University of Zambia Biomedical Research Ethics Committee

Proposal for Ethical Review

Title: Human challenge with live-attenuated rotavirus to assess next-generation rotavirus vaccines in Africa

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ABBREVIATIONS AND ACRONYMS

AE	Adverse Events
ASC	Antibody Secreting Cells
CIDRZ	Centre for Infectious Disease Research in Zambia
CoP	Correlate of Protection
CRF	Case Report Form
DMP	Data Management Plan
DNA	Deoxyribonucleic acid
DTP	Diphtheria, Tetanus, Pertussis
EC	Ethics Committee
EPI	Expanded Program on Immunization
GCP	Good Clinical Practices
HBGAs	Histo-blood group antigens
HepB	Hepatitis B
Hib	*Haemophilus influenzae type b*
HIC	Human infection challenge
ICH	International Conference on Harmonisation
IMNCI	Integrated Management of Neonatal and Childhood Illnesses
LIC	Low income country
LMICs	Lower middle-income countries
MCH	Mother and Child Health
MTA	Material Transfer Agreement
NHRA	National Health Research Authority
ORV	Oral Rotavirus Vaccines
OPD	Outpatient Department
PBMCs	Peripheral blood mononuclear cells
PIDC	Post-Immunization Diary Card
PCR	Polymerase Chain Reaction
RCT	Randomised controlled trial
RNA	Ribonucleic acid
RVGE	Rotavirus Gastroenteritis
SAE	Serious Adverse Event
SOP	Standard Operating Procedure (s)
SRVGE	Severe Rotavirus Gastroenteritis
UNZABREC	University of Zambia Biomedical Research Ethics Committee
VLP	Virus Like Particle
WHO	World Health Organisation

Protocol summary

Title:	*Human challenge with live-attenuated rotavirus to assess next-generation rotavirus vaccines in Africa*
Population:	*Healthy infants randomised at 6 weeks of age followed up for between 16 and 20 weeks.*
Study Site:	*Single site trial conducted at Chawama First Level Hospital*
Study Duration:	*12 months from enrollment of the first participant through completion of the final study visit for the last participant*
Participation Duration:	*16-20 weeks*
Description of Agents or Interventions:	*Live-attenuated oral human rotavirus vaccine (Rotarix) manufactured by GlaxoSmithKline containing at least 10^6 $CCID_{50}$ (median cell culture infective doses) of G1P[8] rotavirus, RIX4414 strain produced in Vero cells. This vaccine is an oral suspension with a single dose (1.5ml) administered using an oral applicator.* *Trivalent P2-VP8 subunit vaccine manufactured by SK Chemicals containing 90ug of each VP8 antigen derived from P[4] (DS-1), P[6] (1076), and P[8] (Wa) rotavirus strains fused to the P2 epitope from tetanus toxoid and adsorbed to aluminium hydroxide. A single dose (0.5ml) of this vaccine is administered intramuscularly through injection.*
Objectives:	***Primary Objective:*** • *To evaluate whether infant immunization with parenteral trivalent P2-VP8 subunit vaccine administered as 3 doses alone or as 1 or 3 doses in combination with 2 doses of live-attenuated oral rotavirus vaccine offers increased protection compared with 2 doses of oral vaccine alone against rotavirus shedding after an oral vaccine challenge dose administered at 18 weeks of age* ***Secondary Objectives:*** • *To compare seroconversion (IgA,IgG) in blood and saliva samples taken at 6 and 18 weeks of age among infants immunized with parenteral trivalent P2-VP8 subunit vaccine, given as 3 doses alone, or as 1 or 3 doses in combination with 2 doses of oral rotavirus vaccine, with seroconversion among infants receiving 2 doses of oral rotavirus vaccine only* • *To compare seroconversion (IgA,IgG) in blood and saliva samples taken at 6 and 14 weeks of age among infants immunized with parenteral trivalent P2-VP8 subunit vaccine,*

given as 2 doses alone or in combination with oral rotavirus vaccine, with seroconversion among infants receiving 2 doses of oral rotavirus vaccine only

- *To evaluate the association of anti-VP5, VP7 and VP8 antibodies (IgA, IgG) induced in blood by oral vaccine with protection against shedding after subsequent challenge*
- *To evaluate the association of anti-VP8 (IgA,IgG) induced in blood and saliva by parenteral subunit vaccine with protection against shedding after subsequent challenge*
- *To evaluate the association of neutralizing antibodies against G1P[8] rotavirus induced by vaccination with protection against shedding after subsequent challenge*

Exploratory objectives

- *To evaluate in a subset of infants whether a dose of trivalent P2-VP8 vaccine at 14 weeks of age boosts rotavirus-specific mucosal immunity compared with infants not receiving this vaccine, as assessed by ELISPOT of gut-homing antibody secreting cells in whole blood collected from infants at 15 weeks*

Hypotheses:

Primary Hypothesis:

A combined infant schedule of Rotarix and parenteral VP8 subunit vaccine will increase protection against shedding of live-attenuated vaccine virus following challenge at 18 weeks of age compared with Rotarix alone

Secondary Hypothesis

Human challenge with Rotarix will allow rapid and efficient investigation of potential immune CoPs against faecal shedding of rotavirus. Identification of novel immune CoPs in this model based on minimally invasive sample collection (saliva, peripheral blood) would facilitate the development and testing of new rotavirus vaccines.

Description of Study Design:

Single centre, open-label randomized controlled trial with 4 groups enrolling a total of approximately 720 infants and their mothers.

Intervention Groups:

Study Group	***Sample Size***	***Description***
Group 1 (Rotarix only)	*180*	*Rotarix will be administered at 6 and 10 weeks of age following the national EPI schedule. A challenge dose of Rotarix will be administered at 18 weeks of age and stool*

		samples collected just before challenge and 5, 7 & 9 days later. Stool samples will also be taken just before vaccination at 6, 10 and 14 weeks of age, and at 11 and 15 weeks, to look for wild-type rotavirus infection and vaccine virus shedding. Blood samples will be taken at 6 and 18 weeks of age. An additional blood sample will be taken at 14 weeks (n=130) or 15 weeks (n=50) of age. Saliva samples will be collected at 6,10,14 and 18 weeks of age.
Group 2 (P2-VP8 only)	*180*	*Parenteral P2-VP8 subunit vaccine will be administered at 6, 10 and 14 weeks of age. A challenge dose of Rotarix will be administered at 18 weeks of age and stool samples collected just before challenge and 5, 7 & 9 days later. Stool samples will also be taken just before vaccination at 6, 10 and 14 weeks of age, and at 11 and 15 weeks, to look for wild-type rotavirus infection and vaccine virus shedding. Blood samples will be taken at 6 and 18 weeks of age. An additional blood sample will be taken at 14 weeks (n=130) or 15 weeks (n=50) of age. Saliva samples will be collected at 6,10,14 and 18 weeks of age.*
Group 3 (Rotarix + 1 dose P2-VP8)	*180*	*Rotarix will be administered at 6 and 10 weeks of age, followed by parenteral P2-VP8 subunit vaccine at 14 weeks of age. A challenge dose of Rotarix will be administered at 18 weeks of age and stool samples collected just before challenge and 5, 7 & 9 days later. Stool samples will also be taken just before vaccination at 6, 10 and 14 weeks of age, and at 11 and 15 weeks, to look for wild-type rotavirus infection and vaccine virus shedding. Blood samples will be taken at 6 and 18 weeks of age. An additional blood sample will be taken at 14 weeks (n=130) or 15 weeks (n=50) of age.*

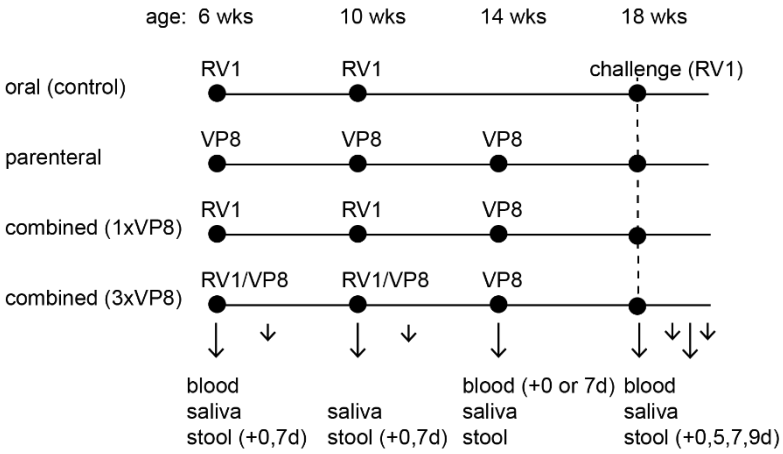
		Saliva samples will be collected at 6,10,14 and 18 weeks of age.
Group 4 (Rotarix + 3 doses P2-VP8)	*180*	*Rotarix and parenteral P2-VP8 subunit vaccine will be co-administered at 6 and 10 weeks of age, with an additional dose of P2-VP8 subunit vaccine administered at 14 weeks of age. A challenge dose of Rotarix will be administered at 18 weeks of age and stool samples collected just before challenge and 5, 7 & 9 days later. Stool samples will also be taken just before vaccination at 6, 10 and 14 weeks of age, and at 11 and 15 weeks, to look for wild-type rotavirus infection and vaccine virus shedding. Blood samples will be taken at 6 and 18 weeks of age. An additional blood sample will be taken at 14 weeks (n=130) or 15 weeks (n=50) of age. Saliva samples will be collected at 6,10,14 and 18 weeks of age.*
Total:	*720*	

Estimated Time to Complete Enrollment: *12 months*

Justification of Sample Size *The primary objective of the study will be to detect a reduction in the proportion of children shedding vaccine rotavirus in stool at any timepoint 5-9 days after challenge, among children immunized with P2-VP8 subunit vaccine alone or in combination with Rotarix, compared with infants receiving Rotarix alone. We estimate that approximately 50% (range 30-70%) of children previously vaccinated with 2 doses of Rotarix will shed Rotarix detected by PCR at any timepoint 5-9 days after challenge. Randomisation of 720 infants across the four study arms therefore gives 90% power (range 56-100%) to detect a 40% reduction in the prevalence of Rotarix shedding after challenge (with adjusted type-1 error, alpha=1.7% to account for 3 comparisons of VP8 schedules vs Rotarix), assuming loss to follow-up of up to 10%.*

Primary Statistical Analyses *The proportion of children with Rotarix detected by PCR at any timepoint 5-9 days after challenge will be compared between Groups 2-4 and Group 1 using a two-sided Fisher's Exact test with Bonferroni corrected significance level alpha equal to 1.7%.*

Flow diagram



1.0 Introduction

Diarrhoea continues to be one of the leading causes of death among children in low-income countries, and rotavirus is a major cause.[1] While the introduction of oral vaccines against rotavirus has significantly reduced rotavirus diarrhoea associated deaths, oral vaccines have frequently been shown to demonstrate lower efficacy and effectiveness in low income countries.[2–4] Rotavirus vaccines specifically have been shown to be significantly less effective in low income countries (LIC) with rotavirus diarrhoea accounting for over 130,000 child deaths every year in these settings.[3,5–8]

The development of parenteral rotavirus vaccines such as the trivalent VP8 subunit vaccine could potentially address challenges associated with oral vaccine administration including, but not limited to elevated maternal antibodies, malnutrition, persistent disease exposure and co-infections in the child's gut [9].

