icddr,b	**RRC APPLICATION FORM**

RESEARCH PROTOCOL **Number:** PR-16023 **Version No.** 5.00 **Version date:** 10 July 2017 **IND Number:** 15335 **Sponsor Number:** VAC 049	**FOR OFFICE USE ONLY**		
	RRC Approval:	☒ Yes	☐ No	Date:12-APR-2016
	ERC Approval:	☒ Yes	☐ No	Date:31-JUL-2016
	AEEC Approval:	☐ Yes	☐ No	Date:
	External IRB Approval	☒ Yes	☐ No	Date:03 MAY 2016
	Name of External IRB: ___WIRB_____			

Protocol Title:* (maximum 250 characters including space)
A Phase 1 Randomized, Double-Blinded, Placebo-controlled, Dose-Escalation Study to Assess the Safety, Tolerability and Immunogenicity of Live Attenuated, Oral Shigella WRSS1 Vaccine in Bangladeshi Toddlers (12 to 24 months old)

Short Title: (maximum **100** characters including space) *Shigella* WRSS1 Vaccine trial in Bangladesh

Key Words:* WRSS1, Shigella sonnei Vaccine, Toddlers, Bangladesh, Safety, Immunogenicity

Name of the Research Division Hosting the Protocol:*

☐ Health Systems and Population Studies Division (HSPSD)
☐ Nutrition and Clinical Services Division (NCSD)
☒ Infectious Diseases Division (IDD)
☐ Maternal and Child Health Division (MCHD)
☐ Laboratory Sciences and Services Division (LSSD)
☐ Other (specify)

Has the Protocol been Derived from an Activity:* ☐ No ☐ Yes (please provide following information):

Activity No. :
Activity Title:
PI:
Grant No.: Budget Code: Start Date: End Date:

icddr,b Strategic Priority/ Initiative (SP 2015-8):* (check all that apply)

☐ Reducing maternal and neonatal mortality
☒ Controlling enteric and respiratory infections
☐ Preventing and treating maternal and childhood malnutrition
☐ Detecting and controlling emerging and re-emerging infections
☐ Achieving universal health coverage
☐ Examining the health consequences of climate change
☐ Preventing and treating non-communicable diseases

Research Phase (4 Ds):* (check all that apply)

☐ Discovery
☒ Development
☐ Delivery
☐ Evaluation of Delivery

Anticipated Impact of Research:* (check all that apply)

☒ Knowledge Production
☐ Capacity Building
☐ Informing Policy
☐ Health and Health Sector Benefits
☐ Economic Benefits

Which of the Sustainable Development Goal This Protocol Relates to?:*
(Please visit: http://www.icddrb.net.bd/jahia/Jahia/..... for selecting SDG Code(s)

☐ Yes (please select SDGs)
☐ No

Does this Protocol Use the Gender Framework:*
(Please visit: http://www.icddrb.net.bd/jahia/Jahia/pid/684 for Gender Alanysis Tool with instructions)

☒ Yes (please complete Gender Analysis Tool)
☐ No

If 'no' is the response, its reason(s) in brief:

Will this Research Specifically Benefit the Disadvantaged (economically, socially and/or otherwise)**:** ☒ Yes ☐ No

Does this Protocol use Behaviour Change Communication: ☐ Yes ☒ No

<table>
<tr><td>Principal Investigator (Should be icddr,b staff):* Sex ☒ Female ☐ Male
Dr. Rubhana Raqib

(Position, phone no, extension no, cell, and email address):
Senior Scientist, Tel: 880-2-9827001-10 Ext:2404, Off: 880-2-9886734, Mobile:880-2-01713-040942; email: rubhana@icddrb.orgDo you have ethics certification? ☐ No ☒ Yes (please attach in your CV below)

Do you have RBM training certification? ☐ No ☒ Yes (please attach the certificate with CV below)</td><td>Primary Scientific Division of the PI
Infectious Diseases Division (IDD)</td></tr>
<tr><td>Co-Principal Investigator(s) Internal: Sex ☐ Female ☒ Male
Dr. K Zaman

(Position, phone no, extension no, cell, and email address):
Senior Scientist, Tel: 880-2-9827001-10 Ext:3806, Mobile:880-2-01713047100; email: kzaman@icddrb.org

Signature or written consent of Co-PI: _____
(electronic signature or email or any sort of written consent)
[if more than one, please copy and paste this row for additional Co-PIs]

Do you have ethics certification? ☐ No ☒ Yes (please attach in your CV below)

Do you have RBM training certification? ☐ No ☒ Yes (please attach the certificate with CV below)</td><td>Primary Scientific Division/ Programme of the Co-PI
Infectious Diseases Division (IDD)

_____
Approval of the Respective Senior Director/ Programme Head

(Signature)</td></tr>
<tr><td colspan="2">Co-Principal Investigator(s) - External: Sex ☐ Female ☐ Male

Address (provide full official address, including land phone no(s), extension no. (if any), cell phone number, and email address).

