A double-blind, randomized controlled trial of the effect of vaccine inoculum on oral rotavirus vaccine (Rotarix, GlaxoSmithKline) take and immunogenicity in Dhaka, Bangladesh

Study protocol
Version 9
24 July 2019

NCT02992197

Table of Contents

☐ Check here if appendix is included

List of Abbreviations

Ab	Antibodies
AE	Adverse event
AR	Adverse reaction
BW	Birth weight
$CCID_{50}$	Cell culture infective dose, 50%
COP	Correlate of protection
CPE	Cytopathic effect
CRF(s)	Case report form(s)
Ct	Cycle threshold
DGDA	Directorate General of Drug Administration
DNA	Deoxyribonucleic acid
DTP	Diphtheria-tetanus-pertussis-vaccine
DSMB	Data and Safety Monitoring Board
DSMP	Data Safety Monitoring Plan
EAE	Expected Adverse Events
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
EPI	Expanded Programme on Immunization
ERC	Ethical Review Committee (ICDDR,B)
FDA	Food and Drug Administration
FFU	Focus-forming unit
FRA	Field Research Assistant
FWA	Federalwide assurance
GCP	Good Clinical Practice
HBGA	Histo-blood group antigens
HC	Head circumference
ICDDR,B	International Centre for Diarrhoeal Disease Research, Bangladesh
ICH	International Conference on Harmonization
ICF(s)	Informed consent form(s)
Ig	Immunoglobulin
IRB(s)	Institutional Review Board(s)
JHU	Johns Hopkins University
LAV	Live-attenuated vaccines
NIH	National Institutes of Health
NIPORT	National Institute of Population Research and Training
NSP	Non-structural protein

OHRP	Office for Human Research Protections
OPV	Oral polio vaccine
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PI(s)	Principal Investigator
PROVIDE	Performance of Rotavirus and Oral Polio Vaccines in Developing Countries
qRT-PCR	Quantitative reverse-transcription polymerase chain reaction
RV-IgA	Rotavirus-specific serum IgA
RRC	Research Review Committee
RT-PCR	Reverse transcription polymerase chain reaction
RV	Rotavirus
RV1	Rotarix, GlaxoSmithKline
RV5	RotaTeq, Merck
SAE	Serious Adverse Event
SAR	Suspected Adverse Reaction
SID	Study identification number
SUSAR	Serious and unexpected adverse reaction
UP	Unanticipated problem
UPnonAE	Unanticipated Problem that is not an Adverse Event
UAE	Unexpected adverse event
UVM	University of Vermont
VE	Vaccine efficacy
VP	Viral protein
WHO	World Health Organization

Project Summary

[The summary, within a word limit of 300, should be stand alone and be fully understandable.]

Principal Investigator: Dr. Rashidul Haque

Research Protocol Title: A double-blind, randomized controlled trial of the effect of vaccine inoculum on oral rotavirus vaccine (Rotarix, GlaxoSmithKline) take and immunogenicity in Dhaka, Bangladesh.

Proposed start date: March 1, 2017 Estimated end date: Dec 31, 2019

Background:

a. **Burden:** Oral rotavirus vaccines (e.g. Rotarix, RV1, GlaxoSmithKline) are highly efficacious in high-income countries, but significantly underperform and/or are unavailable in low-income countries (including Bangladesh) where rotavirus remains the leading cause of diarrheal disease in children.
b. **Knowledge gap:** The reasons for rotavirus vaccine underperformance remain unclear. Inadequate vaccine inoculum leading to diminished immunological effect is an unexplored possibility; improved immunogenic markers are needed to accelerate vaccine studies.
c. **Relevance:** Improved vaccine performance and immunogenic markers will substantially impact rotavirus disease burden, vaccine research, and delivery in low-income countries.

Hypothesis: Increased RV1 inoculum will increase rates of vaccine take (i.e. post-vaccination faecal RV1 shedding or antibody seroconversion); antibodies targeting RV proteins VP4, VP5*, VP7, or NSP4, and post-vaccination antigenemia/viremia will be superior immunogenic markers vs the current standard, anti-RV serum IgA (RV-IgA).

Objectives:

Primary (General) Objective

1. To evaluate the effect of increased RV1 inoculum on vaccine take among infants in Dhaka.

Secondary (Specific) Objectives

1. To separately evaluate the effect of increased RV1 inoculum on:
    a. RV1 shedding and viral burden.
    b. RV-IgA seroconversion and concentration.

Exploratory Objectives

1. To evaluate alternate rotavirus immunogenic markers:
    a. VP4, VP5*, VP7, and NSP4 antibodies.
    b. Antigenemia/viremia.
2. To explore the relationship between histo-blood group antigens (Lewis, secretor status) and: RV1 shedding, immunogenicity, antigenemia/viremia.
3. To explore RV1B and T cell responses.

Methods: We will perform a double-blind, randomized (1:1), controlled trial among 220 infants in Dhaka to evaluate vaccine take and immunogenicity following standard- (10^6 FFU) vs high-dose ($10^{6.3}$ FFU) RV1.Infants will be followed through 14 weeks of life and evaluated pre- and post-vaccination for RV1 fecal shedding, serum RV-IgA, RV-VP4, VP5*, VP7, and NSP4 Abs, and antigenemia/viremia.

Outcome measures/variables: Vaccine take; RV1 shedding; RV-IgA seroconversion and concentration; VP4, VP5*, VP7, and NSP4 antibody seroresponse and titre; RV1 antigenemia/viremia.

Description of the Research Project

Hypothesis to be tested:

In a hypothesis testing research proposal, briefly mention the hypothesis to be tested and provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

Does this research proposal involve testing of hypothesis: ☐ No ☒ Yes (describe below)

The hypothesis to be tested is that increasing the dosage of the oral, live-attenuated rotavirus vaccine Rotarix (RV1, GlaxoSmithKline) will lead to increased vaccine take and immunogenicity compared to standard dosing.

It is now well-established that oral vaccines, including oral rotavirus (RV) vaccines, show significantly diminished efficacy in low-income countries. One possibility is that inhibitory factors in the infant gut, such as inflammation from enteric co infections or high levels of maternally-derived antibodies, might rapidly neutralize the vaccine and hinder vaccine "take." Vaccine take refers to the ability of the vaccine to exert a biological effect on the host, and for oral vaccines is generally defined as seroconversion or detection of faecal vaccine shedding(1). Faecal shedding demonstrates replicating infection by the vaccine strain virus, a requirement for host recognition and subsequent immune response and long-term protection. Factors proposed to inhibit vaccine responses in the gut are prevalent in the developing world but might be overcome with an increased inoculum of vaccine.

Specific Objectives:

Describe the specific objectives of the proposed study. State the specific parameters, gender aspects, biological functions, rates, and processes that will be assessed by specific methods.

Primary (General) Objective

1. To determine whether the proportion of infants in Dhaka, Bangladesh with successful vaccine take is increased following high-dose RV1 vaccine administration compared to standard-dose RV1. Vaccine take is defined as detection of either post-immunization faecalRV1 shedding or rotavirus-specific serum IgA (RV-IgA) seroconversion.

Secondary (Specific) Objectives

2. To separately evaluate the effect of increased RV1 inoculum on:
    a. Frequency of post-vaccination vaccine shedding and vaccine-strain virus burden in stool
    b. Frequency of RV-IgA seroconversion and RV-IgA concentration.

Exploratory Objectives

3. To evaluate alternate measures of rotavirus immunogenicity as candidate correlates of protection:
    a. Antibodies against RV1 outer capsid proteins VP4, VP7, and VP5* and RV1 non-structural protein NSP4.
    b. RV1 antigenemia and viremia following vaccination.
4. To explore the relationship between the Lewis histo-blood group antigens (HBGA) and secretor status and RV1 faecal shedding, immunogenicity, and antigenemia/viremia.
5. To explore B and T cell responses to RV1 immunization.

Background of the Project including Preliminary Observations:

Provide scientific validity of the hypothesis based on background information of the proposed study and discuss previous works on the research topic, including information on sex, gender and diversity (ethnicity, SES) by citing specific references. Critically analyze available knowledge and discuss the questions and gaps in the knowledge that need to be filled to achieve the proposed aims. If there is no sufficient information on the subject, indicate the need to develop new knowledge.

Diminished efficacy of oral rotavirus vaccines in low-income countries

Rotavirus remains the leading cause of diarrhoeal disease in children worldwide and is responsible forup to a quarter of a million deaths yearly in children<5 years of age, primarily in Asia and sub-Saharan Africa (3, 4).Global introduction of oral live-attenuated vaccines (LAV) for rotavirus, such as RV1 and RotaTeq (RV5, Merck) have had remarkable success in reducing RV morbidity and mortality, particularly in high- and middle-income countries (5). RV vaccination is now recommended by the WHO in all national immunization programs (6), specifically at 6 and 10 weeks of life in the Expanded Programme on Immunizations (EPI) schedule (7). However, for unclear reasons, vaccine efficacy (VE) is significantly diminished in low-income countries, where disease burden is highest (Figure 1). For example, in Europe, Latin America, and the United States, one-year VE against severe RV diarrhoea exceeded 95%, compared to approximately 50% in sub-Saharan Africa and Asia (8-11).

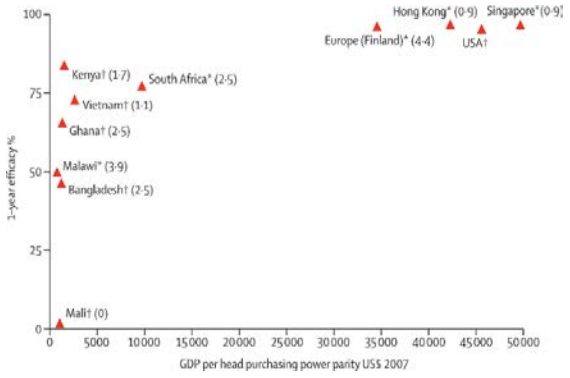

Figure 1. Point estimates of Rotarix* and RotaTeq† vaccine efficacy, and cases of severe rotavirus gastroenteritis prevented per 100 vaccinated infants by gross domestic product (GDP) per head. Ref: (2)

Poor vaccines take as a potential factor in diminished oral vaccine efficacy

Two issues currently frustrate efforts to improve RV vaccine performance: incomplete understanding of the variables impacting VE, and lack of reliable markers of host immune responses to vaccination (immunogenicity) in developing countries. Among variables impacting VE, one possibility is that inhibitory factors, such as maternally-derived antibodies, gut inflammation due to enteric coinfections, or environmental enteric dysfunction (12) might be limiting vaccine take in the infant gut, which is an absolute requirement for host recognition, subsequent immune response, and long-term protection (i.e. effective vaccination).

Paucity of data regarding faecal vaccine shedding and lack of dose-ranging in low-income countries

Faecal shedding of vaccine-strain virus has previously been evaluated, almost exclusively in high- and middle-income countries with good VE (13). In these studies, shedding was detected by RV-specific stool ELISA in approximately 60% of vaccinated infants 7 days following first RV1 dose (Table 1). One previous

Table 1. Selected studies showing rates of RV1 shedding by stool ELISA 7 days after first dose of RV1.

Reference	Study location	Dose of RV1	Percent shedding
Vesikari 2004 (14)	Finland	$10^{5.8}$ FFU	55%
Dennehy 2005 (15)	USA and Canada	$10^{6.4}$ FFU	58%
Phua 2005 (1)	Singapore	$10^{6.1}$ FFU	80%
Ruiz-Palacios 2005 (16)	Mexico	$10^{5.8}$ FFU	61%
Hsieh 2014 (17)	China	10^{6} FFU	24%

study in Bangladesh evaluated RV1 shedding in a small number of infants following vaccination (N=76); less than 15% of vaccinated infants shed RV1 7 days following the first dose (18). Similarly, initial dose-ranging studies for RV1 were conducted in Europe, Latin America, and East Asia, where VE is excellent; in these settings a range of doses ($10^{4.7}$– $10^{6.4}$ FFU) of attenuated RV1 virus was shown to be safe (15, 19). Phase II trials in these countries assessed faecal RV1 shedding at a range of doses; in general, higher doses yielded slightly increased rates of shedding but the effects were extremely modest (1, 15, 20). Large-scale VE trials utilized $10^{6.5}$ $CCID_{50}$ of RV1, which is similar to the current commercial preparation of Rotarix and is equivalent to 10^6 FFU (17, 19).

Significantly, the effects of vaccine inoculum on vaccine take, post-vaccination faecal RV1 shedding, or immunogenicity have not been evaluated in low-income countries, where current vaccines are less effective. Factors proposed to inhibit vaccine responses in the gut are prevalent in the developing world but might be overcome with an increased inoculum of vaccine. This strategy has been successfully used with live oral cholera vaccines (21) but remains unexplored for RV.