The recent WHO prequalification of the typhoid conjugate vaccine Typbar TCV® based on evidence from several studies including a controlled human infection challenge (HIC) study in Oxford illustrates the potential benefits of HIC as a vaccine assessment tool.[10]

This protocol aims to evaluate the efficacy of a novel, injectable trivalent VP8 subunit rotavirus vaccine by use of Rotarix- a live attenuated oral vaccine, as a challenge infection agent in Zambian Infants.

2.0 Statement of the problem

Rotavirus is the most common cause of severe dehydrating diarrhoea among children under 5, with the majority of cases occurring in LIC.[8] Sustained efforts including improvements in child nutrition, equitable access to healthcare services and improved sanitation have resulted in substantial reductions in mortality rates associated with rotavirus infection. In addition, several countries including Zambia have introduced rotavirus vaccines in national health programs further reducing rotavirus associated morbidity and mortality.[11–13]

Despite these efforts, ORV have repeatedly demonstrated lower efficacy and effectiveness in regions with the largest burden of rotavirus morbidity and mortality[14].

Methodologies focusing on streamlining vaccine assessment by advancing promising rotavirus vaccine candidates could help address challenges. Traditional clinical trials to test new vaccines in the development pipeline are challenging because: 1) RCTs of vaccine efficacy with a placebo arm are no longer considered ethical and demonstration of non-inferiority compared with existing vaccines requires very large, time-consuming and often prohibitively expensive trials (>8,000 infants) [15] and, 2) robust immune correlates of protection (CoPs) against rotavirus infection and disease in humans that could enable licensure without an efficacy trial have not been identified. [16,17]

3.0 Rationale/Justification

Rotavirus gastroenteritis causes approximately 130,000 deaths annually and imposes substantial costs associated with the care of sick children by parents.[8] Over 99% of these deaths occur in LIC including more than half in sub-Saharan Africa. The global roll-out of ORVs, Rotarix and RotaTeq, in >35 sub-Saharan African countries to date, is starting to have an impact on this morbidity and mortality burden.[18] Unfortunately, it is precisely in these countries with greatest burden where the immunogenicity and efficacy of these ORVs is lowest, a phenomenon that has been observed for other oral vaccines.[19] Efficacy against

severe rotavirus gastroenteritis (SRVGE) in Africa has been reported between 39-61%, compared with >90% in high-income countries.[20,21] In Zambian children, the effectiveness of Rotarix was estimated at 56% (95% CI,−34% to 86%) against RVGE resulting in hospitalization.[5] Thus, even with high vaccine coverage, reductions in hospitalization for RVGE after the introduction of these vaccines in LIC have been ~50% and rotavirus remains the leading cause of hospitalised diarrhoea in Zambia and in Africa.[11,22] The phenomenon of suboptimal ORV performance in LIC is well established underscoring the urgent need to develop new vaccines or explore alternative dosing schedules to improve the performance of existing vaccines. However, traditional clinical trials required to assess these newly developed vaccines are challenging, often requiring large sample sizes to answer study questions. Controlled human infection or human infection challenge (HIC) studies are increasingly used as a tool to assess promising candidate vaccines and improve understanding of infectious disease immunology. By using a routinely administered live attenuated vaccine as a challenge agent, we can take advantage of the HIC model to characterize immune responses to acute infection, and thereby develop a model for broader assessment of rotavirus vaccines.

We propose to use human challenge with live-attenuated vaccine (Rotarix) to assess protection against rotavirus infection and investigate immune correlates of protection following vaccination with a novel trivalent VP8 subunit rotavirus vaccine used alone or in combination with ORV. The relevance of a live attenuated oral challenge model to clinical protection against disease is supported by recent analyses of natural infection cohorts that show acquired immunity to rotavirus protects against RVGE by preventing infection, rather than reducing the probability of disease given infection [23]. It is a model that has proven useful in the comparison of poliovirus vaccines and immunisation schedules, including identification of more effective immunisation schedules that have subsequently been recommended by WHO.[24,25]

This study will evaluate combined immunisation schedules with Rotarix and trivalent VP8 subunit vaccine in comparison with single vaccine schedules using shedding after Rotarix challenge as our primary outcome.

4.0 Literature review

4.1 Burden of rotavirus globally and in Zambia

Diarrhoea is one of the leading causes of childhood morbidity and mortality worldwide resulting in approximately 750,000 deaths each year [26] Rotavirus attributable diarrheal disease accounts for a substantial proportion of severe diarrhoea cases among children under 5 with over 130,000 attributable deaths [8].
In Zambia, diarrhoea is an important cause of under 5 deaths with a large proportion of diarrheal disease hospitalizations associated with rotavirus[27]. Following the nation-wide introduction of Rotarix in 2013; all-cause diarrhea, rotavirus diarrhea hospitalizations and in-hospital diarrhea deaths declined at a large referral hospital in Lusaka, Zambia [28]. However, while ORV have reduced diarrheal morbidity and mortality in many countries including Zambia, Rotavirus continues to be the leading cause of hospitalised diarrhoea in LICs. Our previous work to determine the causes of moderate-to-severe diarrhoea among children under 5 in Lusaka following the introduction of rotavirus vaccines found that rotavirus

remains the most common pathogen isolated among children with diarrhoea at an outpatient facility in Lusaka Zambia [22] .

4.2 Prevention and treatment

Parallel efforts to refine diarrhoea case management, improve childhood nutrition and increase access to water, sanitation, and hygiene (WASH) facilities in LICs have improved overall diarrheal disease fatality rates[29,30] . In many countries, oral rehydration therapy and zinc supplementation are now routinely used in the management of diarrheal diseases with better treatment outcomes[29,31]. Programs seeking to improve childhood nutrition by providing supplementary feeds and health education have also been helpful in reducing the burden of diarrheal diseases [32,33]. Efforts to sustain these programs and expand to include new areas/districts may be hindered by financial and human resource constraints which are not uncommon in LICs[34,35]. Vaccination provides an opportunity to complement these efforts by preventing thousands of childhood deaths and reducing costs associated with hospitalization and treatment of sick children[36].

4.3 Current vaccine options and potential alternatives

Vaccination is an important public health strategy in the prevention and control of infectious diseases including diarrhoea. Several WHO prequalified vaccines are currently available for the prevention of rotavirus diarrhoea including - Rotarix® (GlaxoSmithKline Biologicals), Rotateq® (Merck Vaccines), ROTAVAC® (Bharat Biotech International Limited) and Rotasiil® (Serum Institute of India Private Limited) .

Rotarix® is a monovalent vaccine containing a single G1P[8] live attenuated human rotavirus strain that induces broad heterotypic protection against diverse rotavirus serotypes circulating globally and in Africa [37]. Rotateq, a pentavalent rotavirus reassortant vaccine was found to be efficacious and well tolerated among infants in Europe and Latin America [38–40]. The more recently WHO prequalified ROTAVAC® is a live, attenuated G9P[11] monovalent vaccine manufactured by BBIL [41]. The frozen formulation of ROTAVAC® requires thawing till fully liquid prior to administration. A new variant, ROTAVAC 5D®, is a liquid formulation of the monovalent attenuated rotavirus vaccine derived from a neonatal 116E strain [41]. Rotasiil® is an oral rotavirus vaccine produced by Serum Institute of India and the first Rotavirus vaccine with heat stable characteristics to receive WHO prequalification[42].

Nationally licensed vaccines include the Lanzhou lamb rotavirus (LLR), a monovalent attenuated vaccine developed by Lanzhou Institute of Biological Products which has been nationally licensed in China since 2000[43] .

Current Zambian EPI guidelines recommend two doses of Rotarix starting at 6 weeks administered one month apart. Immunisation with 2 doses of this vaccine protects about 50% of infants in low-income countries against SRVGE, comparable with that observed for other licensed oral vaccines [44].

4.4 Investigational subunit P2-VP8 vaccine

The Trivalent P2-VP8 subunit vaccine (SK Chemicals, South Korea) is comprised of three recombinant fusion proteins containing the P2 tetanus toxin universal T-cell epitope linked to the VP8 subunit of VP4 rotavirus structural protein expressing either the P4, P6 or P8 types with a molecular weight of approximately 22 kDa[45].

Nonclinical studies assessing the safety and immunogenicity of the trivalent P2-VP8 vaccine showed the vaccine was safe and immunogenic when administered in rats and guinea pigs[46]. Three doses of vaccine at bi-weekly intervals evoked robust immune responses in Guinea pigs[46]. Further animal studies showed the vaccine was not only immunogenic but also well tolerated. In challenge studies of gnotobiotic piglets using virulent human rotavirus, three intramuscular doses of the vaccine significantly delayed the onset of diarrhea and reduced the duration of diarrhea[47].

Clinical studies assessing safety and immunogenicity of the P2-VP8 vaccine have been conducted in the USA and South Africa. A phase 1 double-blinded, randomized, placebo-controlled dose-escalation study conducted among 18-45 year old American adults demonstrated the monovalent P[8] vaccine was safe, well tolerated and capable of eliciting robust neutralizing antibody responses [48].

Follow on phase I/II age descending dose escalation trials of the monovalent P[8] vaccine among South African adults and toddlers showed the vaccine was well tolerated and immunogenic but elicited poor responses against heterotypic rotavirus strains [45]. Neutralizing antibody responses to heterologous rotavirus strains were most robust to P[8] strains, moderate to the P[4] strain and fairly limited to the P[6] strain [45].

Subsequent studies of a trivalent formulation of the vaccine among South African adults, toddlers and infants confirmed the vaccine was well tolerated and immunogenic [49].Trivalent formulation of this vaccine includes P[4], P[6] and P[8] genotypes (which constitute >95% of strains infecting children globally) and induces strong homotypic antibody responses.

Promising results from early studies of the P8 subunit vaccine show that parenteral vaccines could potentially address challenges associated with oral vaccine administration. However, the impact of this parenteral vaccine on mucosal immunity in the absence of previous exposure to live rotavirus remains unclear [50].

4.5 Immunology of rotavirus vaccines

Rotaviruses show significant genetic and serotypic diversity and are classified according to their surface glycoprotein VP7 (G type) and attachment protein VP4 (P type). These are encoded by different segments of the double stranded RNA genome and occur in different combinations, with G1P[8] the most commonly detected strain globally and in Africa, although other G/P type combinations are also common[51].