Signature or written consent of Co-PI: _____
(electronic signature or email or any sort of written consent)
[if more than one, please copy and paste this row for additional Co-PIs]</td></tr>
<tr><td>Co-Investigator(s) - Internal: Sex ☐ Female ☒ Male
Dr. Nur Haque Alam

(Position, phone no, extension no, cell, and email address):
Senior Scientist, Tel: 880-2-9827001-10 Ext:2346, Mobile:880-2-01713093851; email: nhalam@icddrb.org

Signature or written consent of Co-I: _____
(electronic signature or email or any sort of written consent)
[if more than one, please copy and paste this row for additional Co-Is

Do you have ethics certification? ☐ No ☒ Yes (please attach in your CV below)

Do you have RBM training certification? ☐ No ☒ Yes (please attach the certificate with CV below)</td><td>Primary Scientific Division of the Co-I
Nutrition and Clinical Services Division

_____
Approval of the Respective Senior Director/ Programme Head

(Signature)</td></tr>
</table>

Co-Investigator(s) - Internal: Sex ☒ Female ☐ Male **Dr. Firdausi Qadri** (Position, phone no, extension no, cell, and email address **):** Senior Scientist, Tel: 880-2-9827001-10 Ext: 2413, Mobile:880-01711595367; email: fqadri@icddrb.org Signature or written consent of Co-I: _____ (electronic signature or email or any sort of written consent) [if more than one, please copy and paste this row for additional Co-Is Do you have ethics certification? ☐ No ☒ Yes (please attach in your CV below) Do you have RBM training certification? ☐ No ☒ Yes (please attach the certificate with CV below)	Primary Scientific Division of the Co-I Infectious Diseases Division (IDD) _____ Approval of the Respective Senior Director/ Programme Head (Signature)
Co-Investigator(s) - Internal: Sex ☐ Female ☒ Male **Dr. Md. Jobayer Chisti** (Position, phone no, extension no, cell, and email address **):** Scientist, Tel: 880-2-9827001-10 Ext: 2317, Mobile: 880-2-01749292703; email: chisti@icddrb.org Signature or written consent of Co-I: _____ (electronic signature or email or any sort of written consent) [if more than one, please copy and paste this row for additional Co-Is Do you have ethics certification? ☐ No ☒ Yes (please attach in your CV below) Do you have RBM training certification? ☐ No ☒ Yes (please attach the certificate with CV below)	Primary Scientific Division of the Co-I Nutrition and Clinical Services Division _____ Approval of the Respective Senior Director/ Programme Head (Signature)
Co-Investigator(s) - Internal: Sex ☒ Female ☐ Male **Dr. Dilruba Ahmed** (Position, phone no, extension no, cell, and email address **):** Head, Clinical Microbiology, Phone: +880-2-9827001-10; Ext: 2446 Mobile: +880-01713093858; email: dahmed@icddrb.org Signature or written consent of Co-I: _____ (electronic signature or email or any sort of written consent) [if more than one, please copy and paste this row for additional Co-Is Do you have ethics certification? ☐ No ☒ Yes (please attach in your CV below) Do you have RBM training certification? ☐ No ☒ Yes (please attach the certificate with CV below)	Primary Scientific Division of the Co-I Laboratory Sciences and Services Division _____ Approval of the Respective Senior Director/ Programme Head (Signature)
Laboratory Investigator(s) – External: Sex ☒ Female ☐ Male **Address** (provide full official address, including land phone no(s), extension no. (if any), cell phone number, and email address): **Dr. Malabi Venkatesan** Bacterial Diseases Branch, Walter Reed Army Institute of Research, Silver Spring, MD 20910, tel nos. 301-319-9764 (w), 301-605-4869 (blackberry), email: Malabi.m.venkatesan.civ@mail.mil, Malabi.venkatesan@us.army.mil Signature or written consent of Co-I: _____ (electronic signature or email or any sort of written consent) [if more than one, please copy and paste this row for additional Co-Is]	

Co-Investigator(s) – External: Sex ☐ Female ☒ Male
Address (provide full official address, including land phone no(s), extension no. (if any), cell phone number, and email address):
Dr. Abdullah Baqui, Professor, Department of International Health, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Room E-8138, 615 N Wolfe St., Baltimore, MD 21205, USA; Phone: 410-955-3850; Email: abaqui@jhu.edu

Signature or written consent of Co-I: _____
(electronic signature or email or any sort of written consent)
[if more than one, please copy and paste this row for additional Co-Is]

Student Investigator(s) - Internal: Sex ☐ Female ☐ Male (Position, phone no, extension no, cell, and email address **):** Signature or written consent of Student Investitor: _____ (electronic signature or email or any sort of written consent) Have ethics certificate? ☐ No ☐ Yes (If Yes, please attach to your CV below)	Students Affiliation _____ Approval of the Respective Senior Director/ Programme Head (Signature)

Student Investigator(s) - External: Sex ☐ Female ☐ Male

Address (provide full official address, including land phone no(s), extension no. (if any), cell phone number, and email address):

Signature or written consent of Student Investitor: _____
(electronic signature or email or any sort of written consent)

Collaborating Institute(s): Please provide full official address

Institution # 1

Country	USA
Contact person	Tushar Tewari, MD
Department (including Division, Centre, Unit)	Senior Medical Officer, PATH
Institution (with official address)	PATH Vaccine Solutions (PVS) A-9 Qutab Institutional Area USO Road New Delhi 110067, India
Directorate (in case of GoB i.e. DGHS)	
Ministry (in case of GoB)	

Institution # 2

Country	USA
Contact person	Dr Malabi M. Venkatesan
Department (including Division, Centre, Unit)	Bacterial Diseases Branch
Institution (with official address)	Walter Reed Army Institute of Research, Silver Spring, MD, United States
Directorate (in case of GoB□i.e. DGHS)	
Ministry (in case of GoB)	

Institution # 3

Country	USA
Contact person	Professor Abdullah Baqui
Department (including Division, Centre, Unit)	Department of International Health
Institution (with official address)	Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Room E-8138, 615 N Wolfe St., Baltimore, MD 21205
Directorate (in case of GoB□i.e. DGHS)	
Ministry (in case of GoB)	

Note: If less than or more than three collaborating institutions, please delete or insert blocks as needed.