Need for improved markers of rotavirus immunogenicity and correlates of protection

Efforts to improve vaccine efficacy are further hindered by lack of a highly reliable marker of immunogenicity for RV in developing countries. A reliable marker of immunogenicity must reflect host immune response to vaccination or natural infection. A marker of immunogenicity that correlates well with VE can be further employed as a correlate of protection (COP), a surrogate endpoint that would render clinical endpoints (i.e. RV diarrhoea) dispensable and greatly accelerate clinical vaccine trials. The standard marker of oral RV vaccine immunogenicity is RV-IgA. Typical assays measure antibodies (Ab) directed against the RV middle capsid structural protein VP6 (Figure 2). Serum RV-IgA has been shown to broadly correlate with VE when assessing population-level data (23). This association disappears, however, when assessing IgA responses specifically in low-income countries (24), as demonstrated in a large clinical trial in Africa where RV-IgA only explained a small fraction (32.7%) of vaccine effect (25). Improved markers of immunogenicity and improved COPs are required to expeditiously evaluate vaccine performance in low-income countries.

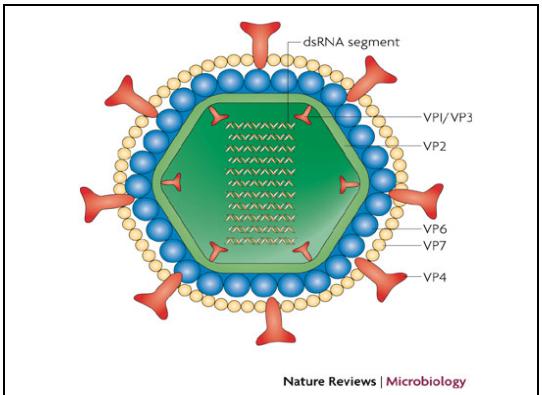

Figure 2. Rotavirus structure. Ref: (22)

A number of alternate markers have been proposed, including serum Ab against the RV outer capsid structural proteins VP4 and VP7 (24), which mediate viral attachment and may be more relevant to clinical immunity (Figure 2). RV antigenemia and viremia have also been noted following natural infection (26), but it is unknown if this occurs following vaccination. It seems probable that extra-intestinal antigen presentation and systemic immune responses would contribute to and enhance host protection from gut infection with RV; antigenemia or viremia could potentially serve as alternate markers of immunogenicity or COPs by identifying subjects more likely to develop these responses. Further, the Lewis HBGA and secretor status have recently been shown to modulate susceptibility to RV infection in a genotype-dependent fashion, with Lewis and secretor-negative individuals protected from infection due to genotype P[8] rotavirus (27, 28). As RV1 is an attenuated G1P[8] virus, it is conceivable that Lewis and/or secretor status could similarly mediate susceptibility to vaccine take and subsequent protection from RV diarrhoea, but this has not been rigorously investigated. Finally, while previous work has demonstrated detection of RV-specific memory B cells and T cells expressing gut homing receptors following natural infection and vaccination, these aspects of RV immunity also remain significantly underexplored (reviewed in (24)).

Preliminary Data

We have preliminary data from the PROVIDE study, an RV1 efficacy trial conducted among infants in Dhaka, Bangladesh (29). In per-protocol analysis, VE against any RV diarrhoea at one year of life was

51%(30). No associations were found between RV-IgA seroconversion (RV-IgA ≥ 20 U/mL following second RV1 dose vs baseline <20 U/mL) and VE, meaning many infants were protected from RV despite no RV-IgA response and vice versa. Overall immunogenicity was poor with only 27% seroconversion among vaccinated infants(30). In this context, there was a complete absence of RV1 shedding as detected by RV-specific stool ELISA following first RV1 dose (N=0/67). These data are in slight contrast with a previous RV1 immunogenicity study conducted in the same region of Dhaka, in which a much higher frequency of vaccine take (73.7%) was detected in a sub-cohort of infants, although total numbers were small (N=38) (18). Only 15% of infants were found to shed RV1 following the first RV1 dose, but a higher seroconversion rate of 56.5% was detected among infants concomitantly administered RV1 and OPV (as in PROVIDE) in the parent cohort. In that study, first vaccine dose was delayed until 12 weeks of life, and RV-IgA was performed using a different ELISA, which may account for some of the observed differences. Despite this, geometric mean concentration among infants vaccinated with concomitant OPV was relatively low at 46.6 U/mL. Overall, even in the study by Zaman et al., rates of RV1 shedding, RV-IgA seroconversion, and RV-IgA concentration remain well below those reported from high- and middle-income countries.

This population, in which vaccine take and immunogenicity were poor, therefore provides an ideal setting in which to test the effects of increased inoculum on vaccine take, immunogenicity, and alternate markers of immune response. Of note, higher rates of RV1 shedding are generally detectable when assessing stool shedding by RT-PCR rather than by ELISA. For example, in a study conducted in China, 10.3% of subjects had detectable RV1 shedding 2-3 days following first dose when assessed by ELISA, compared to 72.4% with shedding detected in the same specimen when assessed by RT-PCR (17). At this time, RT-PCR evaluation of vaccine shedding from PROVIDE is in progress but data are not yet available.

Using the PROVIDE study specimen bank, we are currently developing assays to interrogate serum Ab against RV outer capsid structural proteins VP4, VP7, and VP5*, and RV1 non-structural protein NSP4, all derived from the attenuated RV1 vaccine-strain virus, to identify a better COP. We are utilizing a baculovirus expression system to express target RV1 proteins in Sf9 insect cell culture; this method ensures antigen presentation in a manner that preserves proper conformation and glycosylation. We will thus have a new platform for evaluating host antibody responses to RV. From PROVIDE, remaining specimen volumes are limited and results will require validation in additional cohorts. This study will allow us to validate the performance of antibodies against these targets as markers of immunogenicity, relative to the standard RV-IgA. If these targets represent improved markers of immunogenicity, they would have significant potential as improved COPs.

Research Design and Methods

Describe the research design and methods and procedures to be used in achieving the specific aims of the research project. If applicable, mention the type of personal protective equipment (PPE), use of aerosol confinement, and the need for the use BSL2 or BSL3 laboratory for different part of the intended research in the methods.. Define the study population with inclusion and exclusion criteria, the sampling design, list the important outcome and exposure variables, describe the data collection methods/tools, and include any follow-up plans if applicable. Justify the scientific validity of the methodological approach (biomedical, social, gender, or environmental).

Also, discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them.

STUDY DESIGN

We will perform a double-blind, randomized controlled trial comparing a standard dose (10^6 FFU) to a high dose ($10^{6.3}$ FFU) of RV1 among 220 healthy infants in Dhaka, Bangladesh. The goal of this study is to evaluate the effect of increased vaccine inoculum on vaccine take and immunogenicity, and to explore new markers of immunogenicity against RV. Infants will be randomized 1:1 to receive 10^6(N=110) or $10^{6.3}$(N=110) FFU of RV1 at 6 and 10 weeks of life. A standard dose of commercially available RV1 vaccine contains 10^6 FFU(17, 19). An inoculum of $10^{6.3}$ FFU can be achieved by simultaneous administration of two standard doses of RV1. Importantly, RV1 doses up to $10^{6.4}$ FFU have been evaluated in the United States and Canada and shown to be safe (15).

STUDY SITE AND POPULATION

Two hundred twenty infants (aged 0-7 days) will be recruited from the urban Mirpur community of Dhaka and enrolled into the study. This population represents the same population studied in PROVIDE. Numerous important studies evaluating oral vaccine performance and enteric diseases have also been previously performed in this population. icddr,b already has an established clinic in Mirpur that continues to serve as an ongoing field site for vaccine trials. It is currently the study site for the Dengue in Dhaka Initiative, conducted in collaboration with the University of Vermont (UVM), Johns Hopkins University (JHU), and the National Institutes of Health (NIH). Due to the strong working relationship between this community and the icddr,b, the presence of an established field site, and a proven history in successful completion of multi-center, collaborative trials, this is an ideal site in which to conduct this study.

ELIGIBILITY CRITERIA

Inclusion criteria

1. Generally healthy infant(as determined by research staff; any infant with potential health concerns will be assessed by a medical officer to determine health status)
2. Age 0-7 days at enrolment
3. Mother willing and able to provide signed informed consent
4. Mother willing to allow infant to be vaccinated according to study schedule
5. Mother willing to allow biological specimens, including blood, stool, and saliva, to be collected from infant according to study protocol
6. Mother willing and able to adhere to study schedule

Exclusion criteria

1. Obvious congenital malformation
2. Birth weight (if known) or enrolment weight (if birth weight unknown) < 2000 gm
3. Known immunocompromising condition in infant
4. Enrolment in other vaccine research trials
5. Other household member enrolled in this study

STUDY PROCEDURES

Recruitment

Recruitment will be conducted in the Mirpur community by trained field research assistants. Potential families will be identified and informed of the study during late pregnancy. In consideration of the literacy rate of this community (65.6% among married women in Dhaka, (31)), written recruitment materials will not be used, but recruitment will rely on word-of-mouth.

Screening and enrolment

Families recruited during the pre-natal periods who indicate an interest to participate will be screened following birth of the infant, within the first 7 days of life. Screening will be conducted by field research assistants in the home of the potential study participant. All potential study participants screened will receive a screening number as an identifier. The screening process begins with a review of the inclusion and exclusion criteria listed above to confirm the newborn's eligibility to participate in the study. If the infant is eligible and the family wishes to participate, informed consent will then be obtained from the prospective study participant's mother. The mother will be encouraged to take as much time as necessary to consult with any and all family members (e.g. husband) or other important individuals before making a final decision. Consenting will consist of a thorough review of the written consent form in a manner appropriate for the mother's literacy level, as well as study-related education, including:

1. Information on the risks or discomforts that may occur and the anticipated benefits of participation in the study;
2. Explanation of the study participant's right to leave the study at any time;

3. Assurance of respect for confidentiality and how the study will protect participants' personal information;
4. Training on the procedure and importance of accurate tracking and reporting of diarrhoea surveillance and infant health events within the household;
5. Demonstration of stool specimen collection and explanation of study requirements for samples;
6. Review of the study calendar and time commitment expected of participants;
7. Instructions on how and when to contact study staff.

Prior to signing the consent form, the mother will have an opportunity to ask questions about the study. If the field research assistant determines the mother has demonstrated adequate comprehension of the study, the consent form will be signed by both the field research assistant and the mother. If the mother is not sufficiently literate to sign the consent form, a thumbprint will be obtained in the presence of a witness to indicate consent. The witness may not be associated with the study. The mother will be provided a copy of the signed consent form.

If the mother demonstrates adequate understanding of the study and indicates consent for participation by signing the consent form, screening continues with the following procedures:

- Collection of study participant's name, date of birth, sex, demographic information, and household socioeconomic information;
- Collection of infant's anthropometric measurements: body weight at birth (BW, if known), and body weight at enrolment, length, and head circumference (HC);

At the conclusion of this process, the study participant is considered to be enrolled in the study. Immediately following enrolment, each study participant will be assigned a unique study identification number (SID).

Randomization and blinding

Prior to study initiation, each SID will be randomly assigned to the control or intervention arm (1:1 assignment) by a designated data manager. Randomization will be performed using random permuted blocks. Only the designated data manager at UVM and research pharmacist or designee at icddr,b will have access to the randomization assignments. Double-blinding is required to minimize the chance for potential bias due to differential intensity of follow-up or evaluation in the intervention vs control arms, and to reduce the risk of potential over-reporting by families of potential adverse events if families recognize that their infant is receiving the study intervention, rather than control. The UVM data manager will have access to the randomization codes as a necessary requirement of generating the randomization. The pharmacist or designee requires access to the randomization assignments to ensure proper dosing according to the study protocol and to manage any events that may require unblinding (e.g. serious adverse event requiring unblinding). Upon enrolment, each study participant will be assigned to the lowest available SID number and corresponding randomization assignment. The child will remain assigned to the treatment group therein for the duration of the child's participation in the study, and study participants and investigators will remain blind to intervention status.

Study Participant Withdrawal or Termination from the Study

Participants may withdraw voluntarily from the study at any time without penalty or consequence.

If any of the following events occur, the child will be withdrawn from the study:

1. The research is terminated by the Principal Investigator (PI) or by any regulatory authority.
2. The child's mother withdraws consent for the child to participate in the study.
3. The child's family moves out of the surveillance area.
4. The child dies.
5. The child misses any RV1 dose, unless dosing beyond the visit window is approved by the PI or designee (e.g. medical officer). Any study participant withdrawn for missing the second dose will

be followed for safety until the end of the study period. Any study participant who is withdrawn for having missed the first dose will not be followed for safety as in this scenario no study procedures will have been performed on the participant.