Although the immune response to rotavirus infection has been studied in detail in animal models, immune CoPs against infection and disease in humans remain elusive [16,17]. Investigations using knock-out mouse models demonstrate the central role of the humoral immune response, with gut-homing B cells and intestinal IgA mediating long term protection against rotavirus infection, whilst CD8+ T cells contribute to clearance of primary infection and offer short term partial protection against reinfection [17]. However, measurement of humoral mucosal immunity has proved difficult in humans and serum antibodies assessed through ELISA or neutralisation assays have shown only modest associations with protection against infection or disease [52–54]. Recent advances in our understanding of rotavirus immunology and reassessment of some of our basic tools has led to new opportunities to investigate the immune response to vaccination and identify novel immune CoPs [55,56]. These can be

compared with immunogenicity results from the phase 3 trial of the clinical efficacy of the trivalent VP8 subunit vaccine given as 3 infant doses compared with the standard 2-dose Rotarix schedule.

We hypothesize that human challenge with live-attenuated rotavirus (Rotarix) will allow rapid and efficient investigation of potential immune CoPs against faecal shedding of rotavirus. Identification of novel immune CoPs in this model based on minimally invasive sample collection (saliva, peripheral blood) would facilitate the development and testing of new rotavirus vaccines.

4.5.1 Immune CoPs following oral vaccination

Recent analysis of rotavirus-specific single cell-sorted intestinal B cells from adults found a substantial number of neutralising antibodies targeted VP5 [55]. These antibodies were found to be broadly neutralising and may contribute to the heterotypic protection observed after immunisation with live-attenuated ORV. More recently, antibodies targeting VP8 isolated from the same study and thought to be non-neutralising were shown to neutralise rotavirus when used in a more sensitive assay based on human enteroid or enterocyte HT-29 cell culture rather than standard MA104 monkey kidney cells [56]. Protection against infection following challenge of adults with virulent G1P[8] virus has been found to correlate with serum IgG to G1 VP7 and P[8] VP4 but antibodies specific to VP5 or VP8 were not investigated [57]. More generally, the association of antibodies against antigens other than VP6 with protection against rotavirus infection or disease have not been investigated in children [58].

We will measure serum IgA/IgG in infants aged 6 and 18 weeks before and after vaccination with 2 doses of Rotarix alone (n=180 infants) using ELISA with commercially manufactured homotypic VP5, VP7 and VP8 recombinant proteins based on the Rotarix genome. We will determine the magnitude of the antibody response to these antigens (and G1P[8] virus cell culture lysate) and the association with protection against rotavirus shedding following challenge. We will also measure serum neutralising antibodies on HT-29 cells and investigate their association with protection against rotavirus shedding after challenge. Neutralisation titres in sera from Rotarix immunised infants in the US and India based on HT-29 cells appear to be significantly higher than when measured on MA104 cells and our challenge model provides an opportunity to investigate the association of neutralisation titres using this more sensitive assay with protection against infection [56]. We will initially use the human G1P[8] Wa strain rotavirus in these assays, extending to other human strains corresponding to the P types included in the VP8 vaccine (DS-1 P[4] and 1076 P[6]) if this assay is promising as a CoP.

4.5.2 Immune CoPs following parenteral immunisation

The VP8 subunit vaccine is just beginning phase 3 efficacy testing and immune CoPs against infection or disease in humans have not yet been investigated. Parenteral vaccination using VP8, VLPs or inactivated rotavirus tested in a variety of animal models does not induce an IgA response but generates high levels of serum IgG, which protects against live oral rotavirus challenge in these models presumably through the transudation of IgG across the intestinal epithelium [46,47,59–62]. Immunisation of human infants with trivalent VP8 subunit vaccine induces a modest serum IgA response, which may in part reflect boosting of pre-existing mucosal immunity resulting from natural infections [45,49]. We will be able to investigate the contribution of natural infection to the IgA response after immunisation with VP8 and whether mucosal IgA is required for protection against infection using our human challenge model. These questions

are important for our understanding of the likely effectiveness and long-term impact of the VP8 vaccine. We will measure IgA/IgG in serum and sublingual saliva from infants receiving VP8 vaccine alone or with Rotarix at 6 and 18 weeks using an oral swab suitable for infants (Salimetrics). Rotavirus-specific IgA in these samples will be assessed using ELISA against G1P[8] cell culture lysate or recombinant VP8 protein (as above). Recent work found that IgA in sublingual saliva correlated with intestinal antibody better than parotid saliva and this may therefore provide a novel non-invasive proxy for measuring intestinal immunity [63]. Total IgA and albumin will be measured to adjust for total salivary IgA content and fluid flow rate, respectively, using commercial ELISA kits. The mucosal (salivary) IgA/IgG response to the different vaccination schedules will be compared, and the association with serum antibodies and protection against challenge rotavirus examined. Comparison of antibody concentrations against P[8] VP8 with those against P[4] and P[6] VP8 will allow investigation of the degree of homotypic versus heterotypic boosting by trivalent VP8 vaccine among infants receiving (G1P[8]) Rotarix. Neutralising antibodies in serum and saliva will also be measured using HT-29 cells if they show promise as a CoP after Rotarix immunisation.

The subunit vaccine is based on a truncated VP8 (amino acids 64-223) that includes all known VP8-specific monoclonal antibody epitopes fused to a universal tetanus toxoid CD4+ T cell epitope [46]. We will determine whether its administration at 14 weeks to infants previously immunised with 2 doses of Rotarix boosts systemic and mucosal immunity to VP8 in blood samples collected 7 days after the booster dose in a subset of infants (n=200). We will assess whether administration of this vaccine recalls a VP8-specific mucosal immune response, as seen following administration of IPV to orally immunised children, by looking for early increases in IgG indicative of an anamnestic response and by using a micro-modified ELISPOT assay for detection of gut-homing (a4b7+) ASCs in small volumes of whole blood from infants [64].

4.6 Shedding as a measure of vaccine effect

Shedding of challenge rotavirus has been used extensively in animal models of rotavirus infection and disease, including mice and gnotobiotic piglets, to assess mucosal protection but has been under utilised in human studies [65]. Adult human infection challenge models with wild-type rotavirus have been used in the past to investigate immune CoPs against infection and disease, but these models are not currently established and their relevance to the infant immune response especially in LIC is likely to be limited [66,67].

Our preliminary work supported by a pump-priming grant from the MRC Human Infection Challenge vaccine network (HIC-Vac), showed that the quantity of Rotarix vaccine detected in stool by quantitative PCR (qPCR) was significantly lower following a second ('challenge') dose compared with a first dose[68]. This reduction in shedding following immunisation supports the concept of using Rotarix challenge to assess intestinal mucosal immunity and indicates sensitivity of the qPCR assay to quantify shedding in immunised infants. In phase 2 trials, trivalent VP8 vaccine was safe and induced a strong homotypic serum neutralising antibody response [45,49] . In an exploratory analysis of a subset of infants it was also found to reduce shedding of Rotarix after challenge. However, the IgA response in infants has been modest compared with adults and toddlers and the impact of this parenteral vaccine on mucosal immunity in the absence of earlier exposure to rotavirus infection remains unclear [69].

4.7 Maternal and infant factors that may affect vaccine responses

Immunological breastmilk components such as rotavirus specific IgA and innate glycoproteins [70,71] have been shown to be associated with rotavirus vaccine immunogenicity. More recently, genetically determined histo blood group antigens (HBGA) have been implicated in rotavirus infection of host epithelial cells and secretion of these HBGAs phenotypes in bodily fluids such as breastmilk, saliva and on the surface of mucosal epithelial cells of the gut has been associated with susceptibility to rotavirus infection by vaccine strains and immunogenicity of current oral rotavirus vaccines [72]. In addition to infant immunity and HBGA status, maternal HBGA and rotavirus-specific IgA secretion in breastmilk may therefore be important determinants of Rotarix shedding after challenge. Therefore a single breastmilk sample will be collected from all consenting mothers at the time of challenge to measure these breastmilk components (18 weeks).

5.0 Research questions

This protocol seeks to address four broad scientific questions:

1. Does the parenteral trivalent VP8 subunit vaccine induce protection against multiplication of the live virus?
2. Does combined vaccination with oral (Rotarix) and parenteral VP8 subunit vaccine boost immunity and offer increased protection against Rotavirus infection?
3. Does vaccine immunogenicity (serum and salivary IgA, gut-homing memory B cells) correlate with protection against shedding following challenge?
4. Is the protective efficacy of parenteral vaccine against Rotarix challenge comparable to clinical efficacy against rotavirus disease as observed in the phase-3 study?

6.0 Research aim(s)/General Objective And Specific Objectives

6.1 Overall Aim

To investigate intestinal immunity induced by combined parenteral and oral rotavirus immunisation in infants in Zambia by measuring shedding after challenge with live-attenuated rotavirus vaccine.

Specific Objective 1. To assess protection against shedding of live-attenuated challenge virus induced by trivalent VP8 subunit vaccine, oral rotavirus vaccine (Rotarix) or a combined schedule

Specific Objective 2. To investigate immune response to combined oral and parenteral immunisation and immune correlates of protection against shedding following challenge.

7.0 Methodology

7.1 Study design

We will use a randomized, open-label clinical trial of Rotarix and trivalent VP8 subunit vaccine given alone or in combined schedules to infants in Zambia at 6, 10 and 14 weeks followed by challenge at 18 weeks used to assess mucosal immunity (Figure 1). The primary outcomes will be shedding of rotavirus (Rotarix) at any timepoint in samples collected 5,7 and 9 days after challenge. A single dose of trivalent VP8 vaccine could boost mucosal immunity induced by ORV, whilst 2 or 3 doses are required to induce significant antibody responses in naïve individuals [44]. We will therefore investigate combined ORV-VP8 immunisation schedules with 1 or 3 doses of the trivalent VP8 vaccine. We will also assess the safety of this vaccine and the use of Rotarix as a challenge agent. Secondary outcomes will be seroconversion between 6 and 18 weeks based on serum and salivary IgA and IgG to whole virus or VP8 (see below). Infants randomized to all arms except the parenteral arm will complete the study at 22 weeks of age. Infants randomized to receive VP8 vaccine alone will be given a second dose of Rotarix to ensure they have completed the recommended 2 dose schedule and close out at 26 weeks of age.

