the following procedures will be followed by the investigator for work-up of the intussusception cases.

Case ascertainment

The diagnosis of intussusception should be documented by radiography. Documentation by ultrasonography will be optional depending on availability of necessary expertise.

Data collection for intussusception cases

The investigator will document all available information regarding any intussusception cases occurring during the clinical studies on the Serious Adverse Event pages and fax within 24 hours (1 calendar day) of his/her becoming aware of the event to the GSK Biologicals Contact for Serious Adverse Event (SAE) Reporting.

The investigator should follow the same procedures for reporting intussusception cases as for other SAEs. To allow for a complete assessment of the intussusception cases, information on the subject's feeding practices, immunisation history, as well as any other information thought necessary for assessment by the study staff should be reported to the SB safety contact ***by using the IS reporting form.***

Serum, throat and stool specimen will be collected from intussusception cases

Idiopathic intussusception is thought to be related to lymphoid hyperplasia in the intestinal sub-mucosa and/or mesenteric adenitis resulting from infections. Infectious agents most clearly linked to intussusception are enteroviruses and respiratory adenoviruses. Human rotaviruses also may cause intussusception, although epidemiological data suggest this must be very unusual. In theory, any agent able to replicate in the small intestine could provoke this condition.

GSK will use a central laboratory to perform RT-PCR on throat swabs and stool samples for enteroviruses and adenoviruses and on stool samples alone for rotaviruses. The physician treating a case of intussusception should submit stool samples to the hospital microbiology laboratory for culture of enteric pathogens such as Salmonella, Shigella, Campylobacter, and Yersinia. If culture results suggest presence of enteropathogenic *E. coli,* representative colonies should be retained for further evaluation. The samples to be collected and their handling are described below

If possible a stool specimen should be collected just prior to as well as samples 24 hours and 48 hours after the reduction. The hospital microbiology laboratory should divide each stool specimen into an aliquot for its own testing and two additional aliquots of at least 2 grams each to be frozen at –20°C to –70°C. The frozen stool samples will be used for RT-PCR and other studies to be arranged by GSK Biologicals, such as virus culture, antigen detection by immunoassay, or electron microscopy for virus-like particles. Accordingly, a complete set of stool specimens will be comprised of 3 specimens submitted for bacterial culture and 6 frozen specimens retained for shipment to GSK Biologicals. In the event that faeces are unobtainable at any of the requested sampling times, 3 separate rectal swab specimens should be collected.

One swab specimen should be submitted for bacterial culture and the other 2 swabs should be placed each in a separate tube of 2 ml of sterile virus transport media and frozen at –20°C to –70°C.
A throat swab should be collected as soon as possible after intussusception is diagnosed. The throat swab should be placed in 2 ml of sterile virus transport media and frozen at –20°C to –70°C.
In case of surgical reduction, a surgical specimen of any enlarged mesenteric lymph will be obtained. If bowel or the appendix is resected, these specimens also should be included in the evaluation. If possible, resected tissue should be divided into 3 aliquots to be processed for routine fixation, for electron microscopy (fixation in 4% paraformaldehyde and 1% glutaraldehyde), and for frozen sectioning for detection of virus antigens by immunofluorescence. Routinely fixed specimens should be examined for histopathologic evidence of acute inflammation and presence of virus inclusions or other diagnostic signs. Additional testing including referral of tissue blocks for outside review and/or tests using immunohistochemistry, in situ hybridisation, or PCR will be arranged by GSK Biologicals in consultation with the Attending Pathologist.
Acute and convalescent blood (at least 2 ml of each) will be collected and stored at –20°C for serologic testing. These specimens will be supplemented by antecedent serum specimens from the patient already collected under this protocol. Testing will be arranged by GSK Biologicals to detect an acute antibody response to any pathogen identified by stool and/or throat swab tests or by histopathologic evaluation of tissue.

