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RESEARCH PAPER

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A randomized Phase I/II study to evaluate safety and reactogenicity of a heat-stable rotavirus vaccine in healthy adults followed by evaluation of the safety, reactogenicity, and immunogenicity in infants

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ABSTRACT

Objectives: To assess the safety and reactogenicity of single oral dose of heat-stable rotavirus vaccine (HSRV) in healthy adults aged 18–45 years followed by assessment of safety, reactogenicity, and immunogenicity of three doses of HSRV in healthy infants aged 6–8 weeks at enrollment.
Trial Design: Single-center randomized controlled, sequential, blinded (adults) and open-label (infants).
Setting: Single site at International Center for Diarrheal Disease Research, Bangladesh (icddr,b).
Participants: Fifty eligible adults randomized in 1:1 ratio (HSRV: Placebo) followed by 50 eligible infants randomized in 1:1 ratio (HSRV: Comparator (RotaTeq®, pentavalent human-bovine (WC3) reassortant live-attenuated, rotavirus vaccine)).
Intervention: Adults received either a single dose of HSRV or placebo and followed for 14 days. Infants received three doses of either HSRV or comparator with a follow-up for 28 days after each dose.
Main Outcome Measures: Solicited and unsolicited adverse events (AEs) along with any serious adverse events (SAEs) were part of the safety and reactogenicity assessment in adults and infants whereas serum anti-rotavirus IgA response rates were part of immunogenicity assessment in infants only. Post-vaccination fecal shedding of vaccine-virus rotavirus strains was also determined in adults and infants.
Results: In this study, HSRV, when compared with placebo, did not result in increase in solicited adverse events (solicited AEs) in adults. In infants, HSRV had a safety profile similar to comparator vis-à-vis solicited AEs. In infants, fecal shedding of vaccine-virus strains was not detected in HSRV recipients but was observed in two comparator recipients. Percentage of infants exhibiting threefold rise in serum anti-rotavirus IgA titers from baseline to 1-month post-dose 3 in HSRV group was 88% (22/25) and 84% (21/25) in comparator group.
Conclusion: HSRV was found to be generally well-tolerated in both adults and infants and immunogenic in infants.

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KEYWORDS
Heat stable rotavirus vaccine; randomized; safety; reactogenicity; immunogenicity

Introduction

Background

Rotavirus is the leading cause of severe, dehydrating diarrhea and related deaths in children worldwide and infects nearly every child by the age of 5 years in areas where vaccination is not routine.[1,2] Rotavirus was responsible for an estimated 128,500 deaths (95% uncertainty interval [UI], 104,500–155,600) among children younger than 5 years throughout the world in 2016; the vast majority of these children live in low-income countries primarily in the regions of sub-Saharan Africa, Southeast Asia, and South Asia.[2] Prioritizing rotavirus vaccine introduction and interventions to reduce diarrhea-associated morbidity and mortality is necessary for the continued global reduction of rotavirus disease. In view of the high global rotavirus gastroenteritis burden, the WHO on 5 June 2009, had recommended the inclusion of rotavirus vaccine in all the national immunization programs.[3]

While progress has been made in reducing diarrhea-related deaths among children, rotavirus is still one of the leading causes of illness among children under 5 years of age in Bangladesh. Between 2012 and 2015, most (86%) of rotavirus acute gastroenteritis (AGE) hospitalizations were among infants aged 6 to 23 months.[4] Rotavirus vaccines could have a powerful public health impact if introduced into Bangladesh's national immunization program as also in other countries with similar rotavirus epidemiology and unmet needs. While India introduced a rotavirus vaccine in its Universal Immunization Programme (UIP) in a phased manner in 2016, Bangladesh is planning to introduce the rotavirus vaccine in its routine immunization program in the near future. Rotavirus vaccines are currently available in limited private facilities in Bangladesh and only accessible to those who can afford them.

The mechanism by which wild-type rotavirus infection induces immunity is not well established. The total serum

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anti-rotavirus IgA level, measured shortly after infection, generally reflects intestinal IgA levels and is considered to be a marker of immunological take of vaccination, albeit not a true immunological correlate of protection.[5]

The five most prevalent rotavirus genotype/serotype combinations worldwide are G1P1A[8], G2P1B[4], G3P1A[8], G4P1A[8], and G9P1A[8][6] (nomenclature used is GxPy[z] where x represents the established G genotype/serotype number, y represents the established P serotype number and z represents the established P genotype number). These account for more than 90% of the cases of human rotavirus disease worldwide. Within the 'Indian Rotavirus Strain Surveillance Network' Rotavirus was detected in approximately 39% of all participants admitted for diarrhea and tested for rotavirus.[6] The most common type of strains were G2P[4] (26%), G1P[8] (22%), and G9P[8] (9%).[6,7] In India, rotavirus detection rates were greatest among children aged 6 to 23 months (37% for age group 6 to 11 months, 39% for ages 12 to 23 months). The detection rate in children less than age 6 months was 13%.[6,7] In Bangladesh, the Directorate General of Drug Administration (DGDA) maintains oversight and monitoring of all vaccine clinical trials. Surveillance of rotavirus diarrhea in rural Bangladesh hospital at Matlab during 2000–2006 revealed that rotavirus was detected in 33% of the children less than 5 years of age.[8] Another study from Bangladesh reported G1P[8] (36.4%) and G9P[8] (27.7%) as the most dominant circulating strains in 2001–2005, and later during the 2005–2006 rotavirus season, G2P[4] (43.2%) appeared as the most prevalent strain and G12P[6] became a more prevalent strain (11.1%) during this season.[9] This indicated a close similarity in the prevalence of rotavirus strains globally and in southeast Asian countries (India and Bangladesh).