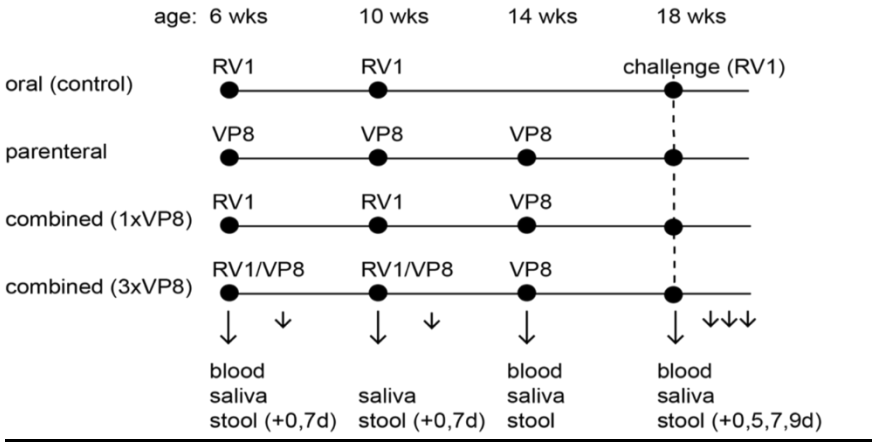


Figure 1: Schematic of study design including immunisation and sample collection visits

7.2 Study site and population/Research materials

The study will be conducted at the CIDRZ Clinical Research Site (CRS) at Chawama first Level Hospital (FLH) within Chawama constituency. Chawama constituency, is in the south west of Lusaka and has a population of over 400,000 people residing in subdistricts including Makeni, Kuku, John Howard, Kamwala south, Msisi, John Laing and Jack compounds. The CIDRZ CRS, maternal child health (MCH) and antenatal care (ANC) departments are co-located within the premises of Chawama FLH where we intend to recruit participants as they attend the healthcare facility. CIDRZ maintains an active presence in several health facilities within Lusaka Province including Kanyama FLH, Chipata FLH and Chainda South clinic. Study infrastructure will be available in all these health facilities, and we may leverage the option of activating additional sites to meet recruitment targets within the timeframe.

As part of the COVID-19 mitigation measures, all daily operations in our clinical sites have been adjusted to reduce transmission risk among study staff and participants seeking healthcare services. Participant flow has been restructured to allow more practical social

distancing. All mothers wear masks when entering facilities and have obligatory temperature checks and hand sanitizing. Children who have fever or are acutely unwell are isolated in a separate room outside the facility. Study staff work in assigned teams on a rotational basis to further reduce minimize infection risk

7.3 Statistical considerations

Sample size determination for Primary outcome The primary objective of the study will be to detect a reduction in the proportion of children shedding vaccine rotavirus in stool at any timepoint 5-9 days after challenge, among children immunized with P2-VP8 subunit vaccine alone or in combination with Rotarix, compared with infants receiving Rotarix alone. We estimate that approximately 50% (range 30-70%) of children previously vaccinated with 2 doses of Rotarix will shed Rotarix detected by PCR at any timepoint 5-9 days after challenge. Randomisation of 720 infants across the four study arms therefore gives 90% power (range 56-100%) to detect a 40% reduction in the prevalence of Rotarix shedding after challenge (with adjusted type-1 error, alpha=1.7% to account for 3 comparisons of VP8 schedules vs Rotarix), assuming loss to follow-up of up to 10%.

Randomisation will be based on a computer-generated blocked randomisation procedure with block sizes of eight implemented by a statistician. The allocation code for each participant will be concealed in sequentially numbered opaque cover which will be opened in order at the time of enrolment of each infant. All biological samples will be given a unique ID linked to the study participant ID, such that laboratory staff and selected investigators are blinded to study arm assignment.

Safety In the absence of any serious adverse events (SAEs) attributed to the VP8 vaccine, we would be able to reject a vaccine-related SAE rate of 0.9% or higher for the 1 and 3 dose combined schedules (alpha=5%).

Sample size considerations for Secondary outcome: Seroconversion After 2 doses of Rotarix in Zambia seroconversion was 60% (130/216) in an earlier study and 45% (10/22) in our MRC-funded pilot of Rotarix challenge, based on IgA to whole virus cell culture lysate (largely anti-VP6 antibodies). Seroconversion based on anti-VP8 antibodies has not been studied after Rotarix administration but may be similar. Seroconversion after administration of monovalent P[8] VP8 subunit vaccine to infants in South Africa was 81% (38/47) based on anti-P[8] VP8 IgA [45], but substantially lower in a clinical trial of the trivalent vaccine[49]. For our planned sample size we would have >99% power to detect an increase in seroconversion from 50% to 80% in either of the combined vaccination arms compared with Rotarix alone, and 70% power to detect an increase to 65% (alpha=2.5% allowing for multiple comparisons).

Investigation of immune response to combined oral and parenteral immunisation and immune correlates of protection against shedding following challenge
Immune CoPs following oral or parenteral vaccination We will measure seroconversion based on serum and sublingual IgA against rotavirus-specific targets (protein antigens and cell culture lysate) among all infants, which will give >95% power to identify even a relatively modest CoP against rotavirus shedding after challenge (area under ROC curve >70%) in each of the study arms, assuming 30-70% or 18-42% of children shed at any time after challenge (depending on whether the single or combined schedule is used; type-1 error of 5%). A similar argument applies to seroconversion determined by neutralising antibodies.

Immune boosting We will compare the number of gut-homing ($\alpha4\beta7$+) IgA and IgG rotavirus VP8-specific ASCs 7 days after the third clinic visit in 50 infants from each study arm (i.e. after the first VP8 vaccine booster dose, after the third VP8 vaccine dose (alone or in combination with Rotarix), or after no vaccine (control) in the Rotarix only arm). Assuming a similar response to a VP8 vaccine booster given to Rotarix immunised infants as seen for poliovirus-specific ASCs after an IPV booster given to OPV immunised children, we would have >95% power to detect an IgA or IgG ASC response compared with infants not given a booster (~60% vs. ~10% response) [64]. This would also give 80% power to identify at least a moderate association with protection against shedding after challenge among the children receiving a booster dose (area under ROC curve 75%).

7.4 Recruitment and sensitisation of expectant women

Expectant mothers attending antenatal in their third trimester will be targeted with a general talk introducing the study (Sensitization talk 1). Interested mothers will be invited to come to the CRS site for a more detailed talk about research, reasons for this study and expected activities for enrolled participants (Sensitization Talk 2). Those motivated to participate will be invited to come with their partner or relative of influence (i.e. Mother in-law or grandmother) for further discussion before they deliver, or before the baby attains 6 weeks age. During this visit, the details of the Informed Consent will be administered. If agreed, the ICF will be signed and the mother booked on specific date for infant vaccination, provided with a stool container and instructed to come with stool on day of the first vaccination (day 0). Approved participant information sheets will be available in English and two major local languages spoken in Lusaka (Nyanja and Bemba), and will be offered to interested mothers in their preferred language. Those unable to read and write will be offered the information with an impartial witness of their choice as required by local ethical guidelines. Before enrolment into the study, each infant's legally acceptable guardian will complete and sign (or witnessed thumbprint) the informed consent document.

A similar procedure will be used for mothers identified during the 6 week routine vaccination visit. Provide sensitization talk 1 to mothers presenting for 6 weeks vaccination at the under 5 clinic. Invite interested mothers to study site for sensitization talk 2, informed consent and ICF signing procedures. Interested mothers will be encouraged to go home and discuss with their families before returning on a specific day with a stool sample for eligibility assessment and vaccination If willing to proceed.

Participant accrual will be cumulative until a total target sample of 720 infants is reached. Prospective infants and their mothers, who sign an informed consent form, will be assessed for eligibility to participate in the study using a test of understanding quiz. Screening for eligibility will also involve clinical assessment including medical history, assessment of vital signs, and physical examination.

7.5 Inclusion and exclusion criteria

Mother-Infant pairs will be eligible for inclusion if they meet the following criteria:

- Healthy infants as established by medical history and physical examination.
- Infants will be 6-10 weeks old at time of enrolment
- parents plan to remain in the area for the duration of the study.

- Parent/guardian understands the study procedures and willing to provide informed consent to participate in the study

Potential participants will be excluded from study participation if

- Infant is acutely unwell at the time of screening
- Infant or infant's mother has syndromic or documented evidence of being immunocompromised (independent of HIV status)
- Infant has a known allergy to any vaccine component
- Infant has previously received rotavirus vaccine
- Infant has received immunosuppressive medication
- Infant has a major congenital or genetic abnormality
- Any condition in the parents/infant that, in the judgment of the investigator, would interfere with or serves as a contraindication to protocol adherence or a participant's parents' ability to give informed consent.
- Participant's parents not available or willing to accept active follow-up by the study staff

Eligibility determination will depend on the results of the medical history and clinical examination, fulfilment of the inclusion criteria, absence of all exclusion criteria, and appropriate understanding of the study as evidenced by appropriate score on testing of understanding. Guardians of enrolled participants will be provided with study appointment cards to track participation, pending study activities, and appointment dates. Parents will be encouraged to bring the child to the study clinic whenever the child is unwell. In the event a child is unwell, medical cover will be available for all enrolled participants to access health care at selected facilities within Lusaka. In addition, qualified clinicians will be available to provide medical care at the research site. Where possible stool samples will be collected from children presenting to the CRS with diarrheal disease.

7.6 Summary of main study activities

Pre-screening: Study staff will identify parents/guardians of potential participants through the mother and child health (MCH) departments of selected study facilities. CIDRZ has well established research sites in the selected facilities and good rapport with staff stationed within the government facilities. Expectant women and postpartum mothers will be identified through the antenatal and child health clinics at the MCH department and provided with study information. Parents/legal guardians expressing interest will be invited to study clinics for prescreening discussions during which study staff will provide detailed study information and answer any questions parents may have. Study staff will be available to discuss key aspects including aims of the study, voluntary participation, potential risks, and benefits. Interested parents/guardians will be requested to return to the study site for screening and enrolment procedures when the child is 6 weeks of age.

Screening: Parents/guardians will be provided with an approved information sheet and undergo informed consent procedures. The ICD will outline study procedures, potential risks and benefits, expected duration of participation, care to be provided during the study and the

planned compensation for participation. Consenting participants will be assigned a unique screening number and assessed for eligibility through medical history and physical examination. Study staff will collect demographic data, medical history and conduct a physical examination. If the child is acutely ill, additional screening visits may be scheduled for any follow-up as needed. If for any reason screening is conducted on days other than the day of enrolment and vaccination, a targeted physical examination will be conducted to confirm the child remains eligible prior to enrolment and vaccination. A study screening log will be used to document all participants undergoing screening with the accompanying outcome including details relating to enrolment and randomization or screen failure. Prior to enrolment, study staff will ensure the following have been completed:

- Obtaining written informed consent
- Assignment of participant ID number
- Medical history and demographics
- Measurement of vital signs
- Full physical exam

HIV status of the mother, and of the infant if known, will be recorded. Mothers with unknown/undocumented HIV status will be offered pre-test counselling and HIV testing according to the national HIV testing and treatment guidelines.