Contribution by the Members of the Scientific Team:

Members' Name	Contribution								
	Research idea/ concept	Study design	Protocol writing	Respond to external reviewers' comments	Defending at IRB	Developing data collection Tool(s)	Data Collection	Data analysis/ interpretation of results	Manuscript writing
Dr. Rubhana Raqib	☒	☒	☒	☒	☒	☒	☒	☒	☒
Dr. K Zaman	☐	☒	☒	☒	☒	☒	☐	☒	☒
Dr. A. Baqui	☒	☒	☒	☒	☐	☐	☐	☒	☒
Dr. Dilruba Ahmed	☐	☐	☒	☒	☒	☒	☒	☒	☒
Dr. Firdausi Qadri	☐	☐	☒	☒	☒	☒	☐	☒	☒
Dr Malabi Venkatesan	☒	☒	☒	☒	☐	☐	☐	☒	☒
Dr. Nur Haque Alam	☐	☐	☒	☒	☒	☒	☐	☒	☒
Dr Jobayer Chisti	☐	☐	☒	☒	☒	☒	☐	☒	☒
	☐	☐	☐	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐	☐	☐	☐
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	☐	☐	☐	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐	☐	☐	☐

Study Population: Sex, Age, Special Group and Ethnicity

Research Subject:
- ☒ Human
- ☐ Animal
- ☐ Microorganism
- ☐ Other (specify): _____

Sex:
- ☒ Male
- ☒ Female
- ☐ Transgender

Age:
- ☒ 0 – 4 years
- ☐ 5 – 10 years
- ☐ 11 – 17 years
- ☐ 18 – 64 years
- ☐ 65 +

Special Group:
- ☐ Pregnant Women
- ☐ Fetuses
- ☐ Prisoners
- ☐ Destitutes
- ☐ Service Providers
- ☐ Cognitively Impaired
- ☐ CSW
- ☐ Expatriates
- ☐ Immigrants
- ☐ Refugee
- ☐ Others (specify): _____

Ethnicity:
- ☒ No ethnic selection (Bangladeshi)
- ☐ Bangalee
- ☐ Tribal group
- ☐ Other (specify): _____

NOTE: It is icddr.b's policy to include men, women, children and transgender in its research projects involving participation of humans, unless there is strong justification(s) for their exclusion.

Consent Process: (Check all that apply**)**
- ☒ Written
- ☐ Oral
- ☐ Audio
- ☐ Video
- ☐ None

Language:
- ☒ Bangla
- ☐ English
- ☐ Other (specify: _____

Project/Study Site: (Check all that apply**)**
- ☐ Chakaria
- ☐ Bandarban
- ☐ Dhaka Hospital
- ☐ Kamalapur Field Site/HDSS
- ☒ Mirpur (Dhaka)
- ☐ Matlab DSS Area
- ☐ Matlab non-DSS Area
- ☐ Matlab Hospital
- ☐ Mirzapur
- ☐ Bianibazar (Sylhet)
- ☐ Kanaighat (Sylhet)
- ☐ Jakigonj (Sylhet)
- ☐ Other community in Dhaka
  Name: _____
- ☒ Other sites in Bangladesh
  Name: ___CTU in icddr,b _____
- ☐ Multi-national Study
  Name of the country_____

Project/Study Type: (Check all that apply)
- ☐ Case Control Study
- ☒ Clinical Trial (Hospital/Clinic/Field)*
- ☐ Community-based Trial/Intervention
- ☐ Cross Sectional Survey
- ☐ Family Follow-up Study
- ☐ Longitudinal Study (cohort or follow-up)
- ☐ Meta-analysis
- ☐ Programme Evaluation
- ☐ Programme (Umbrella Project)
- ☐ Prophylactic Trial
- ☐ Record Review
- ☐ Secondary Data Analysis
  Protocol No. of Data Source: _____
- ☐ Surveillance/Monitoring
- ☐ Systematic Review
- ☒ Other (specify): ____Vaccine Trial_____

***Note**: International Committee of Medical Journal Editors (ICMJE) defines Clinical Trial as "*Any research project that prospectively assigns human participants to intervention and comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome*".

PI of the RRC- and ERC-approved Clinical Trials should provide necessary information to IRB Secretariat (Research Administration) for registration and uploading into relevant websites (usually at the https://register.clinicaltrials.gov/). They should also provide relevant information to the IRB Secretariat in the event of amendment/modification after their approval by RRC and ERC.

Biological Specimen:	
a) Will the biological specimen be stored for future use?	☐ Yes ☒ No ☐ Not applicable
b) If the response is 'yes', how long the specimens will be preserved?	____N/A_____ years
c) What types of tests will be carried out with the preserved specimens?	N/A
d) Will the consent be obtained from the study participants for use of the preserved specimen for other initiative(s) unrelated to this study, without their re-consent?	☐ Yes ☐ No ☒ Not applicable
e) Will the specimens be shipped to other country/ countries? If yes, name of institution(s) and country/ countries?	☐ Yes ☐ No ☒ Not applicable
f) If shipped to another country, will the surplus/unused specimen be returned to icddr,b? If the response is 'no', then the surplus/unused specimen must be destroyed.	☐ Yes ☐ No ☒ Not applicable
g) Who will be the custodian of the specimen at icddr,b?	PI of the study
h) Who will be the custodian of the specimen when shipped outside Bangladesh?	N/A
i) Who will be the owner(s) of the specimens?	icddr,b
j) Has a MoU been signed with regards to collection, storage, use and ownership of specimen? If the response is 'yes', please attach a copy of the MoU.. If the response is 'no', appropriate justification should be provided for not signing a MoU.	☐ Yes ☐ No ☐ Not applicable An MoU is not required as specimens will not be shipped outside icddr,b.