Currently, WHO prequalified rotavirus vaccines are a pentavalent human-bovine (WC3) reassortant live attenuated, rotavirus vaccine (RotaTeq®, Merck & Co., Inc., Kenilworth, NJ, USA), a live-attenuated monovalent human rotavirus vaccine (Rotarix®, GlaxoSmithKline, Belgium), a live-attenuated monovalent human rotavirus 116E vaccine (Rotavac®, Bharat Biotech, India) and a live-attenuated pentavalent bovine-human rotavirus reassortant (Rotasiil™, Serum Institute of India, India). Both RotaTeq® and Rotarix® are stable for the recommended duration at a storage temperature between 2°C and 8°C. Rotavac® is stable for its entire shelf life when stored at −20°C or until expiry of its vaccine vial monitor (VVM2), a heat-sensitive label, when stored between 2°C and 8°C.[10–13] The majority of licensed vaccines possess VVM 7 or VVM 14 category of thermostability which may not address some concerns like vaccine stability in developing countries especially in regions of extreme climatic conditions, reaching up to 40°C and beyond for weeks. Thus, there is a potential need for development of heat-stable vaccines. With one recent heat-stable product (Rotasiil™) pre-qualified for WHO procurement, it augurs well for development of more heat-stable vaccines against rotavirus.

The Hilleman Laboratories manufactured heat-stable rotavirus vaccine (HSRV) is a lyophilized vaccine comprising of five live human-bovine (WC3) rotavirus reassortants (G1, G2, G3, G4 and P1[8]) as in liquid RotaTeq®, the comparator vaccine used in this study. Lyophilized cake of HSRV and placebo also comprised of additional generally recognized as safe (GRAS) excipients: 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), polyvinyl pyrrolidone K-25 (Kollidon 25), L- Arginine and calcium chloride dihydrate which are not present in the comparator.

Considering the similarity in the type of rotavirus strains prevalent in developing world settings and rotavirus strain composition of HSRV together with heat-stable vaccine needs, Hilleman Laboratories' HSRV seems very well suited for use in such settings.

Objectives

Primary objectives

Adult cohort

To assess the safety and reactogenicity of a single oral dose of HSRV as compared to placebo, in terms of solicited or unsolicited AEs, in healthy adults aged 18–45 years.

Infant cohort

To assess the safety and reactogenicity of three-dose series of HSRV as compared to the comparator vaccine, in terms of solicited or unsolicited AEs, in healthy infants 6 to 8 weeks of age at enrollment.

Secondary objectives

Adult cohort

To assess the frequency and duration of shedding of vaccine-virus rotavirus strains in stool samples collected on Days 3, 5 and 7 after a single dose of HSRV or placebo (or for any episode of diarrhea during the study period).

Infant cohort

To assess the frequency and duration of shedding of vaccine-virus rotavirus strains in stool samples collected on Days 3, 5 and 7 after each dose of HSRV or comparator vaccine (or for any episode of diarrhea during the study period).

Another objective was to assess the immunogenicity of HSRV in terms of serum anti-rotavirus IgA antibody titers at one-month (~4-weeks) post-dose 3 as compared to baseline.

Methods

Trial design

This study was a single-center-randomized controlled trial performed sequentially in two age cohorts (Figure 1). Cohort 1 comprised of 50 healthy adults who were randomized in a 1:1 ratio to receive a single dose of either HSRV or placebo in a double-blinded manner only on Day 0 (site-visit 1). Enrollment of participants in cohort 1 was sequential. All participants were dosed and followed up as per requirements of Phase I study. Adult participants were identified by trained field workers visiting door to door in the community. The study period of adult cohort was 14 days. Adult participants were provided diary cards at site-

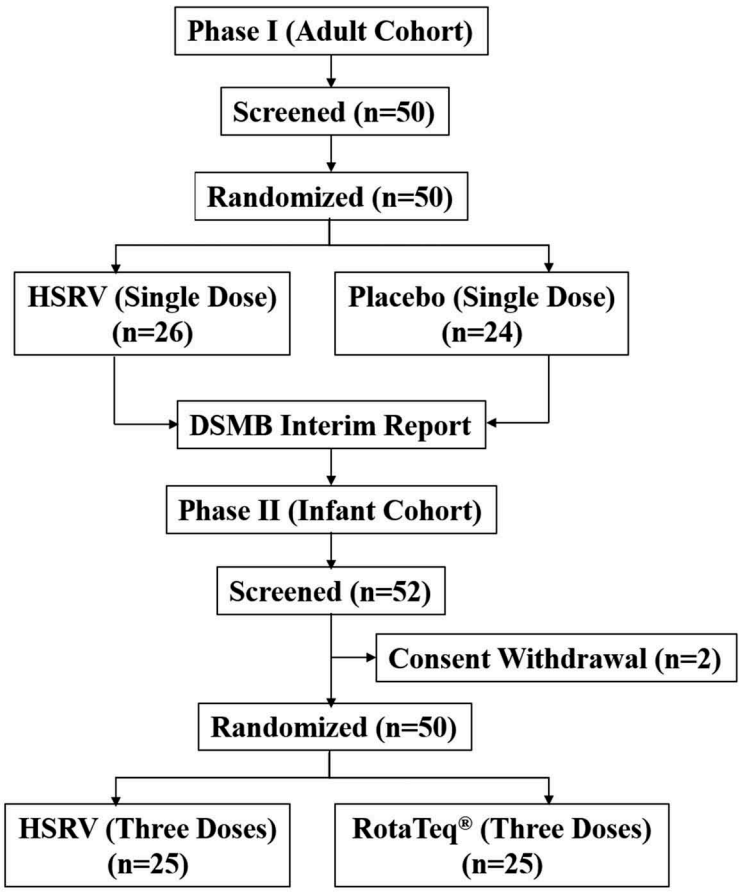

Figure 1. Study design and plan.

visit 1 on Day 0 to record any solicited and unsolicited AEs and gastroenteritis episodes (GE) experienced up to the next 7 days. Diary cards were collected by study staff from each participant's home on Day 8. Participants were instructed to report any unsolicited AEs and GE that they experienced from Day 8 up to site-visit 2 (on Day 14). Any SAEs were to be reported throughout the study period. An interim report comprising of safety data from the adult cohort was submitted to Data and Safety Monitoring Board (DSMB) for approval for initiating the infant cohort study. Cohort 2 was comprised of 50 healthy infants aged 6 to 8 weeks at enrollment, to receive three doses of HSRV or RotaTeq® (open label, however immunogenicity analysis was blinded) with the first dose administered at 8 weeks of age (Day 0; site-visit 1) and subsequent doses administered at 4-week intervals at the site. The study period of infant cohort was 98 days. Diary cards were issued to the parents or legally authorized representatives (LARs) of participants on Day 0, 28, 56 and 84 (site-visit 1, 2, 3, and 4, respectively) to record any solicited (first 7-day period) and unsolicited AEs and GE (28-day period) experienced by the participants and return the completed diary cards during next site-visit for dosing. Any SAEs were to be reported throughout the study period. The last follow-up period for infants was 14 days.