First vaccination clinic visit (Day 0)

For study purposes, the day the first study vaccination is administered will be considered day 0 and the following procedures will be conducted.

1. Confirm eligibility: study staff will confirm the child meets all study eligibility criteria and all documentation is complete and accurate.
2. Randomisation: Children meeting study eligibility will be randomized to one of the four available study arms using simple randomisation in block sizes of 8.
3. Sample collection: Baseline samples including blood, stool and saliva will be collected from all enrolled children prior to administration of the study vaccine.
4. Vaccination: Participants will be vaccinated according to their respective arms following randomization. Prior to vaccine administration, study staff will assess all children to ensure the child is healthy and eligible to receive the study vaccine. Children who are not eligible for vaccination will have their vaccination date deferred with study staff clearly documenting the reason for deferral. Study staff will document all details relating to vaccine administration in the CRF.
5. EPI Vaccinations: All EPI vaccinations will be administered according to national guidelines. Details relating to EPI vaccine administration will be documented in the CRF.
6. Observation: All children will be observed within the study site for 30 minutes following vaccine administration. After 30 minutes, vital signs will be checked, and a targeted physical examination conducted to document any clinically significant abnormalities
7. Post immunisation diary card (PIDC) completion training: Parents will be provided with a thermometer, stool collection container, plastic ruler, and a post immunisation diary card (PIDC) to record solicited reactions from the day of vaccination. Parents will also be given an opportunity to ask questions before they leave the study site and requested to bring the child to the clinic for medical attention each time the child is unwell.

A Research assistant will accompany the participant home and complete participant locator information in the participant file. Study staff will contact the parent by phone and visit the participant's home on day 3 to determine the child's health status and review accuracy of PIDC. Seven days after the vaccination research assistants will visit the participant home to collect the PIDC and a stool sample to test for vaccine shedding and natural rotavirus infection.

Research assistants will confirm the date of the second visit is indicated in the participants appointment card. Similar visits will be conducted for all vaccination time points. For the duration of the study, research assistants will also conduct weekly phone calls or home visits to check on the child's health and answer any questions parents may have.

Second vaccination clinic visit (Day 28)

The next clinic visit will be conducted 28 days after the first vaccination visit. In advance of this visit mothers will be contacted by phone or home visit and asked to collect a stool sample in the evening or on the day of the visit and to bring this sample to the clinic. Participants who do not return to the clinic will be contacted and encouraged to return to the facility for follow up visits as scheduled. Children missing their scheduled appointments will be able to receive their vaccination up to 28 days after their scheduled date. During the second visit, the following study procedures will be conducted:

- Interim medical history assessment to document any adverse events.
- Confirm collection and review of PIDC.
- Physical examination and eligibility assessment for vaccination.
- Sample collection: saliva and stool collection as outlined under sample collection.
- Vaccination: Participants will be vaccinated according to their respective arms following randomization. Prior to vaccine administration, study staff will assess all children to ensure the child is healthy and eligible to receive the study vaccine. Children who are not eligible for vaccination will have their vaccination date deferred with study staff clearly documenting the reason for deferral. Study staff will document all details relating to vaccine administration in the CRF.
- EPI Vaccinations: All EPI vaccinations will be administered according to national guidelines. Details relating to EPI vaccine administration will be documented in the CRF.

3 and 7 days after this visit participants will be visited and contacted by phone to solicit adverse events.

Seven days after this visit a stool sample will be collected from the infant to test for vaccine shedding and natural rotavirus infection.

Third vaccination clinic visit (Day 56)

The next visit will be conducted 28 days after the second vaccination visit. In advance of this visit mothers will be contacted by phone or home visit and asked to collect a stool sample in the evening or on the day of the visit and to bring this sample to the clinic. Participants who do not return to the clinic will be contacted and encouraged to return to the facility for follow up visits as scheduled. Children missing their scheduled

appointments will be able to receive their vaccination up to 28 days after their scheduled date. During the third visit, the following study procedures will be conducted:

- Interim medical history assessment to document any adverse events.
- Confirm collection and review of PIDC.
- Physical examination and eligibility assessment for vaccination.
- Sample collection of blood, saliva and stool as outlined below. Applicable to all participants excluding a subset of 200 infants recruited sequentially irrespective of study arm, who will have a blood sample taken 1 week later (day 63) to determine the antibody secreting cell (ASC) response.
- Vaccination: Participants will be vaccinated according to their respective arms following randomization. Prior to vaccine administration, study staff will assess all children to ensure the child is healthy and eligible to receive the study vaccine. Children who are not eligible for vaccination will have their vaccination date deferred with study staff clearly documenting the reason for deferral. Study staff will document all details relating to vaccine administration in the CRF.
- EPI Vaccinations: All EPI vaccinations will be administered according to national guidelines. Details relating to EPI vaccine administration will be documented in the CRF.

3 and 7 days after this visit participants will be visited and contacted by phone to solicit adverse events.

Whole blood sample collection (subset of 200) (Day 63)

In a sequentially chosen subset of 200 infants divided equally across the 4 arms a blood sample will be collected at 15 weeks of age to determine the ASC response to the 14-week dose (or no vaccine as a comparison group). During this visit, an interim medical history and targeted physical examination will be conducted prior to sample collection.

Rotarix challenge visit (Day 84)

This visit will be conducted 28 days after the third vaccination visit. In advance of this visit mothers will be contacted by phone or home visit and asked to collect a stool sample in the evening or on the day of the visit and to bring this sample to the clinic. Procedures conducted will include:

- Interim medical history assessment.
- Physical examination.
- Collection of a breast milk sample from the mother.
- Challenge dose: All eligible children will have a challenge dose of Rotarix administered
- Blood and saliva samples will be collected from the child prior to administration of the challenge agent. Participants will be observed for 30 minutes following challenge administration and a physical examination conducted to document immediate reactions.

3 and 7 days after this visit participants will be visited and contacted by phone to solicit adverse events.

At 5, 7 and 9 days after this visit a stool sample will be collected at home from the infant to test for vaccine shedding and natural rotavirus infection.

Visit 5 (Day 112)

Except for participants in the parenteral arm, all participants will have close out procedures conducted at 22 weeks (visit 5). Participants in the parenteral arm (VP8) will have the second dose of Rotarix administered to ensure they have completed the recommended 2 dose schedule. Visit 5 procedures will include:

- Interim medical history assessment.
- Physical examination
- Verification of all study details documented in the SDW and CRF for participants closing out.
- Rotarix dose 2 administration for parenteral arm
- Day 3 and 7 home visits to solicit adverse events among participants receiving a second dose of Rotarix.

Visit 6 (Day 140)

Participants in the parenteral arm will have a close out visit conducted 28 days after administration of the second Rotarix dose. The following procedures will be conducted during this visit:

- Interim medical history assessment
- Physical examination
- Verification of all study details documented in the SDW and CRF.

Withdrawal/Early termination from the study or vaccination

Participants may be withdrawn from the study for any of the following reasons:

• Parental withdrawal of consent
• Permanent relocation
• Significant non-compliance with protocol requirements
• Intercurrent illness or disease or medical treatment that occurs during the trial and in the opinion of investigator might influence the study results or ability to continue to comply with trial procedures
• Loss to follow-up

In the event of withdrawal from the study, reasonable efforts should be made to review and collect PIDC and update any ongoing AEs/SAEs.

Study schedule:

Children meeting study eligibility criteria will complete study activities as outlined in Table 1. Day 0 is the day of enrolment.

Table 1: Study activity summary

Visit number	**1**				**2**				**3**		**3.5**		**4**					**5**			**6**
Study day	**0**	3	7	21	**28**	31	35	**49**	**56**	**59**	**63**	**77**	**84**	**87 (D3)**	**89 (D5)**	**91 (D7)**	**93 (D9)**	**112**	**115**	**119**	**140**
Infant age (wks)	**6 wks.**				**10 wks.**				**14 wks.**				**18 wks.**					**22 wks.**			**26 wks.**
#Screening ,consent, enrolment and provision of stool container	**X**																				
Eligibility assessment and Randomisation	**X**																				
Vaccination (according to arm)	**X**				**X**				**x[a]**									**x[b]**			
Reactogenicity assessment	**X**	**X**	**X**		**X**	**x**	**x**		**X**	**X**	**x**										
Safety assessment (Home visit or phone call by RA (AE+PIDC)		**X**	**X**	**x**		**x**	**x**	**x**		**X**	**x**	**x**		**x**	**x**	**x**	**X**		**x**	**x**	
Blood collection	**X**								**x[c]**		**x***		**x**								
Saliva Collection	**X**				**X**				**X**				**x**								
Stool Collection	**X**		**x**		**X**		**x**		**X**				**x**		**x**	**x**	**X**				
Breastmilk collection													**x**								
Rotarix challenge													**x**								
Adverse Event surveillance	**X**	**X**	**X**	**x**	**X**	**x**	**x**	**x**	**X**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**X**	**x**	**x**	**x**	**x**
Home contact tracing ¥	**X**		**X**		**X**		**x**		**X**		**x**		**x**		**x**	**x**	**X**	**x**			**x**

[#]Informed consent will be written and filed at enrolment, but at each visit, enquiries on willingness to continue to participate will be done.
[¥]Home tracing will be done at the start of the study, and each time a study visit is missed, the patient tracker will follow up at home for as long as the child remains within the acceptable time frame for study visits.
* Applicable to children in ASC immunology subset (200 participants)
[a] Applicable to children randomised to all other arms **except** the control arm
[b] Applicable to children in the parenteral arm receiving a second Rotarix dose to complete vaccination
c Applicable to children **not** randomised to ASC immunology arm (520)

7.7 Study sampling procedures

Following enrolment blood, saliva and stool samples will be collected from infants at specific timepoints including immediately before administration of the first dose of study vaccine.

Blood collection

Following visual inspection, blood will be collected from selected sites by trained study staff. The standard blood draw from each participant will be 3-5ml; this volume is safe as per WHO guidelines [73][43]. Peripheral blood mononuclear cells (PBMCs) will be isolated from heparinised venous blood using ficoll density gradient centrifugation and cryopreserved . Separate plasma samples will be aliquoted and stored at -80^{o}C for subsequent testing.

Saliva collection

Saliva will be collected using Salimetrics infant swabs and storage tubes according to manufacturer's instructions. In summary, cotton swabs will be placed under the infant's tongue to collect sublingual pooling saliva before being placed in a storage tube and will be transported to the laboratory on ice where they will be processed and stored at -80°C.

Stool collection

Stool samples will be collected in universal stool collection containers and transferred to the laboratory on ice for processing, aliquoting and storage at -80°C prior to testing for vaccine virus shedding. If no stool sample was collected prior to attending a scheduled visit, study staff will collect a rectal swab and encourage the participant parent/guardian to collect the next available stool sample and bring it to the clinic.