Proposed Sample Size:
Sub-group (Name of subgroup e.g. Men, Women) and Number

Name	Number
(1) Healthy Male and Female Children (12-24 mo)	64
Total sample size	64

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

☐ Human exposure to radioactive agents?
☐ Foetal tissue or abortus?
☐ Investigational new device?
Specify: _____
☐ Existing data available from Co-investigator?
☐ Human exposure to infectious agents?
☒ Investigational new drug?
☐ Existing data available via public archives/sources?
☐ Pathological or diagnostic clinical specimen only?
☐ Observation of public behaviour?
☐ New treatment regime?

Will the information be recorded in such a manner that study participants can be identified from the information directly or through identifiers linked to the study participants?	Yes ☒	No ☐
Does the research deal with sensitive aspects of the study participants' sexual behaviour, alcohol use or illegal conduct such as drug use?	Yes ☐	No ☒

Could information on study participants, if available to people outside of the research team:

a) Place them at risk of criminal or civil liability?	Yes ☐	No ☒
b) Damage their financial standing, reputation or employability, or social rejection, or lead to stigma, divorce etc.?	Yes ☐	No ☒

Do you consider this research: (check one)

☒ Greater than minimal risk	☐ No more than minimal risk	☐ Only part of the diagnostic test

Note: Minimal Risk: The probability and the magnitude of the anticipated harm or discomfort to participants is not greater than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or tests, e.g. the risk of drawing a small amount of blood from a healthy individual for research purposes is no greater than when the same is performed for routine management of patients.

Risk Group of Infectious Agent and Use of Recombinant DNA	
a) Will specimens containing infectious agent be collected?	☒ Yes ☐ No ☐ Not applicable
b) Will the study involve amplification by culture of infectious agents?	☒ Yes ☐ No ☐ Not applicable
c) If response to questions (a) and/or (b) is 'yes', to which Risk Group (RG) does the agent(s) belong? (Please visit http://www.icddrb.net.bd/jahia/Jahia/pid/684 to review list of microorganism by Risk Group)	☐ RG1 ☒ RG2 ☐ RG3 ☐ RG4
d) Does the study involve experiments with recombinant DNA?	☐ Yes ☒ No ☐ Not applicable

Does the study involve any biohazards materials/agents or microorganisms of risk group 2, 3, or 4 (GR2, GR-3 or GR4)?

☒ Yes ☐ No

[If the response is 'yes'] I, (print name of the PI) affirm that we will use the standard icddr,b laboratory procedures for biosafety of the hazardous materials/agents or microorganisms in the conduction of the study.

_____ _____

Signature of the Principal Investigator **Date**

Dissemination Plan: [please explicitly describe the plans for dissemination, including how the research findings would be shared with stakeholders, identifying them if known, and the mechanism to be used; anticipated type of publication (working papers, internal (institutional) publication, international publications, international conferences/seminars/workshops/ agencies. [Check all that are applicable]

Dissemination type	Response		Description (if the response is a yes)
Seminar for icddr,b scientists/ staff	☐ No	☒ Yes	Will be shared with icddr,b scientists and staff through Centres' Scientific forum
Internal publication	☒ No	☐ Yes	
Working paper	☒ No	☐ Yes	
Sharing with GoB (e.g. DGHS/ Ministry, others)	☐ No	☒ Yes	Will be shared with government officials in meetings/workshops
Sharing with national NGOs	☒ No	☐ Yes	
Presentation at national workshop/ seminar	☐ No	☒ Yes	Data will be shared in meetings in form of presentation
Presentation at international workshop/ conference	☐ No	☒ Yes	In the event of a presentation at an international workshop, icddr,b, WRAIR and PVS will collaborate accordingly on all presentation materials including PowerPoint slides and/or submitted abstract
Peer-reviewed publication	☐ No	☒ Yes	Potential publications will be written collaboratively between PVS, WRAIR and icddr,b; PVS requires draft manuscripts be reviewed and approved internally prior to journal submission.
Sharing with international agencies	☒ No	☐ Yes	
Sharing with donors	☐ No	☒ Yes	icddr,b and PVS will disseminate generated data as appropriate to donors, including the BMGF
Policy brief	☒ No	☐ Yes	
Other			
Other			

Funding:

Is the protocol fully funded?	☒ Yes	☐ No
If the answer is yes, please provide sponsor(s)'s name	1. PATH Vaccine Solutions (PVS) 455 Massachusetts Avenue NW, Suite 1000 Washington, DC 20001	
	2.	
Is the protocol partially funded?	☐ Yes	☒ No
If the answer is yes, please provide sponsor(s)'s name	1.	
	2.	

If fund has not been identified:

Is the proposal being submitted for funding?	☐ Yes	☐ No
If yes, name of the funding agency	1.	
	2.	

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

☒ No ☐ Yes (please submit a written statement of disclosure to the Executive Director, icddr,b)

Proposed Budget:

Dates of Proposed Period of Support

(Day, Month, Year - DD/MM/YY)

Beginning Date : January 2017

End Date : 1 year from start

Cost Required for the Budget Period ($)

Years	Direct Cost	Indirect Cost	Total Cost
Year-1	750,135	109,970	860,105
Year-2			0
Year-3			0
Year-4			0
Year-5			0
Total	750,135	109,970	860,105

Certification by the Principal Investigator:

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept the responsibility for the scientific conduct of the project and to provide the required progress reports including updating protocol information in the NAVISION if a grant is awarded as a result of this application.

I also certify that I have read icddr,b Data Policies and understand the PIs' responsibilities related to archival and sharing of research data, and will remain fully compliant to the Policies. (Note: The Data Policies can be found here: http://www.icddrb.org/who-we-are/data-policies)

_____ _____

Signature of PI **Date**

Approval of the Project by the Division Director of the Applicant:

The above-mentioned project has been discussed and reviewed at the Division level.