The study was conducted according to the principles of Good Clinical Practice (GCP), the Declaration of Helsinki, and all applicable regulatory requirements. Two independent committees at icddr,b (Research Review Committee and Ethical Review Committee) sequentially reviewed and approved the protocol and other study-related documents. The DSMB was comprised of independent group of experts as constituted by the Ethical Review Committee (ERC) at icddr,b.

Identity of investigational products

HSRV was formulated as a lyophilized cake comprising of GRAS (generally regarded as safe) excipients as described previously.[14] A series of formulation compositions, differing in buffering agents, bulking agents, cryoprotectants, amino acids, and divalent cations, were screened for their ability to provide stability to rotavirus serotypes during lyophilization and when stored under-elevated temperatures for extended periods before arriving at the final lead formulation[14] HSRV – used in this study. This lyophilized cake was reconstituted using a reconstitution buffer just prior to administration. The placebo formulation was also composed of the same chemical ingredients as HSRV but without any rotavirus strain. The Good Manufacturing

Practice (GMP) grade bulk of reassortant rotaviruses were procured directly from Merck &Co. Inc, Kenilworth, NJ, USA. GMP grade HSRV, placebo, and reconstitution buffer were contract manufactured at a Contract Manufacturing Organization (CMO) – Omnia Biologics Inc., Rockville, MD, USA. Commercially available RotaTeq® was procured from Bangladesh. All the study vaccines were supplied by the sponsor. The HSRV vaccine/placebo doses were provided to the study center with blinded labels in 3 mL vials at 2–8°C, while respecting the randomization block size. Each 3 mL vial containing the lyophilized cake was sufficient to formulate five doses of either HSRV or placebo. For HSRV or placebo dose administration, 2 ml out of total 10.75 mL of the supplied reconstitution buffer was transferred into the vial of the lyophilized product (HSRV vaccine or placebo) using a sterile graduated syringe without needle. The HSRV vaccine or placebo vial was shaken well to re-suspend the product. The entire volume of the re-suspended product was withdrawn into the same syringe and added to the remaining volume of reconstitution buffer. The final re-suspended product was then shaken and only 2 ml from it was withdrawn in to the syringe and administered promptly as a single oral dose as per the randomization list. The remaining volume in the same buffer vial was not used for any further administration. The stability profile of lyophilized HSRV is represented in Table 1. Lyophilized HSRV vaccine vials from the same batch were kept in stability chambers (Memmert, Germany) at various temperature conditions of 2–8°C, 25°C, 37°C, and 45°C for more than 36 months and assessed periodically for stability by multivalent qPCR-based rotavirus potency assay (M-QPA) as described previously.[14] The composition concentration per dose (2 mL) of the reconstituted HSRV is listed in Table 2. Each of the five constituent rotavirus serotypes of HSRV were viable upon reconstitution as determined by M-QPA assay. The dose levels of each rotavirus serotype in a single dose of reconstituted HSRV (2 mL) were similar to rotavirus serotypes in a single dose of the comparator, RotaTeq®, (2 mL) in terms of Infectious Units (IU).[11]

Table 1. Stability profile of heat-stable rotavirus vaccine (HSRV) at various temperature storage conditions.

Storage Condition	Stability
2–8°C	> 36 months
25°C	> 24 months
37°C	20 months
45°C	7 months

Stability profile of comparator vaccine (RotaTeq®) as per its product insert is up to 24 months at storage condition of 2–8°C.

Table 2. Composition per dose (2 mL) of the reconstituted heat-stable rotavirus vaccine.

Components/Serotypes	Amount
Rotavirus serotype G1	not less than 2.20×10^6 IU
Rotavirus serotype G2	not less than 2.80×10^6 IU
Rotavirus serotype G3	not less than 2.20×10^6 IU
Rotavirus serotype G4	not less than 2.00×10^6 IU
Rotavirus serotype P1[8]	not less than 2.30×10^6 IU

Abbreviations: IU = International units

Participants

Study eligibility criteria

Adult cohort. Healthy adult participants of either sex, between 18 and 45 years of age, who were available for the entire period of the study and reachable by study staff for post-vaccination follow-up, were included in the study.

Infant cohort. Healthy infants of either sex, 6 to 8 weeks of age at the time of enrollment, who were born between 36 and 42 weeks of gestation with birth weight ≥2 kg, were included in the study. Infants with a history of congenital abdominal disorders, intussusception, abdominal surgery, signs of severe malnutrition or known or suspected impairment of immunological function(s) in participant or his/her immediate family were excluded from the study.

Study location

The study was performed at a single center at International Center for Diarrheal Disease Research, Bangladesh (icddr,b).

Interventions

For adults

The participants received only a single oral dose (2 mL) of reconstituted HSRV vaccine or placebo and were observed closely for at least 30 min following the administration of the vaccine. The volume of each test item was measured using a sterile-graduated syringe.