Annex III

Composition of RIX 4414 Vaccine

The vaccine composition is as follows:

89-12 HRV strain (RIX4414)	10^5 or 10^6 ffu
Dulbecco's Modified Eagle Medium (DMEM)	3.7 mg
Sucrose	9 mg
Dextran	18 mg
Sorbitol	13.5 mg
Amino acids	9 mg
CaCO3	60 mg
Starch	

Reviewer # 1

Title: A randomised, placebo-controlled study of the safety, reactogenicity and immunogenicity of an orally administered human rotavirus vaccine (RIX4414) in healthy children in Bangladesh.

Summary of Referee's Opinions: Rank Score

	High	Medium	Low
Quality of project	x		
Adequacy of project design	x		
Suitability of methodology	x		
Feasibility within time period	x		
Appropriateness of budget	x		
Potential value of field of knowledge	x		

CONCLUSIONS

I support the project proposal

a) without qualification	x
b) with qualification	
c) on technical grounds	x
d) on level of financial support	x

I do not support the project proposal _____

Please briefly provide your opinions of this proposal, giving special attention to the originality and feasibility of the project, its potential for providing new knowledge and the justification of financial support sought; include suggestions for modifications (scientific or financial) where you feel they are justified.
(Use additional pages if necessary)

This application is designed to investigate the safety and immunogenicity of a new rotavirus vaccine. Rotavirus is the leading cause of dehydrating diarrhea and results in significant morbidity and mortality. Thus, the need for a safe and effective rotavirus vaccine, in Bangladesh and worldwide, is clearly demonstrated.

The vaccine under consideration is RIX 4414. It is derived from an attenuated human strain. Although this vaccine has undergone safety and immunogenicity testing in other countries, the investigators wish to study a less well nourished group of children in Bangladesh. The application would have been improved by a listing of the previous safety and immunogenicity data.

This is a dose escalation study and is well justified. There are uncertain dose requirements in the target population; the vaccine has undergone additional processing and passaging since previous testing; the effect of routine EPI vaccines will be evaluated during this study and finally, to warrant shelf life, the commercial lots will have a higher release titer than minimally required.

Safety considerations include the observation of fever, irritability and local swelling in a previous live rotavirus vaccine. Reactogenicity and adverse events will be assessed after a single dose of each virus concentration or placebo through daily home visits for the first 2 weeks and then twice weekly home visits for another 4 weeks, and thereafter monthly active surveillance to complete 6 months of follow up. However, the exact criteria for active surveillance are not clearly identified.

In addition, the increased risk of intussusception (IS) due to rotavirus vaccines is well considered in this application. The reasons that RIX4414 is unlikely to cause IS include the following. Wild type rotavirus does not appear to be a cause of IS since the seasonality with rotavirus is so marked in industrialized countries, yet there is no corresponding seasonality for IS. Secondly, RIX4414 is a human strain, typical of the viruses that every child will become infected with early in life, so vaccination does not induce an exposure that would not otherwise occur, unlike administration of rhesus rotavirus vaccine which represented a virus that would not normally infect children. Thirdly, the risk of IS is primarily during the age 4 to 12 months, and this study will complete vaccination prior to this time period. Nevertheless, all subjects receiving RIX4414 during clinical trials will be informed of the risk of IS, and the investigators will ensure that any potential case of IS is rapidly identified and treated. Moreover, given the incidence of IS (approx. $1:10^4$) due to RotaShield, the previous rotavirus vaccine, there is unlikely to be a statistically significant increase in IS in the studied population.

The statistical and power analyses appear adequate and a DSMB is to be established.

It is unclear whether the support budget comes at all from GSK Biologicals, the manufacturer of the vaccine. It is difficult for me to interpret the budget in light of my lack of knowledge about local costs and customs, e.g. furniture, rent for the clinic space, etc.. Overall, the budget appears well justified except for the consultant position ($25,000). This is to "advise and guide smooth running of the project." If this includes costs associated with a DMSB and travel and is required, then it is acceptable. Otherwise, it should be better justified.