_____

Name of the Division Director Signature Date of Approval

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☒ Check here if appendix is included

TABLE OF FIGURES AND TABLES

Project Summary

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

Principal Investigator: Rubhana Raqib, PhD
Co-Principal Investigator (Internal): K. Zaman, MD

Research Protocol Title: A Phase 1 Randomized, Double-Blinded, Placebo-controlled, Dose-Escalation Study to Assess the Safety, Tolerability and Immunogenicity of Live Attenuated, Oral Shigella WRSS1 Vaccine in Bangladeshi Toddlers (12 to 24 months old)

Proposed start date: January 2017 Estimated end date: 12 months from start

Background (brief):

a. **Burden:** Shigellosis remains a major public health problem and a major cause of morbidity in children under 5 years old in impoverished Asian populations. In a large multicenter study of *Shigella* diarrhoea in six Asian countries, *Shigella* was isolated from 5% of all diarrhoea episodes detected. *S. flexneri* was the most frequently isolated species followed by *S. sonnei* and *S. boydii*. *S. sonnei* is a significant cause of diarrhoeal infection in Bangladesh and is gradually increasing in prevalence with economic improvements. In a recent analysis of diarrheal bacterial isolates obtained over 4 years (Jan 2005 to Dec 2008) from patients admitted at the International Centre for Diarrheal Diseases, Bangladesh (icddr,b) in Dhaka, 20% were *Shigella* spp [1]. *Shigella* isolation rates were second only to *V. cholera,* which accounted for ~40% of the isolates. Of the *Shigella* isolates, *S. flexneri* and *S. sonnei* made up 59% and 11.2%, respectively [1]. The Dhaka Hospital surveillance data shows that *S. sonnei* is 2nd to *S. flexneri* cases and have been steadily increasing over the years (from 4-9% in the period 1980-2008 to about 27% in 2011-2012). Among children under the age of 5, the prevalence also increased from 11% in 2008 to 40% in 2011. In the Mirpur Treatment Centre, among children <5, the rate of *S. sonnei* (53%) was much higher compared to *S. flexneri* (18%) in 2011. In the GEMS study conducted in Mirzapur by Dr ASG Faruque, *S. sonnei* isolation rate was as high (25%) as *S. flexneri* (21%) in children <5 year. More recently, studies on stool specimens from GEMS and MAL-ED studies using molecular assays showed increased burdens for *Shigella spp* then shown by conventional methods among under 5 year children.
b. **Knowledge gap:** Testing the potential of live *Shigella* vaccines in populations at need is a critical step to understanding their safety, attenuation level, and ability to generate a protective immune response.
c. **Relevance:** *Shigella* appears to be more ubiquitous in impoverished Asian populations than previously thought; various new serotypes and antibiotic-resistant strains have emerged. Nearly all shigellae are resistant to nalidixic acid and cotrimoxazole. In recent studies at icddr,b, resistance to ciprofloxacin in *Shigella* isolates increased from 1% in 2005 to 34% in 2008. Intra-familial transmission of *Shigella* carries a high risk for diarrhoea and persistence in the community. Immunization with an efficacious *Shigella* vaccine will prevent shigellosis, substantially reduce overall diarrhoea burden in the community and block the spread of multidrug-resistant *Shigella* strains. Cross-protective *Shigella* vaccines will be needed to prevent shigellosis in Asia.

WRSS1 Vaccine: A live attenuated *S. sonnei* WRSS1 vaccine was constructed from parent strain Moseley by making a 212-bp deletion in *virG* (also known as *icsA*). *S. sonnei* WRSS1, like its parent strain Moseley, is invasive in the HeLa cell invasion assay. Unlike Moseley however, the vaccine is negative in the Sereny test and does not form plaques in tissue culture monolayers, indicating loss of ability to spread intracellularly and intercellularly. *S. sonnei* WRSS1 develops predominantly form I colonies (>90%) and agglutinates with *S. sonnei* O-specific antiserum, indicating retention and expression of the virulence plasmid. The WRSS1 is at the most advanced stage of development of the *Shigella* vaccines. In an earlier trial completed in 2010, WRSS1 was tested in a single, low, oral dose in Thai adults and was shown to be safe, immunogenic, and a promising vaccine candidate when given to healthy adults in a range of 10^3 to 10^4 CFU. In a follow-up study (PR#12054) carried out in Bangladesh, the WRSS1 vaccine was evaluated in healthy adults (18-45 years) and children (5-9 years). The new study in toddlers aims to confirm increased colonization rates and improved immunogenicity of WRSS1 by administering 3 doses at gradually increasing dose levels.

The study at icddr,b will evaluate up to four dose levels of vaccine to assess the potential for enhancement of immune responses when booster doses are given at one month intervals after the initial priming dose.

Hypothesis:
Primary Hypothesis: WRSS1 will be safe and tolerable in the toddlers.

Secondary Hypotheses:

- WRSS1 will generate a positive (4-fold rise) serum/plasma immune response detectable in one or more assays in at least 50% of vaccinees.
- WRSS1 will be able to replicate in the intestine

Objectives:
Primary Objectives:

1. To evaluate the safety and clinical tolerability of oral *Shigella sonnei* WRSS1 vaccine in Bangladeshi toddlers by monitoring the occurrence and severity of clinical signs and symptoms after administration of up to three vaccine doses.