6. The mother is not available to answer questions for more than 14 days and her whereabouts are unknown.
7. An investigator, in consultation with the medical officers, determines that continued participation in the study jeopardizes the health or well-being of the child. In this case, the study team will ask permission to contact the family at the end of the study to confirm the health and well-being of the withdrawn child.

Replacement of Study Participants

Children who withdraw or are terminated from the study will not be replaced, as expected attrition has been incorporated into sample size and target enrolment calculation.

Intervention

All children enrolled in the study will receive oral RV1 vaccine. Children will be randomized 1:1 to receive either a standard dose of vaccine (10^6 FFU = control) or double the standard dose ($10^{6.3}$ FFU = intervention) of RV1 vaccine at 6 and 10 weeks of life, according to the WHO-recommended EPI schedule.

Vaccine:

RV1 (Rotarix, GlaxoSmithKline) is an oral, live-attenuated rotavirus vaccine. It is provided as a clear, colourless liquid formulation, containing 1.5 mL of vaccine in a pre-filled applicator syringe or tube. Vaccine must be stored at 4ºC. Each vial contains 10^6-$10^{6.5}$$CCID_{50}$ (corresponding to 10^6 FFU) of RIX4414, an attenuated G1P[8] strain of group A human rotavirus(17, 19). A double dose of vaccine thus represents up to $10^{6.3}$ FFU of vaccine ($10^{6.3}/10^6 = 1.995$). All vaccine and placebo doses will be transferred to sterile, generic-appearing syringes prior to administration by the research pharmacist or designee at the central pharmacy, labeled with the SID, and transported cold to the study clinic. For study participants randomized to receive 2 doses of vaccine, 2 syringes of vaccine, totaling 3 mL volume will be administered. Study participants randomized to receive a standard dose of vaccine will receive one syringe of vaccine and one syringe containing 1.5 mL of sterile, pharmacy-grade water, for 3 mL total volume. In this manner, all study participants and study staff will thus remain blinded to intervention arm.

Study Event Schedule

A detailed work plan for the study is presented below (Table 2), including: the study immunization plan, schedule of clinic visits, and timetable of laboratory tests for all study indicators. Full description of home visits and clinic visits are provided after the summary table. A summary of the study participant participation timeline is provided (Figure 3).

Specimen Collection

Mothers will be provided specimen cups and trained in how to save stool specimens for retrieval at designated time points by field research assistants for faecal RV1 shedding analysis. In addition, mothers will be trained to save at least one diarrhoeal stool specimen for retrieval by field research assistants for evaluation of RV diarrhoea during each diarrhoeal episode (if any) for her infant. Per the discretion of the mother and the assessment of the field research assistant, mothers may bring stool specimens to scheduled clinic visits, as each visit requires collection of stool, or may request that the specimens be retrieved by field research assistants. For all clinic visits except week 14, the specimen must be obtained within the 24 hours prior to the visit. All blood specimens will be collected at clinic visits by trained personnel.

Scheduled clinic visits

RV1 vaccine will be administered at the week 6 and week 10 clinic visits. It has been described that OPV has a mild inhibitory effect on RV1 immunogenicity, as measured by RV-IgA, when administered

simultaneously versus when given in staggered fashion (18, 32). No effect of RV1 on OPV immunogenicity has ever been observed and we have no reason to suspect an increased dose of RV1 will have an effect. To ensure that there is no interference between OPV and RV1, and at the request of the Mirpur EPI clinic, each child will receive EPI vaccines (including OPV) in our study clinic or at an EPI office at least 7 days following each RV1 dose. This will ensure that each child receives all vaccines on a similar schedule, without interference from OPV, but that EPI vaccines and RV1 are all received during approximately the 7thand 11thweeks of life for all infants.

Table 2. Summary of work plan

Week of life	Pre-natal	0	1	2	3	4	5	6		7	8	9	10		11	12	13	14	
Study Visit #								1		2			3		4			5	
Study Activity																			
Recruitment of families	x																		
Informed consent, enrollment, randomization		x																	
Collection of demographic, socioeconomic information		x																	
Anthropometric measurements		x						x					x					x	
EPI vaccines										x					x				
Weekly home visits[1]		x	x	x	x	x	x			x	x	x			x	x	x	x	x[2]
Daily home visits[3]								x	x				x	x					
Intervention																			
Rotarix								x					x						
Blood collection																			
Venipuncture								3 mL										3 mL	
Heelstick										0.5 mL			0.5 mL		0.5 mL				
Blood measures																			
RV-IgA								x										x	
RV-IgG								x										x	
Zinc								x										x	
VP4, VP7, VP5*, NSP4 Ab								x										x	
Rotavirus EIA (antigenemia)								x		x			x		x				
Rotavirus PCR (antigenemia)								x		x			x		x				
Viral culture (viremia)								x		x			x		x				
PBMC collection								x										x	
Stool collection																			
Diarrheal surveillance		Continuous throughout study following enrollment																	
Stool collection for vaccine shedding assessment			x					x	x	x	x		x	x	x	x			
Stool measures																			
Stool PCR, Rotarix shedding								x	x	x	x		x	x	x	x			
Gut inflammatory markers								x		x									
Stool PCR, gut pathogens								x											
Stool EIA for vaccine shedding assessment								x	x	x	x		x	x	x	x			
Stool EIA for rotavirus diarrhea		Continuous testing of diarrheal specimens throughout study following enrollment																	
Other																			
Saliva collection (Lewis/secretor status)																		x	

1.) AE screening, health and safety assessment, reminder of scheduled stool collections, visits, and procedures

2.) 72 hours following the final clinic visit (week 14) to perform final safety assessment, conclude study participation

3.) For 7 consecutive days following vaccination; telephone contact is acceptable alternative, unless a safety concern requires evaluation

Every reasonable attempt should be made to collect scheduled specimens within the designated visit window for each scheduled clinic visit; however, scheduled specimens may be collected outside the visit window at the investigator's discretion in cases where doing so will not affect study participant safety or the scientific objectives of the study.

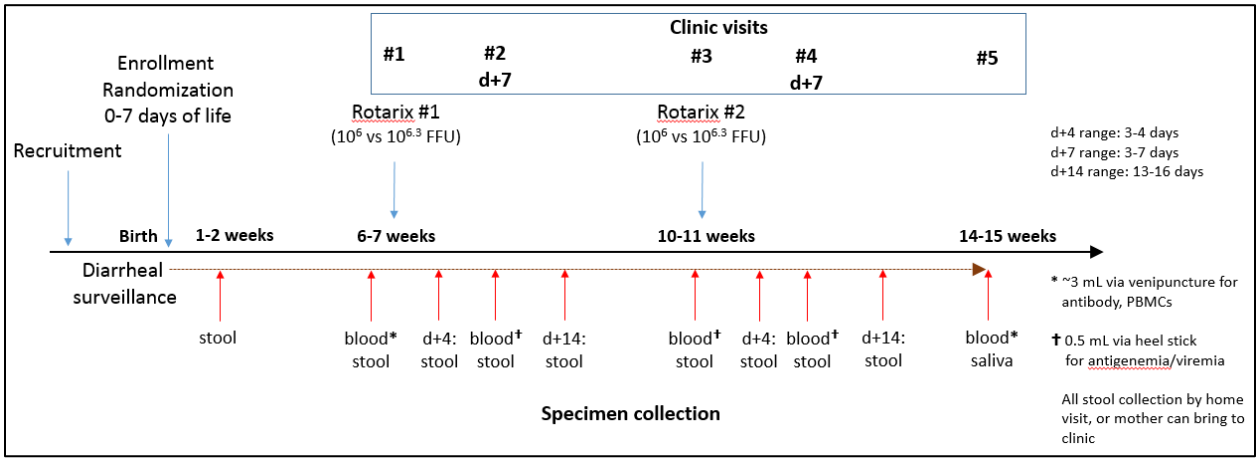

Figure 3. Summary of study participant participation

Visit 1: Age 6 weeks (+7 days)

1. Confirm that correct, signed informed consent form is on file
2. Measure infant's weight, length, and HC
3. Verify eligibility and that all inclusion and exclusion criteria have been met, and confirm that no contraindications to vaccination are present (per vaccine manufacturer instructions)
4. Prior to vaccination, confirm stool sample for baseline faecal RV1 shedding analysis has been collected
5. Prior to vaccination, collect approximately 3 mL of blood by venipuncture for the following:
    a. RV-IgA and RV-IgG
    b. RV VP4, VP7, VP5*, and NSP4 Ab
    c. Rotavirus plasma antigenemia/viremia
    d. PBMCs
    e. Zinc
6. Administer oral RV1 vaccine, unless contraindications identified on screening

Visit 2: Age 7 weeks (week 6 visit date +3-7 days):

1. Perform safety assessment (for AEs)
2. Confirm stool sample for faecal RV1 shedding analysis has been collected
3. Collect approximately 0.5 mL of blood by heelstick for rotavirus antigenemia/viremia analysis
4. If visit occurs on day +7, EPI vaccines can be given at that time if available; if visit occurs on day +3-6 or EPI vaccines unavailable in the study clinic, family may be escorted on or after day +7 to an EPI clinic for EPI vaccines

Visit 3: Age 10 weeks (week 6 visit date +28-35 days)

1. Perform safety assessment (for AEs)
2. Measure infant's weight, length, and HC
3. Confirm that no contraindications to vaccination are present (per vaccine manufacturer instructions).Prior to vaccination, confirm stool sample for faecal RV1 shedding analysis has been collected
4. Prior to vaccination, collect approximately 0.5 mL of blood by heelstick for rotavirus antigenemia/viremia analysis
5. Administer oral RV1 vaccine, unless contraindications identified on screening

Visit 4: Age 11 weeks(week 10 visit date +3-7 days):

1. Perform safety assessment (for AEs)
2. Confirm stool sample for faecal RV1 shedding analysis has been collected
3. Collect approximately 0.5 mL of blood by heelstick for rotavirus antigenemia/viremia analysis
4. If visit occurs on day +7, EPI vaccines can be given at that time if available; if visit occurs on day +3-6 or EPI vaccines unavailable in clinic, family may be escorted on or after day +7 to an EPI clinic for EPI vaccines

Visit 5: Age 14 weeks (week 10 visit date +28-35 days)

1. Perform safety assessment (for AEs)
2. Measure infant's weight, length, and HC
3. Collect approximately 3 mL of blood by venipuncture for the following:
    a. RV-IgA
    b. RV VP4, VP7, VP5*, and NSP4 Ab
    c. Zinc
    d. PBMCs
4. Collect saliva for Lewis/secretor status testing.

Home visits

Weekly home visits

Home visits will be performed throughout each study participant's participation in the study to perform safety assessments, perform study-related procedures, and help ensure protocol adherence. Any scheduled clinic visit can substitute for a home visit if the schedule for clinic visit coincides with need for home visit. Following enrolment and randomization, each study participant will be visited at home by a field research assistant at least once weekly for the duration of the study. Weekly home visits will consist of the following activities:

- Record diarrhoea and health surveillance data to screen for adverse events following RV1 vaccination (safety assessment).
- Refer to the clinic, if indicated, for acute medical conditions.
- Remind mother about stool specimen collection time points, and ongoing sample collection for diarrhoea surveillance, as indicated.
- Remind mother of all scheduled clinic visits and procedures, as described elsewhere in the protocol.

Daily home visits

In addition to the weekly home visits listed above, for 7consecutive days following each dose of vaccine (with the exception of day +3-7 following each dose, which is a scheduled clinic visit), each study participant will be visited at home by a field research assistant to perform vaccine safety assessment and record signs or symptoms of possible adverse events (as described below in "Adverse Events"). If a home visit cannot be arranged, telephone contact will be an acceptable alternative, unless a safety concern is raised that requires evaluation (e.g. temperature measurement to rule out fever and need for medical evaluation).

Retrieval of stool specimens for RV1 faecal shedding evaluation

Retrieval of stool specimens for faecal RV1 shedding analysis will occur at the time of weekly or daily home visit at the following time points (see Figure 3):

- Pre-vaccination: 1) Age $\leq$14 days
  2) Age 6 weeks, immediately (0-3 days) prior to Clinic Visit #1

- Post-RV1 dose #1: 3) Age 6 weeks, 3-4 days after Clinic Visit #1
  4) Age 7 weeks, 6-7 days after Clinic Visit #1
  5) Age 8 weeks, 13-14 days after Clinic Visit #1
  6) Age 10 weeks, immediately (0-3days) prior to Clinic Visit #3
- Post-RV1 dose #2: 7) Age 10 weeks, 3-4 days after Clinic Visit #3
  8) Age 11 weeks, 6-7 days after Clinic Visit #3
  9) Age 12 weeks, 13-16 days after Clinic Visit #3

For stool collections that coincide with clinic visits, mothers may choose to bring stool specimens to the clinic visits if desired, rather than having an FRA retrieves it from the home. Pre-vaccination and post-RV1 dose #1 specimens will also be evaluated for gut inflammatory markers and for additional enteric pathogens, including but not limited to enteroviruses.