For infants

The participants received three doses of either test HSRV (2 mL of reconstituted vaccine) or comparator, RotaTeq® (ready-to-use 2 mL liquid dose), via oral route 4-weeks apart and were observed closely for at least 30 min following the administration of each vaccine dose. The volume of reconstituted HSRV was measured using a sterile-graduated syringe. For RotaTeq® recipients, the dose administration followed the instructions provided by the manufacturer. The intended dose regimen was decided to be three-dose based on the prescribed three-dose series in infants for RotaTeq® since HSRV constituent rotavirus bulks are similar to RotaTeq®. From a historical perspective, it has been previously demonstrated that three-dose regimen of RotaTeq® induce a significantly higher immune response (i.e., a ≥ threefold rise in antibody titer from Pre-dose 1 to Post-dose 3) in a larger proportion of infants than a two-dose regimen.[15] In Bangladesh, childhood vaccines are recommended beginning 6 weeks of age. All infant participants were screened and enrolled at 6 weeks of age and administered commercially available routine Expanded Program on Immunization (EPI) vaccines such as Pentavalent vaccine, (diphtheria, pertussis, tetanus, Hep B vaccine, Hib vaccine), Pneumococcal conjugate vaccine (PCV), Inactivated polio vaccine (IPV) and Oral polio vaccine (OPV) according to the manufacturer's instructions. Routine childhood vaccines were given at least 14 days prior to

administration of HSRV or RotaTeq® dose at 6, 10 and 14 weeks of age. Accordingly, study vaccines were administered to infants at 8, 12 and 16 weeks of age. According to the protocol, breast-feeding of infants was allowed *ad libitum* around the time of their study vaccination.

Outcome measures

The primary outcome measures were frequency, severity, and causality of solicited/unsolicited AEs and serious adverse events (SAEs) following administration of investigational rotavirus vaccine (HSRV) or placebo in the adult cohort up to 14 days after a single dose; and following administration of HSRV or RotaTeq® in the infant cohort up to 28 days after each dose. Solicited events included protocol-listed symptoms (fever, cough, runny nose, diarrhea, vomiting, nausea (adults only), abdominal pain (adults only), loss of appetite (infants only) and fussiness/irritability (infants only)) reported within 7 days after any study vaccination administration. Unsolicited events included protocol-listed events reported after 7 days after study vaccination administration up to the follow-up period or any other event that was not listed in the protocol. Any untoward medical occurrence that was life-threatening, resulting in disability or death or considered serious as per medical/scientific judgment or any case of intussusception (resulting in hospitalization) during the study period was considered as a serious adverse event. The intensity grades of severity ranged from normal, mild, moderate to severe (i.e. grade 0 to 3, respectively) as assessed by the study principal investigator. All AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 18.1. Diary cards were provided to the participants (adult cohort); parents or LARs (legally authorized representatives) (infant cohort) to record body (axillary) temperature and solicited and unsolicited AEs occurring after each dose administration during the specified follow-up period for either cohort. The collected information was transcribed into the appropriate sections of the electronic case report form (eCRF) in English.

Serum anti-rotavirus IgA response rates, defined as the proportion of participants with a threefold rise in titers from baseline to 28 days after administration of third dose, were also determined but only in the infant cohort. Blood sample (2 mL) was collected from each infant participant at baseline and one-month (~4-weeks) post-dose 3. Serum anti-rotavirus IgA responses were measured using an ELISA/Enzyme Immunoassay (EIA) in a blinded manner at Laboratory for Specialized Clinical Studies (LSCS), Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA using the reagents and procedure as per standard operating protocol (SOP) on "Quantitative Determination of Serum Anti-Rotavirus IgA by EIA" (LSCS SOP No. 110 Version 03). Usually, a fourfold increase in seroconversion is used for demonstrating sero-response for vaccines which have not established immunological correlation of protection, therefore, a fourfold seroconversion data was also included. In this study threefold rise as sero-response was used, since RotaTeq® studies have used similar criterion in previous clinical studies.[16–18]

Other outcome measures also included frequency and duration of post-vaccination shedding of vaccine-virus rotavirus strains in stool samples as determined by genotyping on Days 3, 5 and 7 after each of the prescribed dose(s) in both adult and infant cohorts. Vaccine-rotavirus replication in the intestinal tract, if at all, is known to peak during the 4- to 6-day period after a dose, with minimal replication occurring after a week.[15] The time-frame evaluated for potential vaccine-rotavirus shedding in this study was based on data obtained in previous clinical trials for RotaTeq®[15,16] since the virus bulks are similar in both HSRV and RotaTeq®. Therefore, in this study, stool samples for both adult and infant cohort were collected on Day 3, 5 and 7 post any dose administration. In addition, stool samples were collected from all gastroenteritis (diarrhea with or without vomiting) cases throughout the study and were considered as AE of special interest. Stool samples were screened for vaccine-rotavirus antigen using a commercially available EIA (ProSpecT™ Rotavirus Microplate Assay, Oxford, UK) at Virology laboratory, Infectious Diseases Division, icddr,b using SOP on Rotavirus Antigen Detection by ELISA (applicable for stool, rectal swab samples) (SOP. Ver.52). If a sample was found to be vaccine rotavirus-positive by EIA, the sample was analyzed for identification of G and P serotypes of the virus confirmed by reverse transcription polymerase chain reaction (RT-PCR) using the SOP on Genotyping of Rotavirus by PCR (SOP.VIR.57) at Virology laboratory, Infectious Diseases Division, icddr,b.

Determination of sample size

The sample size was determined using Power Analysis and Sample Size (PASS) 2005, one–sided test for an inequality test of independent proportions, alpha = 2.5%, power = 80% (Reference study: GSK 113552 (Rota-073)). The estimated drop-out rate was 12%. The target sample size of 50 participants in each cohort (25 participants in the HSRV group and 25 participants in the placebo or comparator group) and 44 evaluable participants (22 participants in the HSRV group and 22 participants in the placebo/comparator group) were anticipated for analysis. With this, a possible increase in incidence of each solicited AE could be detected in the HSRV group as compared to placebo or comparator with 80% power.