Secondary Objectives:

1. To evaluate the immune response to WRSS1 antigens in blood and stool following ingestion of WRSS1 vaccine
2. To assess the ability to replicate and duration of fecal shedding of WRSS1 following ingestion

Methods: This is a single site, Phase 1, double-blind, randomized, placebo-controlled, dose-escalation study in toddlers (12-24 months old). The study will enroll approximately 64 toddlers (12-24 months old) to receive three doses of $3x10^3$, $3x10^4$, $3x10^5$ or $3x10^6$ CFU WRSS1 vaccine or placebo. In each cohort, the first dose and immediate safety evaluation will be conducted at the Clinical Trials Unit (CTU), an inpatient facility located on the icddr,b campus, where the volunteers will be admitted for observation for 24 hours post-vaccination. Second and third vaccinations for the applicable cohorts will take place on an outpatient basis in the CTU. Before enrolling participants in subsequent cohorts to receive a higher vaccine dose, the safety data from the previous cohort(s) (through Study Day 7) will be evaluated and reviewed by the Internal Protocol Safety Team (IPST) comprised of the study physician, a medical monitor from the CRO (The Emmes Corporation), the principal investigator, and the Medical Monitor from PVS. Evaluations of safety via clinic visits, physical exams, solicited symptoms and laboratory evaluations will be conducted. Shedding of vaccine strain will be assessed by culture and PCR methods in collected stool specimens. Immunogenicity will be studied by determining *S. sonnei* LPS and invaplex antigen-specific antibody titers and cytokine responses in lymphocyte supernatant (ALS), and serum/plasma. Serum bactericidal response against *Shigella* as a functional indicator of protection will be also studied. Antigen specific IgA response and cytokine responses in stool will be measured.

Outcome measures/variables:

1. Safety and tolerability of the WRSS1 vaccine
2. Immunogenicity of the WRSS1 vaccine
3. Intestinal replication of the WRSS1 vaccine

KEY ROLES

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LIST OF ABBREVIATIONS

AE	Adverse Event
CTU	Clinical Trials Unit, icddr,b
DDSS	Diarrhoeal Disease Surveillance Systems
DSMB	Data Safety Monitoring Committee
ERC	icddr,b Ethical Review Committee
FDA	US Food and Drug Administration
GEMS	Global Enteric Multicenter Study
HEENT	Head/Ears/Eyes/Nose/Throat
icddr,b	International Centre for Diarrhoeal Disease Research, Bangladesh
INT	Immunobiology, Nutrition and Toxicology
IPST	Internal Protocol Safety Team
IRB	Institutional Review Board
ISF	Investigational Study File
LDC	Less Developed Countries
mL	Milliliter
MM	Medical Monitor
ORS	Oral Rehydration solution
OTC	Over the Counter
PATH	Program for appropriate technology in health
PI	Principal Investigator
PVS	PATH Vaccine Solutions
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
WIRB	Western Institutional Review Board
WRAIR	Walter Reed Army Institute of Research

DESCRIPTION OF THE RESEARCH PROJECT

1.0 STUDY HYPOTHESIS

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

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

1.1 Primary Study Hypothesis

- Orally administered WRSS1 will be safe and tolerable in toddler recipients and will cause no major side effects in vaccinees.

1.2 Secondary Study Hypotheses

- WRSS1 will generate a positive (4-fold rise in serum/plasma) immune response detectable in one or more assays in at least 50% of vaccinees.
- WRSS1 will be able to replicate in the intestine as determined by fecal shedding.

2.0 OBJECTIVES

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

2.1 Primary Study Objective

To evaluate the safety and clinical tolerance of WRSS1 in Bangladeshi toddlers (12-24 months old) by monitoring the occurrence and severity of clinical signs and symptoms after administration of three vaccine doses.

Safety will be assessed by analyses of the following primary endpoints (events), where the unit of analysis in each case will be the proportion of participants with at least one event:

- any solicited reactogenicity
- any unsolicited AEs
- any SAEs
- unsolicited AEs and SAEs judged as having a reasonable possibility that the study product caused the event

2.2 Secondary Study Objectives

- To examine the immunogenicity of WRSS1.
- To assess the ability to replicate and duration of fecal shedding of WRSS1 following ingestion.

The secondary immunogenicity endpoints are chosen to further assess the mucosal and systemic immunogenicity. This will be done by assessing:

- Frequency and magnitude of antigen specific serum/plasma IgA, IgG and IgM antibody titers to *S. sonnei* LPS, and *S. sonnei* Invaplex antigens after administration of three vaccine doses
- Frequency and magnitude of antigen-specific fecal IgA antibody titer to *S. sonnei* LPS and *S. sonnei* Invaplex antigens after administration of three vaccine doses
- Frequency and magnitude of antigen (LPS and Invaplex)-specific IgG, IgA, and IgM antibody titers in lymphocyte supernatent (ALS) to *S. sonnei* LPS and Invaplex antigens

Finally, fecal shedding of vaccine will be measured by the frequency and duration of detectable fecal presence of WRSS1 by culture and PCR.

2.3 Exploratory Objectives

- To evaluate serum bactericidal activity and cytokine profile against WRSS1 in toddlers before and after vaccination.

3.0 BACKGROUND OF THE PROJECT INCLUDING PRELIMINARY OBSERVATIONS AND SCIENTIFIC RATIONALE

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

3.1 Clinical Significance and Pathogenicity of Shigella[1]

Shigellosis is a highly infectious acute diarrheal disease that is transmitted through direct fecal-oral contact or through contaminated food or water. Infection with *Shigella sp* results in a wide range of clinical symptoms from mild watery diarrhea to severe diarrhea with dehydration, general colonic invasion and generalized toxic inflammation. More than one quarter of these patients progress to overt dysentery manifested as multiple liquid stools often containing frank blood, with systemic symptoms such as fever and/or abdominal pain [2]. In the nineteenth century and early twentieth century, outbreaks of dysentery were often accompanied by >20% mortality rates from dehydration, shock, and/or hemolytic uremic syndrome [3]. A review of scientific literature published between 1966 and 1997 projected an annual incidence of over 160-million shigellosis cases worldwide with over 1 million deaths [4]. February 2009 estimates from the WHO estimate 90 million episodes of shigellosis cases worldwide and 108,000 deaths [5] although data from most of Africa and many parts of SE Asia were not available. Even though the fatality rate for shigellosis has decreased in recent decades, infections are still common in areas with substandard sanitation and continue to be significantly underestimated. The increasing spectrum of *Shigella* antimicrobial resistance mandates continued public health interventions and vaccine development efforts [2,6].