Ongoing

- Diarrhoeal surveillance, including:
  - Diarrhoea sample collection when infant is reported to have 3 or more abnormally loose stools in a 24 hour period, for testing for presence of rotavirus antigen (i.e. rotavirus diarrhoea) and potentially other pathogens; and
  - Appropriate treatment/medical management for diarrhoea, as indicated.

Conclusion of study participation

A home visit will be conducted 72 hours following the final clinic visit to perform a final safety assessment following the week 14 clinic blood draw. If no concerns are raised, the study participant will be discharged from the study at that time. If a home visit cannot be arranged, communication via telephone will be an acceptable alternative, unless a concern is raised that requires evaluation.

Maternal participation

Recent data suggest that maternal histo-blood group antigen phenotype may also be important in infant susceptibility to RV. In a prior study involving the MAL-ED cohort in Dhaka, researchers demonstrated that maternal secretor and Lewis status had a much greater effect on infant susceptibility to RV infection than infant status (33). In a study from India, infants of mothers whose milk had higher levels of secretor-dependent antigens were more likely to have symptomatic neonatal RV infection (34). Similarly, in our previous PROVIDE cohort, we have determined that infants of mothers whose breast milk was secretor-negative had significantly higher odds of Rotarix seroconversion (manuscript in preparation). Therefore, newer data indicate that maternal secretor and Lewis status is an important variable in RV vaccine response and susceptibility. Therefore, we will request saliva sample collection from all mothers of infants who completed the study for saliva Lewis and secretor phenotyping and genotyping to further extend these findings.

Laboratory assays (see Table 3 for summary)

Table 3. Summary of Tests/Assays

Test/Assay	Type of sample	Location performed
Nucleic acid extraction	Vaccine shedding stools	icddr,b
Rotavirus PCR	Stool nucleic acid extraction	icddr,b
EIA for rotavirus antigen	Vaccine shedding, diarrhoeal stool	icddr,b
EIA for rotavirus antigen	Plasma	icddr,b
Zinc	Plasma	icddr,b
Rotavirus gene sequencing	Stool nucleic acid extraction	UVM
RV-IgA, RV-IgG ELISA	Plasma	UVM

Nucleic acid extraction	Plasma	UVM
Rotavirus PCR	Plasma nucleic acid extraction	UVM
Viral culture (viremia)	Plasma	UVM
RV antibody immunocytochemistry assays (VP4, VP5*, VP7, NSP4)	Plasma	UVM
EIA for gut inflammatory markers	Stool	UVM
PCR (or other molecular methods) for gut pathogens	Stool, stool nucleic acid extraction	UVM
Multi-color flow cytometry (B & T cell phenotyping)	PBMCs	UVM
Lewis antigen and secretor status by ELISA	Saliva	icddr,b
Lewis and secretor genotyping	PBMCs or stool nucleic acid extracts	UVM

Stool total nucleic acid extraction for vaccine shedding detection and gut pathogen detection. Total nucleic acid extraction will be performed on all stool specimens collected during the study period, with the exception of the week 1-2 collection, for the purpose of vaccine shedding detection using the QIAmp Fast DNA stool Minikit (QIAGEN), similar to previously described methods (35). Extraction controls using MS2 bacteriophage (ATCC) will be added to each extracted specimen, as well as a blank specimen on each day of extraction to serve as a batch control. The 1-2 week stool will only be assessed for rotavirus positivity if the study participant is positive for rotavirus at the week 6 visit, before vaccination, to estimate how long prior to vaccination the participant was first exposed to rotavirus.

Real-time quantitative RT-PCR and gene sequencing for vaccine shedding detection. Due to our previous experience that demonstrated poor sensitivity of stool ELISA to detect faecal vaccine shedding in this population (PROVIDE, unpublished data,(18)) shedding will be assessed by RT-PCR. Total nucleic acids extracted from stool specimens will be tested for the presence of RV using real-time quantitative RT-PCR (qRT-PCR) targeting the highly conserved NSP3 gene sequence using previously described primers and probes(36). Detection of MS2 extraction controls will be conducted as previously described (36). Absolute quantitation of viral burden will be performed based on analysis of target Ct values relative to a standard reference curve. PCR reactions will be performed using the UltraPlex 1-Step Toughmix PCR kit (Quanta Biosciences) and detection will be performed using the CFX96 real-time PCR detection system (Bio-Rad). Specimens that test positive for RV will have genotyping performed by the Sanger method for the RV VP4 gene to confirm presence of vaccine-strain virus (RIX4414). Alternatively, specimens may be directly assayed for vaccine-strain virus using a primer/probe set specific for the NSP2 gene segment for the Rotarix vaccine strain (37).

PCR for gut pathogen burden. Stool total nucleic acid specimens will be evaluated for other gut pathogens, including but not limited to enteroviruses, by conventional or real-time PCR (38)or other molecular methods.

Stool RV-specific EIAfor detection of RV antigen in diarrhoeal stool specimens. All diarrhoeal stool specimens collected during the study period will be tested for the presence of rotavirus antigen using a commercial EIA kit (ProSpecT Rotavirus Microplate Assay, Oxoid), per manufacturer's instructions.

Stool RV-specific EIA for vaccine shedding detection in high-dose RV1 recipients. After initial analysis, EIA for detection of RV antigen will be performed on all stool specimens collected during the study period for the purpose of vaccine shedding detection that test positive by PCR, and on a subset of PCR-negative specimens. While ELISA was previously demonstrated to have poor sensitivity to detect faecal vaccine shedding in this population following standard-dose RV1 (PROVIDE, unpublished data,(18)), it is conceivable that vaccine shedding may be detected following high-dose RV1. If so, detection of shedding in future studies would be greatly simplified by use of EIA rather than real-time qRT-PCR.

Plasma rotavirus antigen and virus detection. Plasma specimens collected 7 days following each vaccine dose will be assessed for presence of rotavirus antigenemia using a commercial kit (ProSpecT Rotavirus Microplate Assay, Oxoid) (39) and by qRT-PCR targeting RV NSP3 following RNA extraction using the QIAmpViral RNA Mini Kit (QIAGEN). Plasma specimens collected 7 days following each vaccine dose will be assessed for the presence of infectious virus via serial passage on MA104 cells followed by antigen-specific immunostaining, similar to previously described methods (26).

*Plasma rotavirus-specific IgA and IgG.*RV-IgG will be assessed in pre-vaccination (week 6) plasma specimens, and RV-IgA will be assessed in pre-vaccination (week 6) and post-vaccination (week 14) plasma specimens by ELISA, as previously described (40). RV-IgA concentration (U/mL) will be calculated via interpolation from a standard reference specimen.

VP4, VP7, VP5, NSP4 antibody immunocytochemistry assays.* IgA and IgG antibodies targeting RV1-specific outer capsid proteins VP4, VP7, VP5*, and RV1-specific non-structural protein NSP4 will be assessed in pre-vaccination (week 6) and post-vaccination (week 14) plasma specimens using an immunocytochemical staining procedure, similar to previously described methods (41, 42). Briefly, recombinant baculovirus expressing epitopes cloned from the RV1 vaccine strain will be used to synchronously infect monolayers of Sf9 cells to yield recombinant protein expression. At the time of peak protein expression, cells will be fixed and permeabilized. Study participants' plasma will be incubated on the fixed cell layers, followed by visual detection using conjugated secondary antibodies against human IgG and IgA.

Determination of Lewis and secretor status. Saliva specimens collected from all study participants (including mothers who agree to provide a sample) will be tested for determination of Lewis antigen and secretor status by ELISA, similar to previously described methods (27). However, phenotyping assays, particularly in young infants, may be imprecise (43); an alternate method to more definitively identify Lewis and secretor status is to confirm the host's genotype for the alleles of the genes that confirm the fucosyltransferase enzymes that determine Lewis (FUT3) and secretor (FUT2) status. Therefore, we will also performe sequence analysis of the FUT3 and FUT2 genes using the PBMC or stool nucleic acid extracts being collected for this study or from DNA extracts of infant and maternal saliva specimens to corroborate the phenotyping results. It is likely that additional, unknown genes in addition to FUT2 and FUT3 may be associated with rotavirus susceptibility or vaccine response. The only way to do this is to perform exploratory genetic analyses, which can be performed on the same specimens.

B and T cell phenotyping. B and T cell phenotyping will be performed on baseline (week 6) and post-vaccination (week 14) PBMCs by multi-color flow cytometry.

Duration of participation

Study participants will be active participants from the time of enrolment (0-7 days of life) until 3 days after the final visit at week 14 of life (approximately 3 months of life). Safety assessment will be performed by home visit (or telephone call, if home visit cannot be coordinated) after the final study visit. If no concerns are identified, the study participant will be discharged from the study and his or her participation in the study is concluded.

Specimen preparation, handling, shipping and storage

Preparation, handling, shipping, and storage of blood, stool, and saliva specimens will be described in the manual of procedures. All samples will be labelled by unique identifiers and not by name.

During the study period, as needed for completion of the proposed assays, specimens may be housed at either at icddr,b or at UVM. After completion of the study, any specimens remaining at UVM will be

returned to icddr,b. Any specimens remaining that are of too low of volume to be usable any more will be destroyed. All specimens will be stored at icddr,b up to 5 years following the completion of the study.

SAFETY MONITORING, ASSESSMENT AND REPORTING

ADVERSE EVENTS: DEFINITIONS AND REPORTING

Table 4 summarizes the definitions and required responses of any safety issues that might arise during this study. All AEs occurring from the time of the first study visit at 6 weeks of life through the end of the study period will be documented, recorded, and reported. All reports will include assessment of severity, relationship to study interventions, actions taken, and outcomes, as described below in "Assessment of adverse events." The study team will not have primary responsibility for reporting safety submissions directly to the Data Safety and Monitoring Board (DSMB), except as specifically described below under Data Safety and Management Plan. Safety reports requiring DSMB attention will be submitted to DSMB by the ERC. All events outlined in Table 4. Adverse Events Definitions and Reporting will be reported to DSMB by the study team at annual review or otherwise directed. For reports to the Directorate General of Drug Administration (DGDA), only those events that are considered to have been possibly, probably, or definitely related to vaccination will be reported.

Table 4. Adverse Events Definitions and Reporting

Event	Definition	Reporting Requirements
Adverse Event (AE)	Any untoward or unfavourable medical occurrence in a study participant, including any abnormal sign, symptom, or disease, temporally associated with the study participant's participation in the research, whether or not the event is determined to be related to the study participant's participation in the research. <u>Solicited AE's.</u> We will specifically query for symptoms listed below. Within 7 days of vaccination: 1. Fussiness 2. Cough or runny nose 3. Fever 4. Loss of appetite (decreased feeding compared to baseline) 5. Vomiting 6. Diarrhoea (defined as 3 or more stools looser than normal per mother's judgement within 24 hours) Within 3 days of blood draw: 7. Bleeding at puncture site 8. Bruising at puncture site 9. Signs of infection (redness, swelling, warmth, tenderness, purulent drainage, or fever) at puncture site <u>Unsolicited AE's.</u> Any of the above symptoms if they should occur outside the specified time period for solicited AE's, or any other signs or symptoms reported by the family.	1. **ERC**: Annual review 2. **DSMB**: Annual review, or as otherwise directed. 3. **UVM:** Annual review 4. **DGDA:** Annual review
Expected Adverse Event (EAE)*	An AE that is a known reaction to the vaccine or phlebotomy, or that is generally common in this population. This includes the symptoms that appear above	1. **ERC**: Annual review 2. **DSMB**: Annual review, or as otherwise directed.