Reviewer # 2

ZAMAN, K. MOHAMMAD --V27/181/143 « A randomised placebo-controlled study of the safety, reactogenicity and immunogenicity of an orally administered human rotavirus vaccine (RIX4414) in healthy children in Bangladesh. »

This protocol proposes to conduct a randomized, placebo-controlled, self-contained study within one community in Bangladesh to assess reactogenicity and immunogenicity of one or two orally administered doses of a live, attenuated human-derived rotavirus vaccine (RIX4414) among young children. Although the vaccine has already undergone safety, immunogenicity studies in other countries, the investigators plan on repeating the safety studies in an older group prior to studies in infants to insure the vaccine's safety in the less well nourished children of Bangladesh. In the first part of this study, safety of escalating doses will be assessed in 150 toddlers (2-4 years old) and 150 infants (6-14 weeks old) in a stepwise manner. Viral shedding in each group will be assessed. Routine EPI vaccines should be administered according to local recommendation (at 6,10 and 14 weeks of age) but not concomitantly with the studied vaccine. The rotavirus vaccine will be separated from the routine vaccines, including OPV by at least 14 days.

The group at ICDDR,B has extensive experience in conducting vaccine studies including a recent trial with the rhesus rotavirus vaccine Dr D. Sack has worked previously in the original studies with the SKB vaccine. This protocol would begin the groundwork of testing an RV vaccine that may arrive at licensure in 3-5 years. Several comments raised :

1. The protocol does not include a consent form or evidence of a local ethical review. Given the issues of IS required in the protocol, the handling of this issue in the consent form would be important to review.

2. The investigators go to great pains to provide active monitoring of the vaccinees for IS in the follow-up period. They do not spell out the criteria their field staff or clinicians might use to triage patients from the field with suspicious symptoms or the diagnostic procedures or algorythm to be followed if a child with these symptoms should appear at the clinic.

3. Follow-up of patients will proceed for 6 months, an extremely long period for simple safety study where the only long term sequella identified to date occurs within 2 weeks of immunization. Is this prolonged follow-up required ? If they want to pursue such a long follow-up, shouldn't they include surveillance for diarrhoeal events (and rotavirus) since perhaps 50% of the rotavirus diarrhoea that these children will experience will occur between the 4^{th} and 10^{th} month of life ?

4. The rationale for two doses is a bit unclear and is unexplained in the background. Why is this group testing different doses ? The original studies with this vaccine indicated very good seroconversion (80-90%) following a single 105 dose in American infants. The trials of the vaccine being planned in the US and Latin America and those under way in Finland apparently are using this dose which may be the dose under consideration for the licensed product. In the immunogenicity study, it would seem that the question to be addressed (and the basis of sample size calculations) should be « Does the vaccine work as well in Bangladesh infants as in American infants ? » If the first 50 children given 2 doses of the 10^5 dose had a 90% seroconversion to the vaccine, would there be any rationale to test the higher 10^6 dose ? And if there was comparable seroconversion to a single dose, would it be still necessary to test a second dose regimen ?

5. The large sample size determined for the immunogenicity study is based upon detecting a 15% difference in the seroconversion rate of the 2 different doses. It seems this assumption needs to be reassessed with real data. The investigators comment that they tested the rhesus vaccine in Bangladesh children already. Did the immune response of Bangladeshis to this vaccine differ from the immune response of American children? If so, this would be a clear rationale to consider raising the dose. If the responses were the same, the rationale for dose changes would need to be reassessed. Furthermore if the 10^5 dose produced high (80-90%) seroconversion in infants but substantial fevers, what would the next step be ? Accept the fevers or decrease the dose to 10^4 ?

6. It is not clear in the stepwise vaccine testing why sera is not being taken as part of the first safety studies in infants. Clearly, safety of the vaccine is key and could be addressed without the collection of sera. At the same time, collection of sera could provide a clear direction for the next study and allow a proper basis for sample size calculations. As stated in the proposal, the simple IgA serology could be assessed in one month and would help define the second phase of immunogenicity studies being planned.