Study randomization

The participants were randomly assigned to the study groups in a 1:1 ratio (Adult Cohort: HSRV group and placebo group; Infant Cohort: HSRV group and comparator group). A randomization list was generated by an independent expert at MSD Wellcome Trust Hilleman Laboratories using a standard computer program – Statistical Analysis System (SAS®) software: SAS (R) 9.3 (TS1M0), Seed:1019785346 – and was used to number the vaccines. A randomization blocking scheme (2 per block) was used to ensure that balance between treatments was maintained: a single treatment number uniquely identified the vaccine doses administered to the same participant. The paper copy of the randomization list was concealed using sequentially numbered, opaque, sealed envelopes (SNOSE) and shared with the designated study independent staff at the site.

Concomitant vaccine and treatment

At each study visit or contact, the participant or parents or legally authorized representative (LAR) were asked about any medication(s) taken. Concomitant medications were coded using the WHO Drug Dictionary (version WHODD201509) and classified by Anatomical Therapeutic Chemical categories.

Blinding

The Adult Cohort study was performed under double-blind conditions and both the participants and the study personnel were unaware of the administered treatment. For the Infant Cohort, the study was open-label. However, all serum and stool samples were sent to the analytical laboratory under blinded conditions for analysis and reporting.

Statistical methods

All analyses were performed using SAS® version 9.2 in a secure and validated environment. The datasets followed the analysis dataset model (ADaM) data specifications. Continuous variables were summarized including number of participants (n), mean, standard deviation (SD), median, minimum, and maximum. Categorical variables were summarized by visit with frequency counts and percentages. Analyses were performed by cohort and treatment arm. The confidence intervals (CI) was 95%, unless otherwise stated. For percentages and seroconversion rates, the CIs were obtained using Clopper–Pearson's method. The analysis populations for safety, immunogenicity and stool analyses included the Total Vaccinated Population (or Cohort) and According to Protocol Population (ATP). The total-vaccinated cohort included all participants who received at least one dose of HSRV, comparator or placebo. The ATP population included all participants from the Total-vaccinated cohort who received the study vaccine or placebo/comparator according to the protocol and fulfilled all study inclusion criteria and whose randomization code was not broken. All original and derived parameters as well as population characteristics were described using summary statistics. Frequency counts and percentages were presented for each qualitative variable. Descriptive statistics (number of participants [n], mean, standard deviation [SD], median, minimum and maximum) were calculated for each quantitative variable (unless otherwise stated). The stool sampling data were presented as the number and percentages of participants with rotavirus in stool samples collected at pre-determined time points and at combined pre-determined time points. For the immunogenicity analysis in infant cohort, descriptive statistics (also including geometric mean and geometric coefficient of variation) was used to describe the absolute serum anti-rotavirus IgA titre levels and the fold-rise above the baseline levels was presented by treatment arm. Confidence intervals at 95% level for the geometric mean titres before and after vaccination as well as the fold-rise were obtained using t-distribution on the logarithmic scale assuming a normal distribution and then back-transforming.

Results

Study participants

In the adult cohort, all 50 enrolled participants (26* in the HSRV group and 24 in the placebo group) were included in the Total Vaccinated Cohort safety and ATP analysis (Figure 1) and all of them completed the study. In the infant cohort, 52 participants consented for the study, of which consent was withdrawn for two participants prior to randomization (Figure 1). All enrolled 50 participants (25 in HSRV group and 25 in comparator group) were included in the Total Vaccinated Cohort safety and ATP immunogenicity analysis and all of them completed the study. The study was started in June 2016 and completed in April 2017.

(*One of the vial label (vial ID 28) was found missing from the supplied vaccine vials. Consequently, the final vaccine supply labeled with ID 1 to 60 was delivered to study team without ID 28. To maintain sequential randomization procedure, 26 participants got randomized in HSRV group instead of 25 and as a result, there were 24 participants in the placebo group).

Demographic and other baseline characteristics

Demographic characteristics

Demographic characteristics were comparable between HSRV and Placebo groups in the adult cohort (Table 3) and between HSRV and comparator groups in the infant cohort (Table 4). The mean (SD) age of adults was 30.8 (4.89) years. The mean (SD) age of infants at first vaccination (childhood EPI vaccination) was 6.15 (0.14) weeks. All study participants in the adult and infant cohorts were of Asian origin.

Medical history at baseline

No medical history (any previous and current medical conditions and medications) was reported either in the Adult Cohort or in the Infant Cohort.

Measurements of compliance in returning diary cards

Compliance in returning filled diary cards was 100% for both the Adult and Infant Cohorts.

Table 3. Summary of demographic characteristics – adult cohort (Total-vaccinated cohort).

Characteristic Category	Statistic	HSRV Vaccine (N = 26)	Placebo (N = 24)	All (N = 50)
Age (years) at vaccination	N	26	24	50
	Mean	31.0	30.6	30.8
	SD	5.67	3.99	4.89
	Median	31.5	30.5	31.0
	Minimum	21	21	21
	Maximum	41	37	41
Gender				
Male	n (%)	11 (42.3)	11 (45.8)	22 (44.0)
Female	n (%)	15 (57.7)	13 (54.2)	28 (56.0)
Race				
Asian	n (%)	26 (100)	24 (100)	50 (100)

Abbreviations: HSRV = Heat-stable rotavirus vaccine; N = Number of participants in the treatment arm; n = Number of participants in the specific category; SD = standard deviation.

% was calculated using the number of participants in the treatment arm as the denominator (n/N*100).

Table 4. Summary of demographic characteristics – infant cohort (Total Vaccinated Cohort).

Characteristic Category	Statistic	HSRV Vaccine (N = 25)	RotaTeq® (N = 25)	All (N = 50)
Age (weeks) at vaccination	N	25	25	50
	Mean	6.16	6.14	6.15
	SD	0.15	0.13	0.14
	Median	6.10	6.10	6.10
	Minimum	6.0	6.0	6.0
	Maximum	6.4	6.3	6.4
Gender				
Male	n (%)	9 (36.0)	14 (56.0)	23 (46.0)
Female	n (%)	16 (64.0)	11 (44.0)	27 (54.0)
Race				
Asian	n (%)	25 (100.0)	25 (100.0)	50 (100)

Abbreviations: HSRV = Heat-stable rotavirus vaccine; N = Number of participants in the treatment arm; n = Number of participants in the specific category; SD = standard deviation.
% was calculated using the number of participants in the treatment arm as the denominator (n/N*100).