	in the list of solicited AE's, even if they occur outside the specified time period for solicited AE's. For example, diarrhea is a very common event in this population, and may be completely unrelated to study participation, and therefore is an EAE. As it is now accepted that RV1 is associated with a very small (1-5 excess cases per 100,000 children immunized) risk of intussusception, this will be categorized as an expected SAE. However, all cases of intussusception will be considered a pausing criteria (see below).	3. **UVM:** Annual review 4. **DGDA:** Annual review
Serious Adverse Event (SAE)	An AE that is determined to be "serious," whether or not the event is related to study participation, including: 1. Death 2. Life threatening event that places a subject at immediate risk of death at the time of the event and does not refer to an event that hypothetically might have caused death were it more severe 3. Inpatient hospitalization or prolongation of existing hospitalization, defined as at least an overnight stay in the hospital or emergency ward for treatment that would have been inappropriate if administered in the outpatient setting 4. Persistent or significant disability or incapacity 5. Other medically important event that may put the study participant at risk of harm or require intervention to prevent harm	1. **ERC***: As soon as possible (goal: within 24 hours of knowledge of their occurrence), including measures taken to mitigate/manage the event. In the event any participant experiences multiple SAEs, each event will be reported separately. If detailed information is not available at the time of submission of the initial report, follow-up report(s) will be submitted as soon as information is available. The first follow-up report will be made as soon as possible (preferably within 72-96 hours whenever feasible) of the initial report. Final outcome of the event will be reported within 72 hours of knowledge of outcome. A follow up SAE report will be submitted to the ERC if SAE persists $\geq$ 2 weeks. 2. **DSMB:** Annual review, or as otherwise directed. 3. **UVM:** To be reported as soon as possible to UVM study staff. If it is determined that the event was unexpected AND related to study participation, the event will be reported to the UVM IRB as soon as possible by the UVM PI. Otherwise, it will be reported at annual review. 4. **DGDA:** Reports of SAEs will be submitted by the PI at the same time they are reported to the ERC for any SAE related to the intervention.
Unexpected Adverse Events (UAEs)	An AE that does not qualify as an EAE, or an EAE that significantly exceeds the severity that has typically been observed. The PI will make this determination on a case-by-case basis, should it arise.	1. **ERC**: As for SAE. 2. **DSMB**: Annual review, or as otherwise directed. 3. **UVM:** As for SAE. 4. **DGDA:** as for SAE

Serious and Unexpected Suspected Adverse Reactions (SUSAR)	A Suspected Adverse Reaction (defined as an AE that for which there is a reasonable possibility that the AE was caused by the vaccine) that is both Serious and Unexpected. SUSARs are reported only if there is evidence to suggest a causal relationship between the vaccine and the adverse event, such as: 1. A single occurrence of an event that is uncommon and known to be strongly associated with the vaccine. 2. One or more occurrences of an event that is not commonly associated with the vaccine, but is otherwise uncommon in the exposed population. 3. An aggregate analysis of specific events observed in a clinical trial that indicates those events occur more frequently in the intervention than control group.	1. **ERC**: As for SAE. 2. **DSMB**: Annual review, or as otherwise directed. 3. **UVM:** As for SAE. 4. **DGDA:** as for SAE
Unanticipated Problem that is not an AE (UPnonAE)	Does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involve risk to the study participant, affect others in the research study, or significantly impact the integrity of research data. Examples might include occurrences of breaches of confidentiality or accidental destruction of study records.	1. **ERC**: As soon as possible but no later than one week following the event 2. **DSMB**: Annual review, or as otherwise directed. 3. **UVM:** To be reported as soon as possible to UVM study staff. If it is determined that the event was unexpected AND related to study participation AND involve harm or have the potential to cause harm to others, the event will be reported to the UVM IRB as soon as possible by the UVM PI. Otherwise, it will be reported at annual review. 4. **DGDA:** Annual review
Protocol deviation or Non-compliance	A protocol deviation is any change, divergence, or departure from the IRB approved study procedures in a research protocol. A protocol deviation is considered severe if it meets the definition of a Serious Adverse Event or compromises the safety, welfare or rights of study participants or others. Non-compliance is defined as failure to comply with applicable IRB or regulatory requirements for protection of human study participants.	1. **ERC**: As soon as possible but no later than one week following the event 2. **DSMB**: Annual review, or as otherwise directed. 3. **UVM:** To be reported as soon as possible to UVM study staff. If it is determined that the event involves harm or has the potential to cause harm to others, the event will be reported to the UVM IRB as soon as possible by the UVM PI. Otherwise, it will be reported at annual continuing review. 4. **DGDA:** Annual review for protocol deviation, as for ERC for non-compliance

Pausing Criteria	Pausing is the suspension of any study interventions or procedures to a single study participant until a decision is made whether or not to resume study participation. The pausing criteria for a single study participant include any of the following: 1. Identification of a previously undiagnosed immunocompromising medical condition which no longer permits vaccination. 2. Development of a chronic disease. 3. Any severe (e.g. anaphylactic) reaction to previous vaccination 4. Intussusception 5. Any safety issue that the PI determines should pause further study intervention or procedures to be performed on a single study participant.	1. **ERC**: As for SAE. 2. **DSMB**: As directed by ERC. 3. **UVM:** To be reported as soon as possible to UVM study staff. The event will be reported to the UVM IRB as soon as possible by the UVM PI. 4. **DGDA:** as for SAE The PI and DSMB will determine if it is safe for the individual to resume study participation, and if so, will notify icddr,b ERC of the decision to resume participation. The UVM PI will be notified at the same time and will report the decision to UVM IRB as soon as possible. If a child meets pausing criteria and is unable to resume participation, the child will continue to be followed for safety until the end of the study.
Stopping Criteria	Stopping the study requires immediate discontinuation of all study interventions and procedures and suspension of further recruitment and enrolment until a decision is made whether or not to continue the study. Either IRB or the DSMB may stop the study at any time following review of any safety concerns. Stopping rules are as follows: 1. Two or more study participants experience the same or similar SAEs that are unexpected and are possibly, probably, or definitely related to the vaccine. 2. Any safety issue that the PI and/or designee determines should halt the study	1. **ERC**: As soon as possible (goal: within 24 hours of knowledge of their occurrence). 2. **DSMB**: As directed by ERC. 3. **UVM:** To be reported as soon as possible to UVM study staff. The event will be reported to the UVM IRB as soon as possible by the UVM PI. 4. **DGDA:** A report will be submitted by the PI at the same time it is reported to the ERC. The PI and DSMB will determine if it is safe to resume the study. If so, the PI will notify icddr,b ERC of the decision to resume participation. The UVM PI will be notified at the same time and will report the decision to UVM IRB as soon as possible.

*Given the demographic profile of the participants for this study (Bangladeshi infants from birth until approximately 4 months of age), we might see mortality and morbidity-related SAEs in the study population. Of note, data from the 2014 Bangladesh Demographic and Health Survey report under-five and infant (< 1 year) mortality rates of 46 deaths per 1,000 live births and 38 per 1,000 respectively for the country as a whole; furthermore, 61% of all under-five deaths occur during the neonatal period in the first month after birth (31). We also anticipate a high baseline of morbidity in this population, particularly from diarrhoeal and respiratory illness, and malnutrition.

MONITORING FOR ADVERSE EVENTS

All study participants will be closely monitored for adverse events during the study. For 7 consecutive days following each dose of RV1, field research assistants will perform a home visit or contact the family by telephone to ask specifically about solicited AEs as described in Table 4. Families will also be encouraged

to report any additional signs or symptoms that may be concerning. Participants will be observed for 30 minutes following each immunization to ensure no allergic reactions have taken place. Safety assessment will also be performed at each scheduled clinic visit and at weekly home visits. Additional assessments may be performed at the request of the family or study medical officer's discretion, as conditions dictate, including assessments to confirm resolution of an adverse event. These assessments may involve specimen collection for the primary purpose of safety assessment (e.g. collection of complete blood counts, serum electrolytes) if it is felt to be indicated by the medical officer. A study clinician will be available to study participants by telephone 24 hours a day during the entire study period, and all participants will be provided with a telephone contact card during the initial recruiting and again at the enrolment visit.

If the family is concerned the child has a fever, the family will be instructed to record the infant's temperature. Families that do not have access to a thermometer will be provided a thermometer by the study clinic. Fever will be defined as axillary or rectal temperature $\geq$38^{o}C on any measurement. If fever is confirmed, the family will be instructed to bring the child to clinic for evaluation and/or referral will be made for medical evaluation and management, according to local standards of care. Families will be provided with a card containing information for how to contact study staff with any concerns; families will have 24/7 access to study staff.

Stable chronic conditions which are present prior to the first clinic visit (pre-existing conditions) and do not worsen are not considered AEs and will be accounted for in the study participant's medical history. Exacerbation or worsening of pre-existing conditions are defined as AEs and are evaluated using the same criteria as for AEs. Given the study population (healthy infants from birth-3.5 months), it is unlikely that any such AEs will be detected as we anticipate that any infants with obvious medical conditions will be excluded.

ASSESSMENT OF ADVERSE EVENTS

All identified adverse events will be assessed for severity, relationship to study interventions, actions taken, and outcomes. Each category for AE assessment will be coded, according to the following grading systems:

Determination of Severity

The PI or designee will assess and grade AE severity using the classifications outlined in Table 5. For solicited AEs, the adverse event intensity scoring system will be adapted from Dennehy et al. (15), as described in Table 6, and used for scoring severity.

Table 5. Adverse event severity definitions

Severity	Definition
Grade 0 (Normal)	No symptoms
Grade 1 (Mild)	Event that is easily tolerated, may require 1 dose of medication/treatment
Grade 2 (Moderate)	Event that interferes with normal activity or requires more than 1 dose of medication/treatment
Grade 3 (Severe)	Event that prevents normal activity and requires medical intervention
Refer to Table 6 for a RV-specific Adverse Event Intensity Scoring System that specifies Grade 0-3 events	
Grade 4 (Life Threatening)	Event which places the study participant at immediate risk of death
Grade 5 (Death)	Event which results in death

Table 6. Adverse Event Intensity Scoring System. Adapted from: (15)	
	Definition
Fever (Rectal or Axillary Temperature)	
0	<38.0°C
1	38.0°C-38.5°C
2	38.6°C-39.5°C
3	>39.5°C
Diarrhoea	
0	Normal (0-2 looser than normal stools/day)
1	3 looser than normal stools/day
2	4-5 looser than normal stools/day
3	≥6 looser than normal stools/day
Vomiting	
0	Normal (no emesis)
1	1 episode of vomiting/day
2	2 episodes of vomiting/day
3	≥3 episodes of vomiting/day
Fussiness/Irritability	
0	Behaviour as usual
1	Crying more than usual/no effect on activity
2	Crying more than usual/interferes with normal activity
3	Crying that cannot be comforted/prevents normal activity
Cough/runny nose	
0	Normal
1	Cough/runny nose that is easily tolerated
2	Cough/runny nose that interferes with normal activity
3	Cough/runny nose that prevents normal activity
Loss of appetite	
0	Normal
1	Eating less than usual/no effect on activity
2	Eating less than usual/interferes with normal activity
3	Not eating at all

Note: The severity grades listed in Table 6 are equivalent to the severity grades listed in Table 5.

Relationship to Study Interventions

The PI or designee will assess the relationship between any study intervention or procedure and the occurrence of each AE. The PI will use clinical judgment to determine the relationship, based on the information available at the time. Alternative causes, such as undercurrent illnesses/infections, non-study related therapies (e.g. EPI vaccines), and other risk factors as well as the temporal relationship of the event to the study intervention or procedure will be considered and investigated. The PI may change his/her opinion of causality based on availability of follow-up information, and may amend/update reports accordingly.

Causality of AEs will be assessed by the PI or designee using the following question, "Is there a reasonable possibility that the AE may have been caused by the intervention or procedure?" Causality will then be assigned to one of the following categories:

1. Related	There is a reasonable possibility that the AE was related to the study intervention or procedure. AEs related to study participation can further be categorized, if necessary, based on strength of relatedness:
1a. Definitely Related:	There is a clear-cut temporal association with the study intervention or procedure and the AE, and there are no other reasonable causes for the outcome in question.
1b. Probably Related:	It is more likely than not that the study intervention or procedure contributed to the AE; the temporal association is suggestive but not definitive, and other causes remain a possibility but are less likely.
1c. Possibly Related:	It is possible that the study intervention or procedure contributed to the AE; the temporal association is not clear cut and other causes remain a possibility.
2. Unrelated:	The AE is not causally or temporally related to the study intervention or procedure. There are other, more likely causes and the study intervention or procedure is not suspected to have contributed to the AE.

Adverse event action taken
The PI/designee will assess the action taken by the study participant or the study staff in relation to the AE using the following classifications:

1. None
2. Therapy required, not requiring hospitalization (e.g. antibiotics for superficial infection at venepuncture site)
3. Permanently discontinued from study participation
4. Hospitalization
5. Other

Adverse event outcome
The PI/designee will assess the outcome of the AE, either at resolution or at the end of the study participant's participation, using the following classifications:

1. Resolved
2. Recovered with sequelae
3. Continuing
4. Death
5. Unknown

SAEs that have not resolved by the end of the follow-up period are followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (e.g., the study participant is lost to follow-up), the reason a final outcome could not be obtained will be recorded by the investigator on the AE case report form.

DATA MANAGEMENT
Source Documentation and Data Collection
Clinical data. Complete source documentation will be required for every study participant for the duration of the study. The participant's study record will record his/her participation in the clinical trial, concomitant medications or other interventions administered, as well as any adverse reactions experienced during the trial.

Information from source documentation will be double data entered by trained, designated study team members into electronic case report forms (CRFs), which will be created using the REDCap software

program. In some instances, the source document may directly mirror the electronic case report form for ease of entry.