Breastmilk collection

A single breastmilk sample will be collected from all consenting mothers during the fourth study visit (RVV Challenge-18 weeks). Mothers will be requested to manually express breastmilk into 50ml falcon storage tubes.

7.8 Laboratory Methods

Stool-NSP2 (Rotarix) and VP6 (wild type) qPCR

We will perform RNA extraction from raw stool samples using the Qiagen RNA Kit as per manufactures protocol and carry out reverse transcription PCR to generate cDNA using random primers. We will quantify the shedding of the challenge agent using qPCR with Rotarix-specific primers targeting the NSP2 gene with a predefined shedding positive ct value cutoff[74]. To assess the potential effect of naturally acquired rotavirus infection on

vaccine response, we will also test for its presence in stool samples collected between 6 and 18 weeks of age using qPCR targeting the VP6 gene [75].

Salivary and breastmilk HBGA and Secretor typing
We will determine histo-blood group antigen (HBGA) FUT2 secretor genotype from infant blood samples using PCR for common single nucleotide polymorphisms found in the FUT2 gene (G428A, C302T, A385T) known to predict secretor phenotype[76] .

HBGA ABH, Lewis and secretor phenotype will also be determined using ELISA. In brief, to determine HBGA ABH and Lewis secretor phenotype, saliva or breastmilk samples will be incubated with monoclonal antibodies against blood group A, B, H, Lewis A and Lewis B antigens followed by peroxide-conjugated detection antibody and developed using 3, 3', 5, 5' Tetramethyl benzidine before reading absorbance at 450nm. Secretor status will also be determined in a similar manner using ELISA by detection of Fucα1-2Gal-R known to be present in secretors but not in non-secretors by incubation with peroxidase-conjugated Ulex europaeus (UEA-1) Lectin.

Enzyme-linked immunosorbent assays for rotavirus antibodies
Rotavirus-specific serum, salivary and breastmilk IgA and IgG will be determined using ELISA with G1P[8] virus from cell culture lysate. These assays are already established at the CIDRZ Laboratory using a standard curve to estimate IgA concentration from an international human standard provided by Gagandeep Kang (CMC, India). [77]This standard assay is largely a measure of anti-VP6 antibody, since viruses in the cell culture lysate tend to be formed of double (VP6 exposed) rather than triple-layered (VP7, VP4 coated) particles. We will therefore additionally measure anti-VP8 antibody responses using commercially manufactured VP8 proteins generated from sequences corresponding to the P[4], P[6], and P[8] genotypes found in the vaccine. We will also measure anti-VP7 and anti-VP5 IgA/IgG using commercially manufactured proteins generated from published sequences.

Enzyme-linked immunospot and immunophenotyping
In brief, the measure of intestinal IgA ASCs will be evaluated by ELISPOT. Using 96-well plates (Millipore) coated with affinity-purified goat anti-human IgA and IgG at a known concentration. After overnight incubation at 4°C, the plates will be blocked for 2 hours at 37°C. A complete medium suspension of B cells and peroxidase-conjugated goat anti-human IgA (Sigma-Aldrich), will be distributed in ELISPOT plates, and incubated at 37°C in 5% CO_2 for 4 hours. The ELISPOT reader will be used to determine the total ASCs per well.[55]

PBMCs will also be used to characterize rotavirus specific lymphocyte phenotypes using flow cytometry-based assays. Briefly, PBMCs will be stimulated using mitogens and rotavirus vaccine antigens and stained using commercially available monoclonal antibodies identifying B and T cell surface markers (CD3, CD19, CD8, CD4), homing markers (e.g. $a_4\beta_7$, CCR9, L-selectin) and cytokines (e.g. IFN-γ) and use ELISPOT and flow cytometry to enumerate and determine their population type.

Neutralization assay.
In summary, serially diluted human serum specimens from infants will be incubated with live RV strains at 37°C for 1 hour and then added to 96-well plates (Costar) containing HT-29 or

MA104 monolayer cells for a 1-hour adsorption at 37°C. Cells will be washed and fixed with 10% formalin. Thereafter stained with rabbit polyclonal anti-RV antibody, followed by peroxidase-conjugated goat anti–rabbit IgG and a colour reaction developed using (AEC) substrate. Neutralization titres will be analysed as the maximum dilution of sera that resulted in at least a 50% reduction in the number of foci as compared with a no-antibody control.[56]

7.9 Data collection plan and tools

Data from study visits will be collected using paper case report forms (CRF) and entered by data entry clerks into the study database. Paper CRF data will be reviewed by a clinical investigator and later quality checked by the QC nurse before being entered electronically into the CDMS and discrepancies reconciled by a data supervisor. All input forms will have live consistency and range checking validation to flag any implausible values during data entry. Laboratory data will be stored and collated initially in Microsoft Excel before linkage with the CDMS.

7.10 Data management and storage

The study will support a data manager who will provide daily oversight of data management and quality assurance. Overall data management, data security and quality assurance will be provided by the CIDRZ head of data management and the Imperial College School of Public Health data protection officer.

For this project, the data team will review the study protocol and all related study documents prior to site activation. A GCP compliant clinical database management system (CDMS) will be set up and piloted by the data team prior to commencement of enrollment. The CDMS will be housed on a secure network server with restricted access. All data entry clerks will be trained by the data manager prior to any entries taking place and the database will be tested thoroughly prior to the start of data entry.

The data manager will conduct periodical data cleaning routines to flag data queries that were not picked up in the earlier stages. These data queries will be generated for resolution by the field teams with oversight from the study investigators. All changes to any CRFs or databases will be governed by a change control SOP.

A team of Quality Control Officers from the CIDRZ regulatory department will periodically review all CRFs and undertake eCRF verification for study monitoring purposes. The Monitor will communicate all monitoring findings to the Head of Regulatory Affairs within CIDRZ and the study investigators to ensure all monitoring findings are resolved in a timely manner thus ensuring data quality and GCP compliance.

Storage of research records will be done in accordance with the CIDRZ policies and procedures for archiving. All CRF and other paper records will be held for at least 5 years in the central archiving facility. Data in electronic format will be held indefinitely on our media servers. Data requests will be handled by the PI, with oversight from co-investigators. Anonymised data allowing reproduction of results will be made available to other researchers at the time of publication of scientific papers resulting from the study. Other data will be made available on a case by case basis upon request in writing to the PI and /or or the Head of Research Operations, Ms. Hope Mwanyungwi (Hope.Mwanyungwi@cidrz.org).with oversight from the PI and co investigators.

Study reports and findings will be submitted for review and approval to the Zambia National Health Research Authority (NHRA) prior to dissemination both locally and internationally.

7.11 Data analysis plan

We will summarize background characteristics of participants by randomization arms using proportions. The primary objective of the study will be to detect a reduction in the proportion of children shedding vaccine rotavirus in stool at any timepoint 5-9 days after challenge, among children immunized with P2-VP8 subunit vaccine alone or in combination with Rotarix, compared with infants receiving Rotarix alone. The presence of rotavirus (Rotarix) shedding after challenge will be determined for each individual on the basis of detection of the Rotarix NSP2 gene in any stool sample collected at 5, 7 or 9 days after challenge. The primary analysis will be based on the intention-to-treat population for infants who received a challenge dose of Rotarix and provided at least one stool sample after challenge to assess shedding of the challenge virus. Infants receiving P2-VP8 vaccine (Groups 2-4) will be compared to infants receiving Rotarix alone (Group 1) using Fisher's exact test with a two-sided significance level, alpha = 1.7% (corrected from 5% to allow for the 3 comparisons).

In secondary analyses, we will calculate a shedding index endpoint (SIE) based on the log virus copy number averaged across the three time points and compare these values between vaccination groups using parametric (t-test) or non-parametric (Wilcoxon rank sum test) methods as appropriate. Outcome measurements at day 5, 7, and 9 after the challenge introduces a panel structure into the data. Therefore, we will also use random-effects logistic regression (with post-estimation margins) to estimate the marginal effects of vaccination status (control, parenteral, combined (1xVP8), or combined (3xVP8)) on the presence of shedding. The exposure of interest (vaccination status) will be handled as a categorical variable coded 0 "control" 1 "parenteral" 2 "combined (1xVP8)", and 3 "combined (3xVP8)" in the estimation model. HBGA secretor status may affect Rotarix take and also shedding after challenge, potentially confounding protection against challenge or acting as an effect modifier on the effects of the vaccine intervention. For example, HBGA secretors (~80% of population) are more likely to seroconvert after immunisation with Rotarix compared with non-secretors [78–80]. Therefore, in a secondary analysis, we will use interaction test to assess whether the effects of the vaccine differs between HBGA secretor status by including interaction term between the vaccination status and secretor status. Likelihood p-value less than 0.05 will be considered statistically significant. Further, we will use random-effects log-normal regression model to estimate marginal effects of vaccination status on virus copy number in a similar way. These estimates will be adjusted for the potential confounding effects of HIV exposure, and wild-type rotavirus detection in stool collected between enrolment and challenge. All analyses will be performed using Stata 16 MP4 (StataCorp, College Station, TX, USA) and the R statistical programming language.

We will compare the seroresponse between study arms, with seroresponse defined as the proportion of infants with at least a 4-fold increase in rotavirus-specific serum IgA concentration between the 6 and 18 week blood samples. We will also compare the IgG seroresponse between study arms, with seroresponse defined as a fourfold rise in rotavirus-specific IgG concentration measured at 18 weeks compared with that at 6 weeks adjusted to account for the expected decay of maternally-derived antibodies (half-life 30 days).

Seroresponse will be measured against whole virus cell-culture lysate (largely anti-VP6) and recombinant VP8, VP7 and VP5 antigens. Where available, we will also compare seroresponse based on neutralizing antibody titres, defined as a fourfold rise in neutralizing antibody titre at 18 weeks compared to that predicted based on decay of maternal antibodies measured in the 6 week sample, or detection of neutralizing antibodies in previously seronegative infants. For all analyses of seroresponse, we will compare the proportion of infants showing a seroresponse using Fisher's exact test with correction for multiple comparison as appropriate. We will also compare antibody concentrations between study arms using parametric (t-test) or non-parametric (Wilcoxon rank sum test) methods as appropriate. Similar analyses will be conducted for salivary antibodies, and correlation with serum antibody response measure using Pearson's correlation coefficient after appropriate transformation of the variables.

In the subset of 200 infants with ASC results, we will compare the proportion showing an ASC response between study arms using Fisher's exact test.