Analysis of safety and reactogenicity

Safety analysis was conducted on the Total Vaccination Cohort. Both solicited and unsolicited adverse events were evaluated. No deaths, SAEs, or withdrawal due to AEs were reported during the study in either of the study cohorts. A summary of other safety findings is presented below:

Solicited adverse events

Adult cohort. A summary of solicited AEs reported during the Day 0–7 post-vaccination period in the Adult Cohort is provided in Table 5. A numerically higher number of participants reported abdominal pain (4 versus 1), nausea (4 versus 1), and vomiting (2 versus 0) in the HSRV group as compared to the placebo group, respectively. However, the difference was considered as statistically insignificant since the 95% CI for risk difference were too wide. Other solicited AEs were reported at a similar frequency between the two study groups.

None of the solicited AEs reported were grade 3 in intensity and no AEs were reported to be related to the HSRV or placebo.

Table 5. Summary of solicited adverse events during the 8-day-post-vaccination period – adult cohort (Total vaccinated cohort).

	HSRV Vaccine (N_1 = 26)	Placebo (N_2 = 24)	95% CI for Risk Difference	
Symptom	n_1 (%)	n_2 (%)	LL (%)	UL (%)
Abdominal Pain	4 (15.4)	1 (4.2)	−4.8	27.2
Nausea	4 (15.4)	1 (4.2)	−4.8	27.2
Diarrhea	2 (7.7)	1 (4.2)	−9.5	16.5
Vomiting	2 (7.7)	0 (0.0)	−2.6	17.9
Cough	1 (3.8)	1 (4.2)	−11.2	10.6
Pyrexia	1 (3.8)	0 (0.0)	−3.5	11.2
Rhinorrhea	1 (3.8)	1 (4.2)	−11.2	10.6

Abbreviations: 95% CI = 95% confidence interval for risk difference; HSRV = Heat-stable rotavirus vaccine; LL = Lower limit; N_1, N_2 = Number of participants dosed in the treatment arm; n_1, n_2 = Number of participants with that type of AE; UL = Upper limit.
Note: % was calculated using the number of participants dosed in the treatment arm as the denominator (n/N*100).
If the same participant had multiple symptoms then he/she was counted for every symptom. Adverse events were coded using Medical Dictionary for Regulatory Activities (MedDRA) Version 18.1.

Table 6. Summary of solicited adverse events – infant cohort (Total-vaccinated cohort).

	HSRV Vaccine (N_1 = 25)	RotaTeq® (N_2 = 25)	95% CI for Risk Difference	
Symptom	n_1 (%)	n_2 (%)	LL (%)	UL (%)
Appetite loss	0	0	0.0	0.0
Cough	6 (24.0)	12 (48.0)	−49.8	1.8
Diarrhea	1 (4.0)	0	−3.7	11.7
Irritability	4 (16.0)	5 (20.0)	−25.3	17.3
Pyrexia	0	0	0.0	0.0
Rhinorrhea	7 (28.0)	10 (40.0)	−38.0	14.0
Vomiting	3 (12.0)	1 (4.0)	−6.9	22.9

Abbreviations: 95% CI = 95% confidence interval for risk difference; HSRV = Heat-stable rotavirus vaccine; LL = Lower limit; N_1, N_2 = Number of participants dosed in the treatment arm; n_1, n_2 = Number of participants with that type of AE; UL = Upper limit.
% was calculated using the number of participants dosed in the treatment arm as the denominator (n/N*100).
If the same participant had multiple symptoms then he/she was counted for every symptom. Adverse events were coded using Medical Dictionary for Regulatory Activities (MedDRA) Version 18.1.

Infant cohort. Cough, rhinorrhea, and irritability were the most commonly observed solicited AEs in both HSRV and comparator groups (Table 6). Cough (6 versus 12) and rhinorrhea (7 versus 10) were reported at a numerically lower frequency in the HSRV group as compared to the comparator group, respectively. Vomiting (3 versus 1) was reported at a numerically higher frequency in the HSRV group as compared to the comparator group, respectively. However, the difference was considered as statistically insignificant since the 95% CIs were wide. Other solicited AEs were reported at a similar frequency in the two study groups. None of the solicited AEs were grade 3 in intensity and none of the AEs in the HSRV group were considered to be causally related to the vaccine. The frequency of AEs was not found to be related to a particular dose.

Unsolicited adverse events

Very few unsolicited AEs were reported in either of the two study cohorts (Table 7). In the adult cohort, gastrointestinal disorders were reported by three participants in the HSRV group and one participant in the placebo group.

In the infant cohort, unsolicited AEs were reported in not more than five participants in either of the study groups. The most frequently reported unsolicited AEs following the three-dose vaccination course in ≥3 participants were retching, cough, and rhinorrhea in the HSRV group and pyrexia and cough in the comparator group. The frequency of AEs was not found to be related to a particular dose.

Based on the sample size and Clopper–Pearson's 95% confidence intervals (95% CIs) the safety profiles appeared generally similar between HSRV and comparator.

Stool sample analyses: vaccine-virus rotavirus shedding

Stool specimens were collected from all the study participants (adults and infants) at pre-determined time points (Day 3, 5 and 7) post-study vaccination. Fecal vaccine-virus rotavirus strain shedding was not observed in any of the study participants at Days 3, 5, and 7 post-vaccination time points in the Adult Cohort. In the infant cohort, at the combined pre-determined post-vaccination time points (after each dose),

Table 7. Unsolicited adverse event by primary system organ class and preferred term – (Total-vaccinated cohort).