Corrections to the paper source documents must be made by striking through the incorrect entry with a single line (taking care not to obliterate or render the original entry illegible) and entering the correct information adjacent to the incorrect entry. Corrections must be initialled and dated by the person making the correction. Source documentation should support the data collected on the CRFs**,** and must be signed and dated by the person recording and/or reviewing the data.

Signed consent forms, demographic data, and other data with personal identifiers will be maintained by the investigators in a secure study binder for all study participants in a secure, locked location. Since this study binder will have documentation with both personal identifiers and SIDs, the chart is one possible link between study data and a personal identifier. Investigators will also maintain individual study charts for each study participant. These study charts will be identified only by the study participant's unique SID and not by personal identifiers. The study chart will contain all source documentation and CRFs.

Information captured on source documents will be double data entered into the appropriate CRFs. QA/QC will be performed according to the manual of procedures. Any discrepancies or errors will be reviewed and clarified by the data manager, the staff who performed data entry, and the staff member who obtained the source documentation.

Laboratory data. Raw laboratory data generated at icddr,b will be entered into pre-designed Excel templates. Local laboratory staff will perform initial QC/QA to ensure proper data entry, and then transferred to the local data manager for final review. A similar process will take place with any raw laboratory data generated at UVM, with completed documents transferred to the primary data manager at UVM.

Database management

The overall data warehouse for the study will reside at UVM. Data can be added via two mechanisms, via REDCap and via direct file transfer. For clinical data, all data will be uploaded via REDCap. Only authorized personnel from the data management teams at icddr,b and UVM will have access to the REDCap database. The primary data manager will be located at UVM and is identified as key personnel. The primary data manager will have primary responsibility for the REDCap database, including overall design, maintenance, troubleshooting, and granting of access and permissions to others. The primary data manager will be off-site and will not perform data entry or be involved in clinical or laboratory assessments, so thus will be unblinded, generate the randomization assignments, and communicate the randomization to the icddr,b research pharmacist (or designee). At icddr,b, a local data manager will be identified as key personnel. The local data manager and designated data entry staff will have access to REDCap to enter and edit data. None of the study PIs, clinic staff, pharmacy staff, or laboratory staff will be able to edit REDCap data.

Laboratory data from icddr,b will be sent to the UVM data warehouse via secure file transfer using the UVM secure file transfer service. Any laboratory data will only include SID as the identifier, and thus no lab personnel will have access to coded data with other identifiers (e.g. DOB). The primary data manager at UVM will thus be able to generate and compile composite reports combining clinical data exported from REDCap with directly uploaded laboratory data. Primary data analysis will take place at UVM, but access to final data files may be granted to PIs and other authorized personnel at icddr,b for analysis as well.

A complete description of how confidentiality will be maintained with respect to data storage, management, and analysis is further provided below under "Ethical Assurance of Human Rights, Adequacy of Protection Against Risks."

Study Documentation
Study-related documentation will be completed as required by each IRB. Continuing review documentation will be submitted by the Investigators to each IRB at least once yearly as specified by each IRB. The PI and designated icddr,b personnel will maintain adequate records to account for the disposition of the vaccines, including dates of receipt and quantity, current inventory and storage, and dispensation to study participants, and destruction of remaining inventory, if applicable.

Retention of Specimens
All specimens will be stored at either icddr,b, or at UVM, according to the assays which will be performed at each institution (Table 3). Once it has determined that all laboratory assays have been completed and that no further use of the biological specimens are required, any specimens being stored at UVM will be returned to icddr,b.

If a study participant becomes lost to follow-up, all specimens already collected will be tested as outlined in the protocol. Any loss or unanticipated destruction of samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) that compromises the scientific integrity of the study will be reported to each IRB.

Access to research samples will be limited using either a locked room or a locked freezer. Samples and data will be stored using codes assigned by the Investigators or their designee. Data will be kept in password-protected computers. Only Investigators or their designees will have access to the samples and data.

Retention of Records
The PI is responsible for retaining all essential documents listed in the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) Guideline. Trial-related documents will be maintained by the PI in a secure storage facility for a minimum of 5 years after completion or termination of the study. Records will also be maintained in compliance with IRB and Bangladesh national, state, and federal medical records retention standards. All trial-related documents will be stored in a way that ensures that confidentiality will be strictly maintained to the extent provided by Bangladesh national, federal, state, and local law.

All study files, source documents, and trial master files will be maintained and stored in a secure manner in the icddr,b in the office of the Clinical Research Coordinator (or equivalent). No study documents will be destroyed without prior written agreement between the PI and the clinical research staff.

Sample Size Calculation and Outcome (Primary and Secondary) Variable(s)

Clearly mention your assumptions. List the power and precision desired. Describe the optimal conditions to attain the sample size. Justify the sample size that is deemed sufficient to achieve the specific aims.

Sample size calculation was performed to ensure adequate power to evaluate our primary outcome measure, vaccine take. As noted above in Preliminary Data, the seroconversion rate in PROVIDE was 27% among vaccinated infants, and no faecal shedding was detected when assessed by ELISA, yielding a frequency of vaccine take of only 27%. RV-IgA antibody responses were very variable in this population and difficult to interpret, similar to RV-IgA responses in other low-income settings. A clearer impact on vaccine take is more likely to be observed by effecting an increase in faecal vaccine shedding. Because of this, we calculated our sample size primarily to detect differences in rates of faecal shedding. No RV1 vaccine shedding was detected in this population when assessed by stool ELISA and results for RT-PCR assessment of stool shedding are not yet available. However, a Chinese study found 72.4% of subjects had detectable shedding by RT-PCR 2-3 days following first RV1 dose, compared to 10.3% of subjects with detectable shedding when assessed by ELISA (17). Our assumption is that while no shedding was detected by ELISA,

some degree of shedding would have been identified by RT-PCR. Guided by the Chinese study data, we estimated that the baseline rate of RV1 shedding as detected by RT-PCR in the Mirpur population would be 50%. To detect a 20% increase in shedding to 70%, we calculate that 93 study participants in each arm will provide 80% power at 2-sided α=0.05. In PROVIDE, an attrition rate of 15% was observed, with the majority of drop-outs occurring very early in the study. Therefore, we anticipate a similar rate, which would require enrolment of 107 study participants per arm. Our target enrolment will thus be 110 study participants per arm for a total of 220 study participants.

Assuming a similar rate of seroconversion among infants who receive a standard dose of RV1 in this study, at2-sided α=0.05, this sample size will also provide 80% power to detect a 20% difference in RV-IgA seroconversion rate (i.e. an increase in seroconversion rate from 27% to $\geq$47% among infants in the intervention arm). Our other outcome variables are contingent upon faecal shedding (e.g. faecal viral burden) or are purely exploratory in nature and therefore were not considered further in sample size calculation.

Data Analysis

Describe plans for data analysis, including stratification by sex, gender and diversity. Indicate whether data will be analysed by the investigators themselves or by other professionals. Specify what statistical software 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 determine further course of the study.

Definitions

Vaccine take: Detection of faecal shedding of RV1 in any non-diarrhoeal stool specimen at any time point following vaccination, OR RV-IgA seroconversion, defined as post-immunization (week 14) RV-IgA $\geq$ 20 U/mL with pre-immunization (week 6) RV-IgA <20 U/mL.

Faecal shedding: Positive qRT-PCR for rotavirus NSP3 with confirmatory gene sequencing result positive for vaccine-strain virus at any time point following vaccination, with negative week 6 pre-vaccination stool testing. Due to the low level of viral shedding anticipated, no threshold Ct value will be set to determine positivity, and all specimens with detection (up to Ct=40) will be considered positive if controls are appropriate. In the event that qRT-PCR is negative for RV but ELISA is positive, the specimen will be considered negative for faecal shedding for primary outcome analysis, but additional analysis will be performed to include ELISA positive specimens as well. qRT-PCR specimens positive for RV but that yields a non-vaccine strain will not be considered positive for faecal shedding. If the strain is untypeable, the specimen will be considered negative for faecal shedding for primary outcome analysis, but additional analysis will be performed to include these outcomes as positive to compare for differences in outcome relative to the more stringent criteria.

RV-IgA seroconversion: RV-IgA ELISA $\geq$20 U/ml post-vaccination (i.e. at 14 weeks) with RV-IgA ELISA <20 U/ml pre-vaccination (i.e. at 6 weeks).

Antigenemia: Detection of RV antigen in any plasma specimen by rotavirus ELISA following vaccination, or detection of RV in any plasma specimen by qRT-PCR following vaccination.

Viremia: Detection of RV from any plasma specimen following vaccination, as evidenced by positive RV-specific immunostaining following serial passage on MA104 cells or demonstration of cytopathic effect.

VP4, VP7, VP5, or NSP4 sero-response and end-point titer*: Sero-response is defined as greater than or equal to four-fold rise in end-point titer post-vaccination (i.e. at 14 weeks) compared to pre-vaccination (i.e. at 6 weeks). End-point titre is defined as the inverse of the highest dilution that gives positive immunostaining detectable above background.

Diarrhoea. Three or more bowels movements looser than normal per mother's judgement within 24 hours.

RV diarrhoea. Detection of RV antigen using commercial EIA kit in any diarrhoeal stool specimen.

Analysis plan

Data analysis will be conducted by the investigators, using Excel (Microsoft), GraphPad Prism (GraphPad Software, Inc.), and/or SAS (SAS Institute).As this study does not assess treatment efficacy, no scheduled interim analysis will be necessary to determine further course of the study. Study participants and investigators will remain blinded throughout the course of the study. Unblinding will not occur until all study-related laboratory procedures required for analysis of primary and secondary outcomes have been performed and results have been finalized after undergoing QA/QC. Study participant or group unblinding might potentially occur however if deemed necessary for safety review by the DSMB.

Primary analysis will compare the proportion of infants with vaccine take (detection of faecal shedding of RV1 in any non-diarrhoeal stool specimen at any time point following vaccination, OR RV-IgA seroconversion, defined as post-immunization (week 14) RV-IgA $\geq$ 20 U/mL with pre-immunization (week 6) RV-IgA <20 U/mL) in the intervention versus control arm in all study participants who completed the study per-protocol. Analysis will be performed by Chi-square test and an estimate of proportion difference with corresponding 95% confidence interval will be calculated. Secondary analysis will compare frequency of faecal RV1 shedding alone and of RV-IgA seroconversion alone between intervention and control groups, using similar methods, and will compare both RV-IgA concentration and faecal viral burden among infants in the intervention arm versus control using independent samples t-test or Mann-Whitney test, as appropriate depending on the data distribution.

Exploratory analyses, including by intention-to-treat, evaluating categorical outcomes according to intervention versus control assignment (e.g. VP4, VP7, VP5*, and NSP4 sero-response, presence of antigenemia, presence of viremia) will be performed by Chi-square or Fisher's exact test, as appropriate. Additional analyses evaluating continuous variables according to intervention versus control assignment (e.g. VP4, VP7, VP5*, and NSP4 titres) will be performed by independent samples t-test or Mann-Whitney test, as appropriate. Associations between categorical and continuous variables (e.g. RV-IgA seroconversion and quantitative RV1 viral burden in stool specimens) will be evaluated by logistic regression analysis. Associations between continuous variables (e.g. RV-IgA concentration and quantitative RV1 viral burden in stool specimens) will be investigated by linear regression analysis. Any final models will be appropriately penalized for multiple testing.

Data Safety Monitoring Plan (DSMP)

All clinical investigations (research protocols testing biomedical and/or behavioural intervention(s)) should include the Data and Safety Monitoring Plan (DSMP). The purpose of DSMP is to provide a framework for appropriate oversight and monitoring of the conduct of clinical trials to ensure the safety of participants and the validity and integrity of the data. It involves involvement of all investigators in periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of trial sites, and other factors that can affect study outcome.

A Data and Safety Monitoring Board (DSMB) will be convened for this study by the Ethical Review Committee (ERC) of the icddr,b. The ERC DSMB reflects the disciplines and medical specialties necessary to interpret the data from the clinical trial and may include expertise in clinical aspects of disease/patient population being studied, biostatistics, and clinical trials conduct and methodology. The ERC selects the DSMB chair and determines the terms of membership. The ERC DSMB will review and evaluate the accumulated study data for participant safety, study conduct, and progress, and make recommendations to the ERC concerning the continuation, modification, or termination of the trial. The ERC DSMB will meet once before the study opens to make recommendations as to whether the study can proceed, and the DSMB schedule for meetings during and at completion of the study will be agreed between the PI and DSMB. Additionally, the DSMB may meet as needed to identify or review any potential safety concerns. The study

investigators will report to the DSMB per the Board's requirements and expectations. All adverse events and unanticipated problems will be reviewed and reported to the respective IRBs of both icddr,b and UVM and/or to the DMSB according to each institution's reporting guidelines and as further directed by the DSMB. AEs that are considered to be related to vaccination will also be reported to the DGDA according to the same reporting guidelines as for the icddr,b ERC.