7. In the protocol, the authors state reasons for exclusion which include malnutrition, hypersensitivity to any of the vaccine components, use of any immunosuppressing drugs etc. These conditions and components should perhaps be spelled out. What are the vaccine components that could lead to hypersensitivity? And which drug used in Bangladeshi infants 2-3 months of age would be considered immunosuppressing? Also, if a mother or other woman in a household were to become pregnant after an infant was enrolled and immunized, would this be a reason to exclude the child? According to the elimination criteria during the study, it would.

8. The rationale for separating administration of the RV vaccine from OPV and the other vaccines is not clear. OPV resides in the gut for up to 6 weeks following administration so separating the RV from the OPV immunization may make little difference. Furthermore programmatically, RV vaccine would likely and logically be administered as part of the EPI programme. Instead of separating the doses and vaccinations, why not administer the vaccines together and simply test the OPV neutralization titers in the final serum? The presence of good and comparable titers in the 2 groups would be expected and would prevent further delay in speeding to an acceptable dosing regimen. If there is serious concern about decreasing the OPV titers from theoretical interference, one could administer to all children a fourth OPV dose at the final visit when sera is collected to ensure that all children are fully protected. Past experience with the RRV allowed these vaccines to be administered together without obvious interference. It would be unlikely to ever have an immunization programme with RV vaccine administered separately from the other vaccines. Why begin this in Bangladesh when the goal is to arrive at an immunization schedule for a large trial and to have a vaccine that could only practically be delivered as part of the EPI programme ? Better to keep the trial simple and merely measure neuts to OPV in the final sera.

In summary this study is high priority for WHO and the global community and should be pursued. At the same time the rationale for testing different vaccine doses, separating the RV and OPV doses, the sample size calculations, and the extensive staging with large numbers should be reassessed, particularly if serology were included in the original 150 infants and if seroconversions to the 105 dose were reasonably high. This is an important trial and the SC felt that this project is a priority to pursue but the protocol should be revised and rethought addressing some of the concerns mentioned. The SC members recommended to support this project after revision at a level of US$40,000.

Response to external Reviewers' comments

Reviewer # 1

Listing of the previous safety and immunogenicity data (para 2, page 1)

Summary results (including safety and immunogenicity data) of the studies conducted with human rotavirus 89-12 strain have been given in table 1 (page 11).

Active surveillance (para 4, page 2)

Reactogenicity and adverse events will be assessed after a single dose of each virus concentration or placebo through daily home visits for the first 2 weeks and then twice weekly home visits for another 4 weeks, and thereafter monthly active surveillance to complete 6 months of follow-up (page 5 and page 15).
During their visits the field workers will measure temperature and record any other symptoms (page 18).

Establishment of DSMB (page 2, para 6)

A Data Safety Monitoring Committee (DSMB) will be formed including pediatricians to evaluate the reactogenicity data before moving from the older age to the younger age group. The committee will prepare a letter with its recommendations for the file at different phases in the study (page 21).

Budget for consultant (page 2, para 7)

This also includes costs associated with DSMB.

Reviewer # 2

Our responses are as follows:

1. We have included the consent form in our revised version (**Annex I**, page 40). It will be reviewed and approved by the Ethical Review Committee (ERC) of the ICDDR,B prior to starting the study. The ERC is a recognized committee for review of research protocols involving human subjects and has a Multiple Project Assurance (MPA) with a US agency (USAID). The consent form will provide information about the study, aim and the characteristics, effectiveness and safety of the vaccine (including IS), and also the advantages and disadvantages associated with the vaccine. This document will also inform the parents or guardians about the rights and responsibilities of the child as a participant in the study. Overall, the study will be conducted according to Good Clinical practice, the Declaration of Helsinki and local rules and regulations of the country.

2. We have reviewed your comments and revised the proposal accordingly. We have spelled out the criteria that our field staff or clinicians will use to detect a case of IS (page 15). The investigator and his staff will be aware of the possible increased risk of intussusception and will consider this diagnosis among children presenting symptoms of intussusception. Symptoms consistent with intussusception are severe colicky abdominal pain, persistent vomiting, bloody stools, abdominal bloating and fever up to 41°C. The investigator and his staff will take appropriate actions to evaluate and treat the condition. A child with suspected intussusception will be referred to a local hospital in Dhaka (Children's hospital, Shishu Hospital).