Associations between immunological endpoints and protection against Rotarix shedding after challenge will be assessed using a t-test with appropriate transformation. The sensitivity and specificity of different threshold values for potential immune CoPs will be assessed using the area under the Receiver Operating Characteristics (ROC) curve. Association of CoPs with the quantity of shedding will be assessed using parametric or non-parametric correlation coefficients. Adjustment for potential confounders (e.g. HBGAs) will be done using regression models. We will also compare potential CoPs with one another (e.g. saliva vs. serum IgA, ASC vs. serum IgA) and test whether joint analysis of these CoPs using multivariable regression offers a stronger prediction of protection than when analysed in isolation. Cross-validation methods will be used to assess out of-sample predictive accuracy of these CoPs individually and combined in a regression model.

Safety will be assessed among all infants who were randomized and received at least one dose of vaccine. The prevalence of AEs and SAEs will be reported with 95% (exact binomial) confidence intervals. Prevalence between study arms receiving P2-VP8 vaccine alone or in combination with Rotarix will be compared with those receiving Rotarix alone using Fisher's exact test, with a two-sided significance level, alpha = 1.7% (corrected from 5% to allow for the 3 comparisons).

8.0 Trial safety

Safety monitoring will be conducted according to guidelines required for an investigational product. We will establish a Study Steering Committee and Data Safety and Monitoring Board (DSMB) to oversee the study and monitor safety among all enrolled infants. Rotarix is licensed for use in Zambia and has been delivered as part of the Zambian routine immunisation programme since 2013. Administration of a challenge dose of this vaccine at 18 weeks remains within the recommended age window for immunisation. For the duration of the trial, vaccine safety will be carefully monitored following ICH-GCP standard SOPs. Infants will be observed for 30 minutes following administration of each vaccine dose. Unsolicited adverse events will be monitored from enrolment until completion of the study. Parents will be instructed to contact the study team if the infant becomes ill during the study and grading and causality of adverse events will be recorded by study clinicians following established procedures. If a study visit is missed, parents will be followed up at home.

Solicitation and reporting of adverse events

Safety assessments will be conducted for all participants from the randomization through to close out. Parents will be encouraged to bring the child to the clinic each time the child is unwell or inform study staff via telephone. All adverse events and serious adverse events will be reported to ethical and regulatory authorities in line with national reporting guidelines.

Definitions

Adverse Event (AE)

An adverse event is any untoward medical occurrence during a clinical trial that does not necessarily have a casual relationship with the investigational product. AEs include any unfavorable and unintended signs, symptoms, physical examination abnormalities, laboratory result abnormalities or disease temporally associated with the use of the investigational vaccine. For purposes of the study, stable pre-existing conditions that do not change in nature or severity during the study will be reported under medical history and not considered AEs.

Solicited AEs are pre-specific local and systemic adverse events that are known to be associated with vaccination and actively monitored as indicators of vaccine reactogenicity. During the study all solicited AEs will be documented daily in the PIDC for 7 days following each vaccination. All solicited adverse events with onset after 7 days since last vaccination will be captured as unsolicited AEs.

In this study, both systemic and local solicited events will be monitored for children in the VP8 and combined arms while only systemic reactions will be monitored in children in the Rotarix arm. Systemic reactions include fever, diarrhoea, vomiting, irritability, decreased activity and decreased appetite (table 2).

Table 2: Systemic adverse event grading

Systemic (General)	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Fever	37.7 – 38.6°C	38.7 – 39.3°C	39.4 – 40.5°C	> 40.5°C
Vomiting	Transient or intermittent vomiting with no or minimal interference with oral intake	Frequent episodes of vomiting with no or mild dehydration	Persistent vomiting resulting in greater than mild dehydration OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Diarrhea (≥3 loose stools/day)	At least 3 looser-than-normal stools without dehydration	diarrhea with some dehydration (per IMNCI definition)	diarrhea with severe dehydration (per IMNCI definition)	diarrhea with hypovolemic shock
Irritability	Crying more than usual but easily consoled	Crying more than usual and somewhat difficult to console	Continuous crying that is inconsolable for 4 hours or more	

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Decreased activity	Slightly subdued, but responds normally to stimuli	Subdued and does not respond as readily as normal to stimuli	Lethargic	Obtunded
Decreased appetite	Loss of appetite without decreased oral intake	Loss of appetite associated with decreased oral intake without significant weight loss	Loss of appetite associated with significant weight loss	Life-threatening consequences OR Aggressive intervention indicated [e.g., tube feeding or total parenteral nutrition (TPN)]

Local injection site reactions to be monitored include pain, tenderness, redness and swelling (table 3).

Table 3: Grading of local injection site reactions

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Injection site pain (pain without touching) OR Tenderness (pain when area touched)	Pain/tenderness causing no or minimal limitation of use of limb	Pain or tenderness causing greater than minimal limitation of use of limb	Pain/tenderness causing inability to perform usual activities	Pain/tenderness causing inability to perform basic functions OR Hospitalization indicated

Injection site erythema or induration	≤ 2.5 cm in diameter	> 2.5 cm in diameter with < 50% surface area of the extremity segment involved (thigh)	≥ 50% surface area of the extremity segment involved (thigh) OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
Injection site pruritus	Itching localized to the injection site that is relieved spontaneously or in < 48 hours of treatment	Itching beyond the injection site that is not generalized OR Itching localized to the injection site requiring ≥ 48 hours treatment	Generalized itching causing inability to perform usual social & functional activities	NA

Unsolicited AEs are any AEs reported by participants' parents, observed by the study personnel during study visits or identified during review of medical records or source documents. All unsolicited AEs will be treated according to national treatment guidelines and referral provided as required.

Adverse drug reaction / Suspected Adverse Reaction

Suspected adverse drug reaction is any adverse event in which the casual relationship to the investigational vaccine is at least a reasonable possibility, i.e., the relationship cannot be ruled out. For the purposes of safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the vaccine and the adverse event. Suspected adverse reaction implies less certainty about causality than adverse reaction, which means any adverse event caused by a drug or vaccine.

Adverse reaction is any adverse event caused by the vaccine. Adverse reaction is a subset of suspected adverse reaction where there is reason to conclude that the vaccine caused the event.

Unexpected adverse drug reaction is an adverse reaction, the nature or severity of which is not consistent with the information in the Investigator's brochure.

Unexpected suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed.

Serious Adverse Event (SAE)

Serious adverse event is any adverse event that:

- Results in Death

- Is life-threatening (life-threatening means that the study participant was, in the opinion of the site PI or Sponsor, at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).
- Requires inpatient hospitalization or prolongation of existing hospitalization (excludes hospitalization for protocol specified procedures, pre-existing conditions or hospitalization for social reasons e.g lodging). Cases of intussusception requiring hospitalization and/or surgery will be reported as SAEs.
- Results in persistent or significant disability or substantial disruption of the ability to conduct normal life functions
- Is a congenital abnormality or birth defect
- Is an Important medical event that may not result in one of the above outcomes but may jeopardize the health of the study participant or require medical or surgical intervention to prevent one of the outcomes listed in the above definition of serious adverse event

Reporting Period and Parameters

Randomized participants will be monitored for unsolicited AEs from first study vaccination to study completion. AEs maybe reported by parents or noted during interim medical history assessment, physical examinations, or review of laboratory tests.
AEs occurring within 30minutes of vaccination will be reported in the post vaccination assessment section of the SDW and treated by the study physician prior to participants leaving the facility. Participant parents will report solicited AEs daily for 7 days following vaccination and document findings in the PIDC. For study purposes, all conditions which are present at time of enrolment will be documented as “pre-existing conditions” and will not be captured as AEs. SAEs will be recorded from first vaccination to study closeout in all participants.

Severity grading of Adverse Events

The severity of all AEs will be assessed by the study physician according to the severity grading scale below (table 4). AEs will be graded with the worst severity grade during the illness/symptoms. All AEs resulting in participant death will be Grade 5 events.

Systemic Illness	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Illness or clinical AE (as defined according to applicable regulations)	No or minimal interference with usual activities; no medical intervention/ therapy required	Greater than minimal interference with usual activities; no or minimal medical intervention/ therapy required	Marked limitation in ability to perform usual activities; medical intervention/ therapy required	Inability to perform basic functions OR Medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death

Causality of Adverse Event

For each reported AE, a causality assessment will be undertaken to determine the relationship between the investigational product and AEs. The causality assessment will be based on information available at the time of reporting and subject to change based on follow-up information. Determination of causality will be based on clinical judgment and should take into consideration the following factors:

- Temporal (time-based) relationship between the event and administration of the study vaccine?
- Evidence of a plausible biological mechanism for the study vaccine to cause the AE?
- Possible alternative etiologies for the AE such as concurrent illness, concomitant medications?
- Previous reports of similar AEs associated with the study vaccine or other vaccines in the same class?

Causality of the AE will be classified as:

Related: There is a reasonable possibility that the study vaccine caused the event. "Reasonable possibility" means that there is evidence to suggest a causal relationship between the study vaccine and the AE.

Not Related: There is no reasonable possibility that the administration of the study vaccine caused the event.

Follow-up of Adverse Event

All reported AEs will be managed according to national treatment guidelines and followed until resolution, stabilization, or until the participant's participation in the study ends. Participants who have an ongoing AEs at study completion or at discontinuation from the study will be followed by the investigator/study physician until the event is resolved or determined to be irreversible, chronic, or stable by the investigator/study physician.

For study purposes, all diarrhoea AEs, SAEs and suspected cases of intussusception will be documented thoroughly. Management of diarrhoeal disease and suspected cases of intussusception will be conducted in accordance with study specific SOPs.The outcome of AEs will be assessed at the time of last observation per the following categories:

- Recovered
- Recovered with sequelae
- Not recovered
- Death/Fatal
- Recovering
- Unknown. The outcome of the AE is not known

General Guidance on Recording Adverse Events

To improve the quality and precision of acquired AE data, the investigator/study physician will observe the following guidelines:

- Use of recognized medical terms when recording AEs on the source document or in the database.

- Recording the diagnosis (i.e., disease or syndrome) rather than individual signs, symptoms and laboratory values (e.g., record congestive heart failure rather than dyspnea, rales, and cyanosis).
- Signs and symptoms that are considered unrelated should be recorded as individual AEs (e.g., if congestive heart failure and unrelated severe headache are observed at the same time, each event should be recorded as an individual AE).
- AEs occurring secondary to other events (e.g., sequelae) should be identified by the primary cause. A "primary" AE, if clearly identifiable, generally represents the most accurate clinical term to record. If a primary serious AE (SAE) is recorded, events occurring secondary to the primary event should be described in the narrative description of the case.

For example:

Acute diarrhoeal disease→ dehydration → shock → multiorgan dysfunction

The primary AE is acute diarrhoeal disease.

- Death is not an event, but, rather, an outcome of an event. The event that resulted in the death should be recorded and reported on the SAE CRF.
- For hospitalizations for surgical or diagnostic procedures, the illness leading to the surgical or diagnostic procedure should be recorded as the SAE, not the procedure itself. The procedure should be captured in the case narrative as part of the action taken in response to the illness.
- Clinically significant laboratory abnormalities will be followed up until they have returned to normal or a satisfactory explanation has been provided.