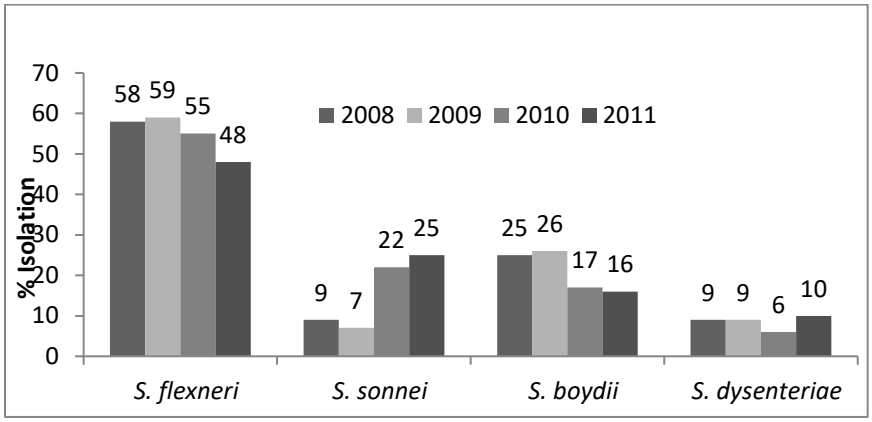
Shigella infections are widespread in less developed countries (LDCs). Studies in Pakistan [7] and Mozambique [8] identified *Shigella* in 4.8% and 6.7%, respectively, of cases of diarrhea compared to 0.4% in healthy controls. Similar estimates have been obtained in studies in 6 Asian countries, where *Shigella* was isolated from 5% of all diarrhea cases occurring in children under 60 months [2]. Using the more sensitive real-time PCR with *Shigella*-specific *ipaH* (the gene encoding invasion plasmid antigen H that is nearly exclusively derived from *Shigella* spp) primers detected *Shigella* spp in 33% of culture-negative stool specimens [2]. Another study in Vietnam using *ipaH* based PCR method also suggested that about 36% of all cases of watery diarrhea in LDCs may be attributable to shigellosis [9], and a more recent similar study in China has produced estimates of about 58% [10]. However, large population based data in Bangladesh comparing PCR method with the stool culture method to detect presense of *Shigella* is not available. Recent studies using molecular methods (PCR-Luminex strategy, multiplex real-time PCR panels, and customised TaqMan array card) revealed much higher infection status of children <5 years of age with Shigella and other enteric pathogens [11-13]. Ongoing multicenter studies of diarrheal disease burden, clinical manifestations, and etiology in sub-Saharan Africa will give an improved picture of the current impact of shigellosis in the most impoverished LDCs.

There are four major serogroups of *Shigella*, namely, *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. There is only one serotype of *S. sonnei*, while each of the other serogroups contain multiple serotypes and subtypes, and at least 54 serotypes or subtypes are currently recognized, while new serotypes continue to be identified. All estimates cited in this study are based on information collected from different sources and different time points, which includes individual studies carried out by icddr,b and the Diarrhoeal Disease Surveillance Systems (DDSS) at the Dhaka and Matlab Hospitals. These estimates may vary from one study to another. In addition, we have tried to gather *Shigella* prevalence data from Bangladesh, neighbouring countries and other regions of the world to emphasize on the importance of this study.

In a large multicenter study of shigellosis in six Asian countries during 2000-2004, *Shigella* was isolated from 5% of all diarrhoea episodes detected, among which *S. flexneri* was the most frequently isolated in 5 countries (68%) followed by *S. boydii* and *S. sonnei*. In Thailand, *S. sonnei* was most frequently detected (about 85 %) [2]. The study found that shigellosis occurs in these 6 Asian sites at a rate 100 times higher than in industrialized countries. Rates of shigellosis were higher in Bangladesh sites followed in declining order by China, Pakistan, Indonesia, Vietnam and Thailand sites.

Over the last 35 years, *S. flexneri* has been the dominant species (61%) followed by *S. boydii* (17%), *S. sonnei* (10%), *S. dysenteriae* (8%), and *Shigella*-like organism (3%) in Bangladesh except during 1993-95 when *S. dysenteriae* was dominant .

Figure 1: Yearly overall isolation of *Shigella* by Sero-Groups in Dhaka Hospital, 2008-2011

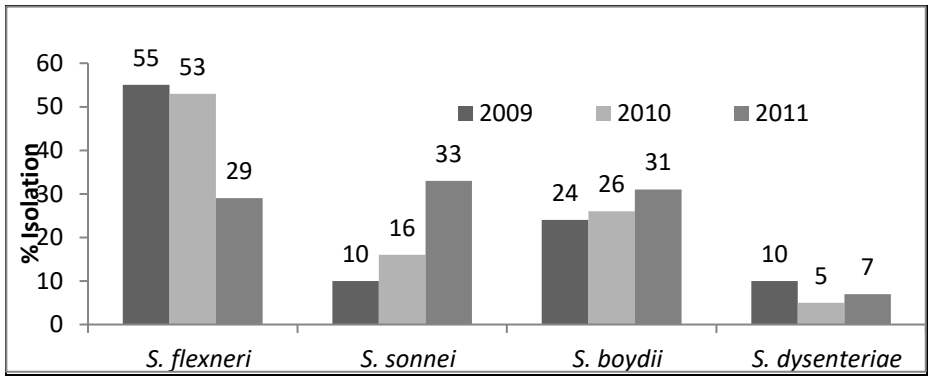


However, in the last decade, the prevalence of *S. sonnei* increased from 8% in 2000 to 27% in 2010 while the prevalence of *S. dysenteriae* drastically reduced to 3% in 2010. Interestingly, since 2004, *S. dysenteriae* type 1 is gradually on the decline and was not found at all in Bangladesh. *S. sonnei* is now a significant cause of diarrhoeal infection in Bangladesh constituting about 27% of all Shigella cases and is second to *S. flexneri* (49%) infections [1,14].