In addition, at the request of the DGDA, post-vaccination solicited AE safety data for approximately twenty children will be submitted to both DSMB and DGDA for initial review. Further enrolment will be paused once a sufficient number of children have been enrolled to ensure that approximately 20 will have completed vaccination for this review period. No additional children will be vaccinated until the initial safety data have been reviewed and approved by both DMSB and DGDA. Children already enrolled will continue additional study procedures according to the protocol schedule.

Ethical Assurance for Protection of Human rights

Describe the justifications for conducting this research in human participants. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how participants' rights will be protected, and if there would be benefit or risk to each participants of the study. 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. Discuss procedures safeguarding participants from injuries resulting from study procedures and/or interventions, whether physical, financial or social in nature. [Please see Guidelines]

Assurance of Protection

This study will be conducted in compliance with the Declaration of Helsinki and in full conformity with the principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR 46, 21 CFR 312, and/or ICH E6; 62 Federal Regulations 25691 (1997). Each participating institution will hold a current FWA issued by OHRP. All key study staff will be trained in Good Clinical Practice. In addition, this study will be registered with ClinicalTrials.gov.

Each participating institution's RRC, ERC or IRB will review and approve this protocol and associated informed consent documents prior to study initiation. The DGDA also will approve this protocol prior to study initiation. Any amendments to the protocol or consent materials will also be approved before they are implemented. The PI will be responsible for obtaining all necessary regulatory approvals. A copy of the study approvals (including approval of the informed consent form) is to be maintained in the Investigator study document binder. During the study, the PI is responsible for providing each regulatory body with all documents subject to review (e.g., Protocol Amendments, informed consent form updates, advertisements, and any written information that may be provided to the participant). Annual Continuing Review Reports on the progress of the study will be submitted by the PI to each regulatory body in accordance with each respective institution's guidelines. A final report will be submitted to each regulatory body at the completion of the study.

Protocol Compliance

The PI will conduct the trial in exact compliance with the written protocol. The PI will not implement any deviation from or change to the protocol without agreement, prior review, and documented approval by each IRB that granted original approval for the study. The DSMB and DGDA will be notified of all protocol revisions (other than administrative) and will review any changes to the protocol that involve DSMB or DGDA oversight or involve changes to the data and safety monitoring plan (DSMP) of the study.

The PI may implement a deviation or change in the protocol in order to eliminate an immediate hazard to study participants without prior IRB, DMSB, or DGDA approval or when the change involves only logistical or administrative aspects of the trial, such as a telephone number change. In the event of a medical

emergency, the PI and/or designated medical officer(s) shall perform any medical procedures deemed medically appropriate.

As soon as possible, the implemented deviation or change, the reasons for the change, and, if appropriate, the proposed protocol amendment(s) should be submitted to each IRB, the DSMB, and DGDA.

Risk to human subjects

Human subjects involvement, characteristics, and design. This is a double-blind, randomized, controlled vaccine immunogenicity trial comparing standard vs high dose oral RV1 vaccine among healthy infants in Dhaka, Bangladesh. The clinical aspects of this study will be performed at the field clinic of the icddr,b in the Mirpur neighbourhood of Dhaka, using the target enrolment goal calculated to ensure adequate power to detect our primary outcome measure. Inclusion criteria include: 1) generally healthy infant; 2) age 0-7 days at enrolment; 3) mother willing and able to provide signed informed consent; 4) mother willing to allow infant to be vaccinated; 5) mother willing to allow biological specimens, including blood, stool, and saliva, collected from infant according to study protocol; 6) mother willing and able to adhere to study schedule. Exclusion criteria include: 1) congenital malformations; 2) known immunocompromising condition in infant; 3) prior rotavirus vaccination; 4) other household member enrolled in the study.

Inclusion of women, minorities, and children. RV gastroenteritis is almost exclusively a disease of infants and young children, and RV vaccine is indicated only for infants ≥6 weeks age. Therefore, by necessity this study will enrol infant children of both sexes, reflecting the racial and ethnic make-up of the local population.

Sources of materials: Vaccines will be purchased in Bangladesh through the local GlaxoSmithKline distributor, and all laboratory materials and reagents will be obtained via commercial vendors. Blood, stool and saliva specimens will be collected from study participants at the indicated time points by trained personnel. Recombinant baculovirus constructs expressing RV1 VP4, VP7, VP5*, and NSP4that have already been constructed by the UVM investigators will be utilized in assessing VP4, VP7, VP5*, and NSP4 antibody responses.

Potential Risks.

Vaccination: The risks of RV1 vaccine to immunocompetent infants is low. Infants with known immunocompromising conditions may be at higher risk since RV1 contains live, attenuated virus; our exclusion criteria should be sufficient to exclude any potentially higher-risk infants. In clinical and post-licensure evaluation, common adverse events following either dose of vaccine were fussiness, cough or runny nose, fever, loss of appetite, vomiting, or diarrhoea, but these rates did not differ from placebo (product insert, Rotarix, GlaxoSmithKline). Further, the rates of SAEs in vaccinated infants and in placebo recipients did not differ. The most severe adverse event following rotavirus vaccination is intussusception, which led to the withdrawal of the first licensed oral RV vaccine (RotaShield, Wyeth) from the market in 1999. Extensive post-licensure surveillance of RV1 has demonstrated a very low increased risk of intussusception following RV1 vaccination of 1-5 excess cases per 100,000 children vaccinated; this incidence rate is substantially lower than the number of deaths prevented yearly due to rotavirus vaccine (5). An evaluation of background intussusception rates in Bangladesh has previously been conducted; in that analysis, the upper limit for estimated incidence of possible intussusception among children <2 years of age was 97 cases per 100,000 children per year (1 per 1030 children per year) (44). Given these numbers, it is extremely unlikely that any participant in this study would experience intussusception.

The intervention group will receive double the standard dose of RV1. A similar dose has been previously evaluated in a phase II trial conducted in the United States and Canada (15). In that study, infants received either $10^{5.2}$ FFU (N=212) or $10^{6.4}$ FFU (N=209) of RV1, or placebo (N=108), whereas our study proposes to use 10^{6} FFU (standard dose) or $10^{6.3}$ FFU (high dose). In the previous study, no differences in either solicited or unsolicited AEs or SAEs were detected between treatment groups. No cases of intussusception

were detected, although the study was not powered for that outcome. One infant had gastroesophageal reflux first noted 7 days after receiving the first dose of $10^{6.4}$ FFU and withdrew from the study. Two others had abdominal tenderness following the second dose of vaccine; imaging studies were negative for intussusception but positive for mesenteric adenitis, and stool specimens for both infants were negative for rotavirus but positive for other enteric viruses (enterovirus and adenovirus, respectively). In summary, both doses of vaccine were safe and well-tolerated. We have no reason to suspect that reactogenicity or safety risks will be increased in our population; on the contrary, given the difficulty in establishing vaccine take with standard doses in this population (potentially due to inhibitory factors in the infant gut), we suspect that infants in this study will "see" a lower inoculum of virus when administered similar doses, compared to children in the U.S. and Canada.

Blood draw: Venipuncture and heelstick will be used to obtain the necessary blood specimens for this study. As both primary and secondary outcome measures rely on plasma antibody measurement, there is no alternative to blood collection in the performance of this study. Potential risks of venipuncture or heelstick include local pain and discomfort, bleeding or bruising at the site of puncture, and infection, but these risks are minimal.

Confidentiality: Breach of confidentiality is another potential risk when collecting and recording personal identifying information.

Adequacy of protection against risks

Recruitment and informed consent. Mothers of potential study participants will be recruited during the prenatal period using community-based field research assistants. Extensive discussion will take place at this time regarding the study design, rationale, risks and benefits. Mothers who express interest in participating will be identified and coordination will take place to ensure that a field research assistant visits the household within one week following the birth of the infant. At that time, the study will be reviewed again, and after confirmation that the family desires to participate and has had all questions answered to their satisfaction, informed consent will be obtained from the mother and the child will be enrolled in the study. Because all study participants will be infants, assent will not be obtained. Consent will be obtained in consideration of the literacy level of the family, and in cases where the mother cannot write, a witnessed thumbprint will be obtained. Witnesses may not be associated with the study. Only after written informed consent is obtained will any study-specific procedures be performed.

Informed consent forms will be revised to reflect any significant amendment(s) which directly affects volunteers, and must also be reviewed and approved by the IRB with the amendment. Study participants will be consented using the most recently-approved informed consent form. Participants already enrolled in the study will be informed about revisions to the protocol and, depending on the impact of the amendment, may be asked to re-consent. Re-consenting may be accomplished by repeating the informed consent process using the approved revised informed consent form, with attention given to the changes, or it may be done using an approved addendum to the informed consent form, clearly stating any revisions or new information and summarizing the potential effect upon participation in the study. The new informed consent form or addendum must be signed and placed into the study record. A signed copy is given to the volunteer.

Protections against risk.

Vaccine safety: To monitor for any potential adverse events and safety concerns during the study period, extensive safety surveillance will be solicited (for 7 days following each vaccine dose and at least weekly thereafter), and mothers will be educated and encouraged to report unsolicited events and concerns whenever they occur. The Mirpur study clinic is capable of providing basic primary care and acute care needs, and these services will be available to all participating infants. If warranted, the study team will

coordinate referral to appropriate facilities for AEs requiring more extensive medical evaluation or hospitalization.

Study withdrawal: Mothers can choose to withdraw their child from the study at any time. Further, pausing criteria have been established as described above in "Pausing criteria."

Blood draw: Blood collection will be performed by trained personnel to minimize risk of pain, discomfort, bruising, bleeding, and infection. The blood volumes proposed for this study are within published standards for safe phlebotomy in children, which is up to 5% of total blood volume per day estimated at 80 mL/kg body weight (i.e. 4 mL/kg/day) and up to 10% of total blood volume over 8 weeks (8 mL/kg) (45).

Confidentiality: Study participant confidentiality is strictly held in trust by the participating investigators, their staff, and their agents. This confidentiality includes documentation, investigation data, participants' clinical information, and all other information generated during participation in the study. To ensure confidentiality and prevent unauthorized breath of confidentiality, study participants will be assigned a unique SID to be used on study forms and in the study database. Whenever feasible, study participants will be identified only via their SID. Each study participant will have a study chart that is labelled only with the SID. This study chart will contain complete source documentation and CRFs. Neither the study forms nor the study database will contain participants' names or other information that could be used to identify them. Any identifying information will be kept in a separate study binder for all study participants. The study binder and study charts will all be kept in a secure, locked office that will not be accessible to unauthorized personnel. In addition, the following will be kept in a locked office and will not be accessible to unauthorized personnel: 1) Informed consent documents, forms, lists, logbooks, appointment books, or any other document linking the SID to participants' names or identifying information; and 2) other study forms, including laboratory reports and administrative forms. All computers containing the study database or electronic records that contain any identifying information will be password-protected will not be accessible to unauthorized personnel. The key linking SIDs to personal identifiers will be kept on a password-protected computer file that will only be available to key personnel. Chart information and information from study records will not be released without written permission of the participant. However, records may be reviewed by representatives from the icddr,b ERC, DSMB, UVM IRB, and DGDA. No identifying information will be disclosed in the process of analysing or publishing results from this study.

Potential benefits of the proposed research to human subjects and others. RV1 vaccine is an FDA-approved, WHO pre-qualified vaccine but has not been incorporated into the Bangladesh EPI and is therefore generally unavailable to this study population. All participants may potentially benefit from participation in this study by receiving RV vaccine and subsequent protection against severe gastroenteritis, dehydration, and/or death. This vaccine is given nearly universally in settings where it is available, and since global introduction, has prevented hundreds of thousands of deaths due to prevention of dehydrating diarrhoea due to RV(4). Importantly, the WHO has recommended that RV vaccine be administered to all infants in spite of the known risks of the vaccine, as the potential risks described above are vastly outweighed by the potential benefits to infants receiving vaccination.

Importance of the knowledge to be gained. This study has the potential to identify a strategy that may improve oral RV vaccine performance in the developing world, and identify candidates for more reliable COPs for RV, which would vastly increase the efficiency of clinical RV research. Either of these outcomes would improve the health of children by leading to improvements in strategies to prevent and study RV infection, which remains the most common cause of diarrhoeal disease in children worldwide. Therefore, the importance of the knowledge to be gained regarding improvement in child health is substantial.

Use of Animals

Describe if and the type and species of animals to be used in the study. Justify with reasons the use of particular animal species in the research and the compliance of the animal ethical guidelines for conducting the proposed procedures.

No animals will be used in this study.

Collaborative Arrangements

Describe 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.