The hospital has the experience and capacity for diagnosis, non-surgical and surgical management of intussusception, and a pediatric surgeon at this hospital is a co-investigator on the protocol to assume responsibility for the care of children suspected of having IS. During the study course, the parents/guardians will be instructed to contact the investigator and his staff immediately should the subject manifest any signs or symptoms they perceive as serious.

Annex II (page 44) provides instruction on the evaluation of intussusception. Each case of suspected or confirmed intussusception will be reviewed by an independent DSMB (data safety monitoring board).

3. We agree that a 6-month follow-up of children will be a long period for simple safety study. We considered the recommendations of the European Committee for Proprietary Products (CPMP) for following up of all vaccinated children for at least 6 months after last immunization considering the safety issue (ref: Committee for Proprietary Medicinal Products (CPMP). Note for guidance on clinical evaluation of new vaccines. The European Agency for the Evaluation of Medicinal Products, Human Medicines Evaluation Unit. London, May 1999).

Since the long-term follow-up does not require any active surveillance for the subjects, and since efficacy is not part of this protocol, the current protocol does not foresee the implementation of an active surveillance for diarrhoeal events.

4. The rationale for the use of several doses and several viral concentrations has been given in details on page 12 and 13 of the protocol. The aim of this dose-ranging study is to identify the most suitable dose and regimen of the vaccine for Bangladeshi infants and to determine if there is a difference in immune responses between the two viral concentrations using two different schedules.

5. We have recalculated the sample size and accordingly changed the design of the protocol. We feel strongly the necessity to test its candidate vaccine at different viral concentration and at different dose schedules. Moreover, there is evidence in the literature of a rather high attack rate of human rotavirus in Bangladesh between 20 to 30% of children that are hospitalized with diarrhea are infected with rotavirus (Albert et al, J Clin Microbiol. 1999; 37:3458-64. Kilgore, et al. Pediatric Infect Dis J 1996, 15:672-7. Haque et al, J Trop Pediatr. 1994 40:351-4). Previous trials conducted with Rotashield in Bangladesh demonstrated that 75% of vaccinees (aged 6-14 weeks) had a 4-fold or greater rise in IgA titer (unpublished result). We have considered this for the calculation of our sample size.

The outcome of this trial in Bangladesh will provide the information necessary to decide the appropriate viral concentration and dose regimen for testing of the HRV vaccine efficacy and safety in the large phase III trial.

With regards to the fevers, the AVANT uncloned P33 89-12 vaccine candidate was shown to be safe in US infants (Bernstein et al, 1998, Bernstein et al, 1999). After administration of the first dose of 10^5 ffu of uncloned P33 89-12, 19 % of the infants had mild fever as compared to 5% of the infants that received the placebo. The Rota-003 trial (Finland study, phase II, double blind, randomized placebo-controlled trial, unpublished) conducted with the GSK cloned RIX4414 candidate vaccine has confirmed the observed safe reactogenicity profile. None of the subjects that received the 10^5 ffu RIX4414 vaccine or placebo had fever after dose 1. Only one of the subjects that received the 10^6 ffu RIX4414 vaccine had fever after dose 1 and one of the subjects that received placebo. It can be concluded that there is no increase in fevers as observed due to the use of a higher viral concentration.

6. An improved study design is outlined in our revised version.

7. For phase II studies, inclusion and exclusion criteria are quite stringent and these have been spelled our clearly (page 16 and 17). **Annex III** (page 46) provides the composition of the vaccine. Any known

hypersensitivity to any of the components should be a contraindication to vaccination. The use of immunosuppressing drugs is known to affect the immune response. Although the likelihood that such drugs are used at the targeted population is remote, we want to collect the data. If the mother or woman of the household were to become pregnant after an infant was enrolled and immunised, this child should be excluded for the administration of the next scheduled dose as there are no transmission data available.

8. We agree to the concern raised by the committee and will allow concomitant administration of OPV.