Reporting of SAE

Investigator Reporting to Sponsor

All SAEs grade≤4 will be reported to the study sponsor within 48 hrs of site awareness as outlined in the safety oversight SOP. All grade 5 SAEs will be reported to sponsor as soon as possible and within 24hrs of site awareness. SAEs occurring within the study period will be reported to all ethical and regulatory bodies in accordance with local reporting guidelines. The initial SAE form should be completed with all available information and including the following:

- Name and contact of the investigator submitting the SAE report
- Participant ID number
- Date participant received study product
- Description of the serious adverse event and date of event onset
- Investigator's preliminary assessment of severity and causality

Where applicable, hospital case records and autopsy reports (including verbal autopsy) should be obtained.

Copies of each report and documentation of IEC/IRB and regulatory notification and receipt will be kept in the study files.

SAFETY OVERSIGHT

The site investigators/study physicians and/or designated site staff will be responsible for continuous close safety monitoring of all study participants. Ongoing safety data reviews will be undertaken by the DSMB which will be guided by a specific Charter. The DSMB Charter will include predefined “Go, No go criteria” for key safety considerations on this study

Overall, the project will be overseen by the Study Steering Committee who will also review the periodic safety reports from the DSMB. Authority to halt the study will be vested in the Steering Committee.

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Overall, the project will be overseen by the Study Steering Committee who will also review the periodic safety reports from the DSMB. Authority to halt the study will be vested in the Steering Committee.

9.0 Ethical considerations

We have considered the criterial for consideration of what makes clinical research ethical as reported by Emmanuel et al [81], and we are certain that this work is scientifically valid and has clear objectives, is designed using acceptable principles, methods and reliable practices.

9.1 Ethics and research governance

This trial will be conducted in compliance with the protocol and standard ethical guidelines including the International Conference on Harmonization’s Good Clinical Practice (GCP) and the Declaration of Helsinki Good Clinical Practice (GCP) guidelines. In keeping with national guidelines of ethical conduct of research in Zambia, ethical and regulatory approval for this study will be sought from the University of Zambia Biomedical Research Ethics Committee (UNZABREC) and Imperial College Research Ethics Committee (ICREC) and the Zambia Medicines Regulatory Authority (ZAMRA) respectively. When UNZABREC, ICREC and ZAMRA approvals are obtained, final authorization to undertake research will be provided by the National Health Research Authority. Product import permits will be requested from ZAMRA once all approvals are in place.

9.2 Informed consent

Participant’s parents/guardians will be informed of the study aims, procedures involved, potential risks and potential benefits associated with participation in the study. Detailed information about the study will be provided and parents/ guardians allowed ample time to review the informed consent document and clarify any questions/concerns with trained study staff. Parents/guardians willing to participate will provide voluntary written informed consent for study participation, sample collection, processing, and shipment/transfer contingent upon appropriate MTA approval. Parents who are unable to sign will provide thumb printed evidence of agreement and an impartial witness will attest to the process as being voluntary. Study staff will emphasize that participation is voluntary and that the parent has the right to decline participation or withdraw from the study at any time without prejudice. The informed

consent documents will be available in English, Nyanja, or Bemba, and caregivers will be offered the option of their preferred language. The original, signed consent documents will be filed in a secure cabinet within the research site and a copy provided to the participant's parents.

9.3 Confidentiality and assurance of privacy

To the extent possible, all study staff will ensure participant privacy and confidentiality is maintained. Each participant will be assigned a unique participant identification number for purposes of identifying all participant documents and samples. Documents containing participant identifiers such as names, phone numbers and addresses will be stored separately in a secure cabinet at the site. All study paper records will be kept in a lockable cabinet and study databases will be password protected with access limited to authorized individuals. Access to study records will be restricted to authorized staff at CIDRZ, Imperial college, ethical and regulatory authorities, and study monitors. After study completion, all paper records will be stored in a secure location for at least 3 years. If findings from this study are published or shared with others, no participant identifiers will be shared during the process of publication and dissemination of results.

9.4 Risks Involved

Extensive safety data exist for Rotarix, which supports its use as a challenge agent in children. Post-marketing data in middle and high-income countries suggests a small risk of intussusception 7-21 days after vaccination (1 to 6 cases per 100,000 doses), although recent data from Africa did not find an increased risk compared with background rates [82].

Trivalent VP8 subunit vaccine was safe in phase 2 testing in South Africa [45] and is now entering phase 3 in a multinational trial enrolling 8,200 infants, including 1,500 in Zambia (NCT04010448). In previous trials, the vaccine was well-tolerated, with mild reactogenicity and no vaccine related SAEs observed[45] .

Risks associated with blood collection including minor pain/discomfort, bleeding and/or bruising at the site of collection will be communicated to caregivers prior to commencement of procedures. To mitigate this risk, study staff will be adequately trained in phlebotomy prior to site activation.

9.5 Anticipated Benefits of the study

It is argued that ethical clinical research should be of value to society [83], and study must evaluate a diagnostic or therapeutic intervention that would lead to improvements in health or tests a hypothesis that can generate important knowledge [81]. Findings of this study will generate new knowledge and improve understanding of rotavirus disease among children in LIC.

No direct benefits can be guaranteed to participants for their participation in this research study. However, the health and well-being of participants will be prioritized throughout the

trial. All participant parents/guardians will be encouraged to call the study staff or come to the research site if they have any health concerns. Qualified study clinicians will attend to all study participants and provide referral for further management if necessary.

9.6 Reimbursements

Participant parents/ guardians will be reimbursed K100 for transportation/travel to the study site. Reimbursement amounts will be clearly outlined in the informed consent document and subject to ethical approval. All study related tests will be provided at no cost to the participant parent/guardian.

9.7 Storage of Specimens

Plasma, stool, breastmilk and saliva samples will be stored for 5 years after study completion and informed consent obtained for further study related to diarrhoeal diseases and the immune response to rotavirus vaccines. Selected samples will be shipped to our collaborators in the United Kingdom for quality control purposes. Aliquots of PBMCs will be frozen at 3-4 million cells/ml and any unused aliquots retained with consent for potential further analyses, including T and B cell phenotyping assays

Where applicable Material transfer agreements (MTAs) will also be sought from the NHRA for transfer of samples.

9.8 Clinical trials registration

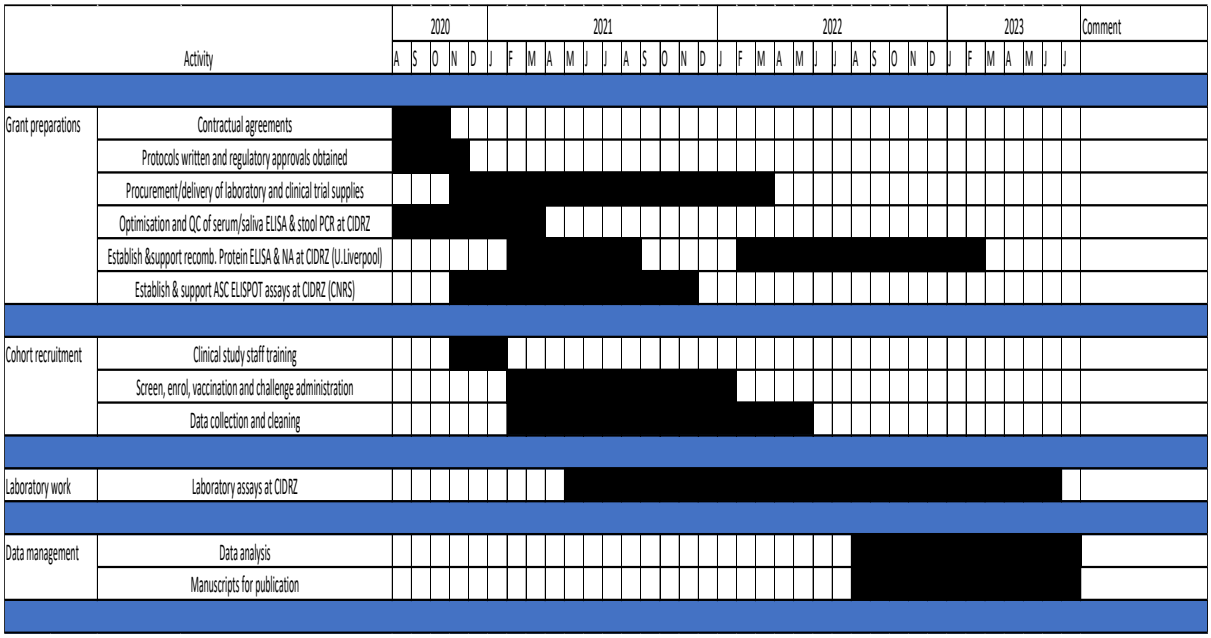
The trial has been registered with clinical trials.gov and a description of the trial is available on: https://www.clinicaltrials.gov/. NCT04658914

9.9 Dissemination of findings

Study findings will be communicated to study participants and their communities prior to wider dissemination. CIDRZ has an ongoing and active community engagement programme. Regular meetings within the community are used to share updates from ongoing work and results from completed projects with participants and other members of the community through short presentations and posters in local languages. At the end of this study, a detailed report outlining study findings will be shared with national ethical and regulatory bodies in keeping with national dissemination guidelines. Following local dissemination, study results will be communicated through peer reviewed scientific publications and conference presentations (e.g. African Rotavirus Symposium, International Rotavirus Symposium, Vaccines for Enteric Diseases). We will seek to maximize awareness of these publications through press releases from Imperial College London, University of Liverpool and CIDRZ, through an online presence (study-specific website) and through Twitter (@VaccineEpi, @cidrzinfo, @MRC_Outbreak). We will also use our role in the MRC-GCRF vaccine networks (HIC-Vac, IMPRINT) to share the results of our study and provide opportunities for collaboration.

10.0 Timelines

Study preparation, participant recruitment and follow-up, data analysis and reporting, is expected to take place over a 3 -year period. Specific timelines are outlined in the Gantt chart below.



11.0 Budget

This work will be supported by the Medical Research Council (U.K) [grant number MR/T030321/1]. The study will be sponsored by the Centre for Infectious Disease Research in Zambia (CIDRZ). The following is a summary of the funding available to conduct the study.

Description	Estimate ZMW
Personnel	6,445,187
Lab consumables and supplies	839,653
Trainings and reimbursements	581,040
Logistics/Regulatory/Communication	873,719
Equipment	1,403,420
Admin/IDC	2,028,604
Total	12,171,625

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13.0 Appendices:

a. **Participant Information Sheet**
b. **Consent forms**
c. **Data collection tools**
d. **Other, e.g. permission letters**
e. **C.Vs**