This study will be carried out in collaboration with the icddr,b and the University of Vermont. The investigative teams for this project have a long-standing collaborative relationship. All necessary arrangements to conduct this study at the icddr,b have been established with the investigators of the collaborating institutions. Any requirements related to financial reporting and scientific progress required by sources of funding for the UVM investigator(s) will be the sole responsibility of the UVM investigator(s).

Facilities Available

Describe the availability of physical facilities at site of conduction of the study. If applicable, describe the use of Biosafety Level 2 and/or 3 laboratory facilities. For clinical and laboratory-based studies, indicate the provision of hospital and other types of adequate patient care and laboratory support services. Identify the laboratory facilities and major equipment that will be required for the study. For field studies, describe the field area including its size, population, and means of communications plus field management plans specifying gender considerations for community and for research team members.

The icddr,b has a 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 observational field studies and clinical trials in the Mirpur community where the field site is located.

Local clinical research study personnel from icddr,b will be used to execute the clinical aspects of the study at the Mirpur field site, an outpatient clinical research clinic with a full complement of support staff. The clinic building includes office and clinic spaces, storage capacity, and appropriate facilities for conducting the study. The catchment area for this site is approximately 100,000 residents.

Laboratory assays will be performed at both UVM and at icddr,b using existing equipment. Personnel at both sites will be experienced in all site-specific procedures proposed herein. All initial specimen processing will take place at icddr,b.

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.

1. Phua KB, Quak SH, Lee BW, Emmanuel SC, Goh P, Han HH, et al. Evaluation of RIX4414, a live, attenuated rotavirus vaccine, in a randomized, double-blind, placebo-controlled phase 2 trial involving 2464 Singaporean infants. The Journal of infectious diseases. 2005;192 Suppl 1:S6-s16.
2. Nelson EA, Glass RI. Rotavirus: realising the potential of a promising vaccine. Lancet (London, England). 2010;376(9741):568-70.
3. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet (London, England). 2013;382(9888):209-22.
4. Tate JE, Burton AH, Boschi-Pinto C, Parashar UD. Global, Regional, and National Estimates of Rotavirus Mortality in Children <5 Years of Age, 2000-2013. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2016;62 Suppl 2:S96-s105.
5. Yen C, Tate JE, Hyde TB, Cortese MM, Lopman BA, Jiang B, et al. Rotavirus vaccines: current status and future considerations. Human vaccines & immunotherapeutics. 2014;10(6):1436-48.
6. Rotavirus vaccines. WHO position paper - January 2013. Releve epidemiologique hebdomadaire / Section d'hygiene du Secretariat de la Societe des Nations = Weekly epidemiological record / Health Section of the Secretariat of the League of Nations. 2013;88(5):49-64.
7. WHO recommendations for routine immunization - summary tables 2015 [Available from: http://www.who.int/immunization/policy/immunization_tables/en/.
8. Madhi SA, Cunliffe NA, Steele D, Witte D, Kirsten M, Louw C, et al. Effect of human rotavirus vaccine on severe diarrhea in African infants. The New England journal of medicine. 2010;362(4):289-98.
9. Vesikari T, Karvonen A, Prymula R, Schuster V, Tejedor JC, Cohen R, et al. Efficacy of human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in European infants: randomised, double-blind controlled study. Lancet (London, England). 2007;370(9601):1757-63.
10. Vesikari T, Karvonen AV, Majuri J, Zeng SQ, Pang XL, Kohberger R, et al. Safety, efficacy, and immunogenicity of 2 doses of bovine-human (UK) and rhesus-rhesus-human rotavirus reassortant tetravalent vaccines in Finnish children. The Journal of infectious diseases. 2006;194(3):370-6.
11. Zaman K, Dang DA, Victor JC, Shin S, Yunus M, Dallas MJ, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in Asia: a randomised, double-blind, placebo-controlled trial. Lancet (London, England). 2010;376(9741):615-23.

12. Glass RI, Parashar U, Patel M, Gentsch J, Jiang B. Rotavirus vaccines: successes and challenges. The Journal of infection. 2014;68 Suppl 1:S9-18.
13. Anderson EJ. Rotavirus vaccines: viral shedding and risk of transmission. The Lancet Infectious diseases. 2008;8(10):642-9.
14. Vesikari T, Karvonen A, Korhonen T, Espo M, Lebacq E, Forster J, et al. Safety and immunogenicity of RIX4414 live attenuated human rotavirus vaccine in adults, toddlers and previously uninfected infants. Vaccine. 2004;22(21-22):2836-42.
15. Dennehy PH, Brady RC, Halperin SA, Ward RL, Alvey JC, Fischer FH, Jr., et al. Comparative evaluation of safety and immunogenicity of two dosages of an oral live attenuated human rotavirus vaccine. The Pediatric infectious disease journal. 2005;24(6):481-8.
16. Ruiz-Palacios GM, Guerrero ML, Bautista-Marquez A, Ortega-Gallegos H, Tuz-Dzib F, Reyes-Gonzalez L, et al. Dose response and efficacy of a live, attenuated human rotavirus vaccine in Mexican infants. Pediatrics. 2007;120(2):e253-61.
17. Hsieh YC, Wu FT, Hsiung CA, Wu HS, Chang KY, Huang YC. Comparison of virus shedding after lived attenuated and pentavalent reassortant rotavirus vaccine. Vaccine. 2014;32(10):1199-204.
18. Zaman K, Sack DA, Yunus M, Arifeen SE, Podder G, Azim T, et al. Successful co-administration of a human rotavirus and oral poliovirus vaccines in Bangladeshi infants in a 2-dose schedule at 12 and 16 weeks of age. Vaccine. 2009;27(9):1333-9.
19. O'Ryan M. Rotarix (RIX4414): an oral human rotavirus vaccine. Expert review of vaccines. 2007;6(1):11-9.
20. Salinas B, Perez Schael I, Linhares AC, Ruiz Palacios GM, Guerrero ML, Yarzabal JP, et al. Evaluation of safety, immunogenicity and efficacy of an attenuated rotavirus vaccine, RIX4414: A randomized, placebo-controlled trial in Latin American infants. The Pediatric infectious disease journal. 2005;24(9):807-16.
21. Levine MM. Immunogenicity and efficacy of oral vaccines in developing countries: lessons from a live cholera vaccine. BMC biology. 2010;8:129.
22. Angel J, Franco MA, Greenberg HB. Rotavirus vaccines: recent developments and future considerations. Nature reviews Microbiology. 2007;5(7):529-39.
23. Patel M, Glass RI, Jiang B, Santosham M, Lopman B, Parashar U. A systematic review of anti-rotavirus serum IgA antibody titer as a potential correlate of rotavirus vaccine efficacy. The Journal of infectious diseases. 2013;208(2):284-94.
24. Angel J, Steele AD, Franco MA. Correlates of protection for rotavirus vaccines: Possible alternative trial endpoints, opportunities, and challenges. Human vaccines & immunotherapeutics. 2014;10(12):3659-71.
25. Cheuvart B, Neuzil KM, Steele AD, Cunliffe N, Madhi SA, Karkada N, et al. Association of serum anti-rotavirus immunoglobulin A antibody seropositivity and protection against severe rotavirus gastroenteritis: analysis of clinical trials of human rotavirus vaccine. Human vaccines & immunotherapeutics. 2014;10(2):505-11.
26. Blutt SE, Matson DO, Crawford SE, Staat MA, Azimi P, Bennett BL, et al. Rotavirus antigenemia in children is associated with viremia. PLoS medicine. 2007;4(4):e121.
27. Nordgren J, Sharma S, Bucardo F, Nasir W, Gunaydin G, Ouermi D, et al. Both Lewis and secretor status mediate susceptibility to rotavirus infections in a rotavirus genotype-dependent manner. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2014;59(11):1567-73.

28. Payne DC, Currier RL, Staat MA, Sahni LC, Selvarangan R, Halasa NB, et al. Epidemiologic Association Between FUT2 Secretor Status and Severe Rotavirus Gastroenteritis in Children in the United States. JAMA pediatrics. 2015;169(11):1040-5.
29. Kirkpatrick BD, Colgate ER, Mychaleckyj JC, Haque R, Dickson DM, Carmolli MP, et al. The "Performance of Rotavirus and Oral Polio Vaccines in Developing Countries" (PROVIDE) study: description of methods of an interventional study designed to explore complex biologic problems. The American journal of tropical medicine and hygiene. 2015;92(4):744-51.
30. Colgate ER, Haque R, Dickson DM, Carmolli MP, Mychaleckyj JC, Nayak U, et al. Delayed Dosing of Oral Rotavirus Vaccine Demonstrates Decreased Risk of Rotavirus Gastroenteritis Associated With Serum Zinc: A Randomized Controlled Trial. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2016;63(5):634-41.
31. National Institute of Population Research and Training (NIPORT) MaA, and ICF International. 2016. Bangladesh Demographic and Health Survey 2014. Dhaka, Bangladesh, and Rockville, Maryland, USA: NIPORT, Mitra and Associates, and ICF International: .
32. Emperador DM, Velasquez DE, Estivariz CF, Lopman B, Jiang B, Parashar U, et al. Interference of Monovalent, Bivalent, and Trivalent Oral Poliovirus Vaccines on Monovalent Rotavirus Vaccine Immunogenicity in Rural Bangladesh. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2016;62(2):150-6.
33. Colston JM, Francois R, Pisanic N, Penataro Yori P, McCormick BJJ, Olortegui MP, et al. Effects of Child and Maternal Histo-Blood Group Antigen Status on Symptomatic and Asymptomatic Enteric Infections in Early Childhood. The Journal of infectious diseases. 2019;220(1):151-62.
34. Ramani S, Stewart CJ, Laucirica DR, Ajami NJ, Robertson B, Autran CA, et al. Human milk oligosaccharides, milk microbiome and infant gut microbiome modulate neonatal rotavirus infection. Nat Commun. 2018;9(1):5010.
35. Liu J, Gratz J, Amour C, Nshama R, Walongo T, Maro A, et al. Optimization of Quantitative PCR Methods for Enteropathogen Detection. PloS one. 2016;11(6):e0158199.
36. Liu J, Kibiki G, Maro V, Maro A, Kumburu H, Swai N, et al. Multiplex reverse transcription PCR Luminex assay for detection and quantitation of viral agents of gastroenteritis. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology. 2011;50(4):308-13.
37. Gautam R, Esona MD, Mijatovic-Rustempasic S, Ian Tam K, Gentsch JR, Bowen MD. Real-time RT-PCR assays to differentiate wild-type group A rotavirus strains from Rotarix((R)) and RotaTeq((R)) vaccine strains in stool samples. Human vaccines & immunotherapeutics. 2014;10(3):767-77.
38. Oberste MS, Penaranda S, Rogers SL, Henderson E, Nix WA. Comparative evaluation of Taqman real-time PCR and semi-nested VP1 PCR for detection of enteroviruses in clinical specimens. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology. 2010;49(1):73-4.
39. Patel M, Rench MA, Boom JA, Tate JE, Sahni LC, Hull JA, et al. Detection of rotavirus antigenemia in routinely obtained serum specimens to augment surveillance and vaccine effectiveness evaluations. The Pediatric infectious disease journal. 2010;29(9):836-9.
40. Ward RL, Bernstein DI, Shukla R, Young EC, Sherwood JR, McNeal MM, et al. Effects of antibody to rotavirus on protection of adults challenged with a human rotavirus. The Journal of infectious diseases. 1989;159(1):79-88.

41. Yuan L, Honma S, Kim I, Kapikian AZ, Hoshino Y. Resistance to rotavirus infection in adult volunteers challenged with a virulent G1P1A[8] virus correlated with serum immunoglobulin G antibodies to homotypic viral proteins 7 and 4. The Journal of infectious diseases. 2009;200(9):1443-51.
42. Yuan L, Ishida S, Honma S, Patton JT, Hodgins DC, Kapikian AZ, et al. Homotypic and heterotypic serum isotype-specific antibody responses to rotavirus nonstructural protein 4 and viral protein (VP) 4, VP6, and VP7 in infants who received selected live oral rotavirus vaccines. The Journal of infectious diseases. 2004;189(10):1833-45.
43. Svensson L, Petersson A, Henry SM. Secretor genotyping for A385T, G428A, C571T, C628T, 685delTGG, G849A, and other mutations from a single PCR. Transfusion. 2000;40(7):856-60.
44. Zaman K, Breiman RF, Yunus M, Arifeen SE, Mahmud A, Chowdhury HR, et al. Intussusception surveillance in a rural demographic surveillance area in bangladesh. The Journal of infectious diseases. 2009;200 Suppl 1:S271-6.
45. Howie SR. Blood sample volumes in child health research: review of safe limits. Bulletin of the World Health Organization. 2011;89(1):46-53.