Adult Cohort		
System Organ Class Preferred Term	HSRV Vaccine (N = 26) n (%) E	Placebo (N = 24) n (%) E
Gastrointestinal Disorders	3 (11.5) 3	1 (4.2) 1
Diarrhoea	2 (7.7) 2	1 (4.2) 1
Vomiting	1 (3.8) 1	0
Infant Cohort		
System Organ Class Preferred Term	HSRV Vaccine (N = 25) n (%) E	RotaTeq® (N = 25) n (%) E
Gastrointestinal Disorders	5 (20.0) 5	3 (12.0) 3
Diarrhea	1 (4.0) 1	1 (4.0) 1
Retching	3 (12.0) 3	1 (4.0) 1
Vomiting	1 (4.0) 1	1 (4.0) 1
General Disorders and Administration Site Conditions	1 (4.0) 1	3 (12.0) 3
Pyrexia	1 (4.0) 1	3 (12.0) 3
Infections and Infestations	3 (12.0) 4	1 (4.0) 1
Fungal Infection	2 (8.0) 2	0
Nasopharyngitis	1 (4.0) 1	0
Rhinitis	1 (4.0) 1	1 (4.0) 1
Respiratory, Thoracic and Mediastinal Disorders	3 (12.0) 10	4 (16.0) 7
Cough	3 (12.0) 5	4 (16.0) 4
Nasal Congestion	0	1 (4.0) 1
Rhinorrhea	3 (12.0) 5	2 (8.0) 2

Abbreviations: N: Number of participants dosed in the treatment arm; n: Number of participants with that type of AE; E: Number of occurrences of an adverse event; %: Percentage, calculated using the number of participants dosed in the treatment arm as the denominator (n/N*100); All adverse events were coded using Medical Dictionary for Regulatory Activities (MedDRA) version 18.1.

none of the HSRV vaccinees showed fecal vaccine-virus rotavirus strain shedding. Fecal vaccine-virus rotavirus strain shedding was detected in two participants from the comparator group after Dose 1 (Table 8). All gastroenteritis (diarrhea with or without vomiting) samples collected, other than predetermined time points, were found to be negative for vaccine-virus rotavirus strains and not related to any investigational product administration in both adult and infant cohorts (*data not shown*).

Table 8. Percentage of participants with vaccine-virus rotavirus strain in stool samples collected at combined pre-determined time points – infant cohort (ATP).

Vaccine-virus Rotavirus Shedding at Combined Time Points					
				95% CI	
Dose	Group	N	n (%)	LL (%)	UL (%)
Dose 1	HSRV vaccine	25	0	0	13.72
	RotaTeq®	25	2 (8.0)	0.98	26.0
Dose 2	HSRV vaccine	25	0	0	13.72
	RotaTeq®	25	0	0	13.72
Dose 3	HSRV vaccine	25	0	0	13.72
	RotaTeq®	25	0	0	13.72

Abbreviations: N: Total number of participants in the treatment arm with an evaluable stool sample at that time point.
Abbreviations: 95% CI = Clopper–Pearson 95% confidence interval; HSRV = Heat-stable rotavirus vaccine; LL = Lower limit; N = Number of participants dosed in the treatment arm; n = Number of participants with Vaccine-Rotavirus strain in stool samples; UL = Upper limit.
% was calculated using the total number of participants in the treatment arm as the denominator (n/N)*100.

Immunogenicity (infant cohort only)

A summary of serum anti-rotavirus IgA titer fold-increase, and seroconversion rates in Infant Cohort (ATP) is presented in Table 9(a-c).

Overall, for a small sample size of 25 infant participants each, HSRV seroconversion was similar to comparator, both for threefold and fourfold rise. The fold rise of Geometric

Table 9. Serum anti-rotavirus IgA seroconversion rates from baseline to 4 weeks post dose 3 and their geometric mean titers in Infant Cohort (a), along with analyses of seroconversion difference (b), and geometric mean titer difference (c).

a)

	Seroconversion		GMT (95% CI)
	≥3-fold (%)	≥4-fold (%)	
HSRV	22/25 (88)	21/25 (84)	178.1 [76.1, 416.5]
RotaTeq®	21/25 (84)	20/25 (80)	89.9 [39.2, 206.1]

Abbreviations: GMTs – Geometric mean titers; 95% CI = 95% confidence interval using t-distribution on log-transformed titers.

b)

	HSRV Vaccine (N_1 = 25)	RotaTeq® (N_2 = 25)		
			95% CI for Seroconversion Difference	
Fold-rise	n_1	n_2	LL (%)	UL (%)
≥3-fold	22	21	−15.2	23.2
≥4-fold	21	20	−17.3	25.3

Abbreviations: 95% CI = 95% confidence interval for seroconversion difference; HSRV = Heat-stable rotavirus vaccine; LL = Lower limit; N_1, N_2 = Number of participants dosed in the treatment arm; n_1, n_2 = Number of participants with the particular anti-rotavirus IgA titer fold rise in serum; UL = Upper limit.
Note: % was calculated using the number of participants dosed in the treatment arm as the denominator (n/N*100).

c)

	HSRV Vaccine (N_1 = 25)	RotaTeq® (N_2 = 25)		
			% CI for Geometric Mean Titer Difference	
	n_1 (SD1)	n_2 (SD2)	LL	UL
Geometric Mean Titer	178.1 (2458.5)	89.9 (1351.2)	−1011.5	1187.9

Abbreviations: 95% CI = 95% confidence interval for geometric mean titer difference; HSRV = Heat-stable rotavirus vaccine; LL = Lower limit; N_1, N_2 = Number of participants dosed in the treatment arm; n_1, n_2 = Geometric mean titer of participants in specific groups; SD1, SD2 = Standard deviation of geometric mean titers in specific groups; UL = Upper limit.

mean titers (GMTs) (from baseline to one-month post dose 3) within each arm indicated statistically similar sero-response for HSRV and comparator. The 95% CIs for seroconversion difference were −15.2, 23.2 for ≥threefold rise and −17.3, 25.3 for ≥fourfold rise in titers.

In terms of titers, anti-rotavirus IgA antibody geometric mean titers at one-month (~4-weeks) post-dose 3 were numerically higher in the HSRV group (178.1 U/mL) as compared with the comparator group (89.9 U/mL), but the CIs ([76.1, 416.5] for HSRV and [39.2, 206.1] for comparator) overlapped hence the titer difference of immune response after three doses of the vaccine was considered similar (CI = 95% confidence interval calculated using t-distribution on log-transformed titers). The 95% CIs for geometric mean titer difference were also too wide (−1011.5, 1187.9).

Discussion

This is the first randomized Phase I/II study to evaluate the safety and immunogenicity of a heat stable formulation of lyophilized live attenuated pentavalent (G1-G4 and P1[8]) rotavirus vaccine in healthy adults and infants.

The HSRV formulation offers a stability profile of 7 months at 45°C (first of its kind stability profile reported anywhere for any rotavirus vaccine)[14] and 20 months at 37° C. Such a heat-stable rotavirus vaccine has the potential to weather the high temperatures encountered in regions where the majority of rotavirus burden exists and has the potential to partially or completely eliminate cold chain dependence and reduce associated costs.

The HSRV has been formulated using the same virus bulks as those used for RotaTeq®. Nonetheless, it is a new formulation (heat stable). Therefore, it was intended to test the safety of the HSRV vaccine in a new phase I/II study. The safety and reactogenicity of the investigational HSRV vaccine was first evaluated in healthy adults aged 18 to 45 years to provide sufficient safety data to allow for the study to proceed in the infant population. Rotavirus vaccine is not indicated for the adult population; hence only a single dose of the new HSRV was administered to assess safety of this vaccine in the Adult Cohort. For the same reason, a comparator vaccine in the adult cohort was not used, a placebo was used instead. The rationale for including a placebo group in the Adult Cohort was to permit the comparison of reactogenicity and safety of HSRV with placebo in terms of any AEs reported in the study Safety assessments were based on gastroenteritis symptoms and other predefined systemic events. None of the AEs in HSRV group in adults were considered to be causally related to HSRV vaccine by the study investigator.

More importantly, in the infant cohort which is also the targeted population, there were fewer gastrointestinal disorders in comparison to other AEs which suggested that post-vaccination HSRV was well tolerated in the targeted population (within-sample size limits). All the AEs (solicited or unsolicited) resolved shortly (mostly within a week) after their onset. There were no serious or severe AEs reported in the participants from HSRV group in this study. Correspondingly, there were no cases of intussusception or death reported in this study. However, even though few, the occurrence of gastrointestinal episodes in HSRV recipients suggests for its close monitoring in future.

Previously, the safety and immunogenicity of the comparator, RotaTeq®, has been evaluated in numerous Phase II and III studies in infants and elderly.[16–19] The sero-response rates (anti-rotavirus serum IgA) for RotaTeq® have been remarkably consistent (>93%) across all populations tested (USA, Europe, Taiwan, Korea, and Latin America) regardless of the race or ethnic origin of the different populations.[17] In this study, immunogenicity results in the HSRV and RotaTeq® groups for the Infant Cohort were comparable with respect to the percentage of participants that attained a threefold or a fourfold rise in serum anti-rotavirus IgA titers. This suggests that the heat-stable rotavirus formulation (HSRV) was immunogenic for the intended use with similar findings reported for the comparator.[16–19]

In terms of fecal shedding, it has been previously reported that for RotaTeq®, vaccine-virus shedding occurs in approximately 10% of the recipients after the first dose and very rarely thereafter.[20] In this study, fecal shedding of the vaccine-virus strains (as assessed by EIA and RT-PCR) was not detected in any of the HSRV recipients from either of the study cohorts and only in two participants (after dose 1) from the comparator group in infant cohort indicating similar findings. Absence of vaccine-related rotavirus shedding in fecal samples of HSRV recipients could be assessed in different ways. Primarily, vaccine-virus shedding and transmission to unvaccinated contacts have been considered as potential adverse events post live virus vaccination especially in developed countries where mortality associated with rotavirus is low, the number of immunocompromised contacts is high, and most regulatory bodies, physicians, and families wish to avoid risk.[20] However, vaccine virus shedding and transmission could have potential benefits as well. In impoverished areas with low immunization rates but high morbidity and mortality caused by rotavirus, this could help stimulate immune response and result in protective immunity in non-vaccinated contacts.[20] However, this study was not powered to evaluate vaccine-virus rotavirus viability in the fecal samples and its transmission.

Correlation between vaccine-virus strain shedding and pyrexia (fever) was also assessed. In the infant cohort, pyrexia was only reported as an unsolicited AE in both HSRV (n = 1) and RotaTeq® (n = 3) groups. In the adult cohort, pyrexia was reported as a solicited AE only in the HSRV group (n = 1). However, fecal shedding of vaccine-virus strains was not detected in any of the HSRV recipients in either infants or adults. Hence, pyrexia could not be considered to be indicative of vaccine-virus replication and subsequent shedding in the stool.

In conclusion, the heat-stable lyophilized rotavirus formulation, HSRV, was found to be generally well tolerated in terms of frequency, severity, and causality of solicited and unsolicited AEs in both study cohorts and immunogenic in the infant cohort. However, the study had a small sample size with usual limitations associated with the analysis of results for statistical significance in terms of 95% confidence intervals; and for deriving any strong conclusions or making significant claims. Therefore, our data should be interpreted with

caution. Overall, the safety profile of HSRV appeared to be generally similar to the comparator vaccine, supporting further evaluation of HSRV in immunogenicity and safety studies in infants in larger Phase IIb-III studies.

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Trial registration

ClinicalTrials.gov Identifier: NCT02728869.

Disclosure of potential conflicts of interest

None perceived or declared. This study was performed under a "research agreement" between MSD Wellcome Trust Hilleman Laboratories Pvt. Ltd, New Delhi and International Center for Diarrheal Disease Research, Bangladesh (icddr,b). Funds were made available to icddr,b in accordance with the approved budget for the conduct of the research as described in the study scope of work. Each party, its investigators and participating organizations in this research were required to adhere to applicable ethical, regulatory and professional standards and also as per ICH-GCP in all matters related to this research.

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