According to the Dhaka Hospital surveillance report of 2011, prevalence of shigellosis is 3% in Dhaka; among the total Shigella cases *S. sonnei* is 2nd to *S. flexneri* cases and is quite high (Figure 1). *S. sonnei* cases have been steadily increasing over the years (from 4-9% in the period 1980-2008 to about 27% in 2011-2012) [15]. Among children under 5 years of age, the prevalence rate has also increased from 11% to 40% in 2011.

Data from study sites in Mirpur (situated about 10.9 km from icddr,b), Matlab (104 km from Dhaka) and Mirzapur (72 km from Dhaka) have been provided below.

Figure 2: Yearly overall isolation of *Shigella* by Sero-Groups in Mirpur Treatment Centre, 2009[14]



In the Mirpur Treatment Centre, the percentage of isolation of *S. sonnei* has been steadily increasing and has superseded *S. flexneri* isolation in 2011 (Figure 2). Among children <5year, the isolation rate was substantially higher for *S. sonnei* (53%) compared to *S. flexneri* (18%) in 2011.

The GEMS study conducted in Mirzapur by Dr ASG Faruque showed that the overall isolation of *Shigella* was 43%. In this study, mild diarrhea cases were excluded while moderate to severe diarrhea cases were enrolled. From 2008 to 2010, isolation of *S. sonnei* steadily increased from 19% in 2008 to 38.5% in 2010. It was second to *S. flexneri* cases which gradually declined from 72% to 57.7% from 2008 to 2010. Among children under 5 years of age, *S. sonnei* isolation rate was higher (25.2%) than *S. flexneri* (21.3%). However, even among moderate to severe diarrhea cases in the GEMS study, the isolation rate of *S. sonnei* was higher than *S. flexneri* in these children.

Another Diarrheal Disease Burden Study (2010-2011) was recently completed by Dr ASG Faruque in Mirzapur, which covered all diarrheal cases (mild, moderate, severe) including watery diarrhea and dysentery among all age groups. This study (Figure 5) also showed that the isolation of *S. sonnei* was quite high (25%) and was 2nd to *S. flexneri* isolation rates which was 53.7% [16]. Similarly, among children <5 years old *S.*

sonnei isolation was high (Figure 3) though interestingly *S. boydii* also showed a dramatic increase within 1 year from 3% to 28%.

Figure 3: Yearly isolation of *Shigella* by sero-groups among children < 5 yrs old, Mirzapur, 2010-2011

The prevalence of shigellosis is 5% in Matlab according to the Matlab Hospital surveillance data of 2011. It shows that the highest isolate was *S. flexneri* (73% to 81%) which remained steady for 4 consecutive years (2008 to 2011) while *S. sonnei* was the 2nd with a lower rate at 7-8%. The prevalance among children <5 years old was similar with slightly increased rates in children <5 (11-15%).

There are no recent surveillance data from field sites. However, data from Dhaka and Matlab Hospitals of icddr,b (Jan. 2013 – Sep. 2015) (Table 1) show that the prevalence of Shigella infection in Dhaka Hospital is 2.3% (total enrolled 3355) while in Matlab Hospital it is 8.6% (total enrolled 7116). Interestingly, the isolation of *S. Sonnei* among patients was highest in 12-24 month old children both in Dhaka and in Matlab.

Table 1A. *Shigella* isolation among different age groups

	Dhaka Hospital				Matlab Hospital			
Age group	*S. flex*	*S. boydii*	*S. sonnei*	*S. dys*	*S. flex*	*S. boydii*	*S. sonnei*	*S. dys*
0-11 m	9 (31.0)	5 (17.2)	14 (48.3)	1 (3.4)	18 (85.7)	2 (9.5)	1 (4.8)	0 (0.0)
12-24 m	16 (51.6)	1 (3.2)	14 (45.2)	0 (0.0)	26 (52.0)	3 (6.0)	20 (40.0)	1 (2.0)
24 – 59 m	15 (57.7)	1 (3.8)	10 (38.5)	0 (0.0)	51 (75.0)	2 (2.9)	15 (22.1)	0 (0.0)
5-9 yr	6 (50.0)	2 (16.7)	4 (33.3)	0 (0.0)	9 (90.0)	1 (10.0)	0 (0.0)	0 (0.0)
> 9 yr	45 (62.5)	11 (15.3)	12 (16.7)	4 (5.6)	90 (70.9)	10 (7.9)	22 (17.3)	5 (3.9)

Table 1B. Isolation of *S. sonnei* among age groups

Age group	Dhaka Hospital n (%)	Matlab Hospital n (%)
0-11 m	14 (25.9)	1 (1.7)
12-24 m	14 (25.9)	20 (34.5)
24 – 59 m	10 (18.5)	15 (25.9)
5-9 yr	4 (7.4)	0 (0.0)
> 9 yr	12 (22.2)	22 (37.9)

The publication by Kathryn Holt in Nature Genetics [17] indicates that antibiotic treatment and better sanitation alone will not be sufficient for controlling shigellosis because antibiotics are driving the evolution of *Shigella* strains and are not a long-term solution for the elimination of this global health problem. These data highlight the socio-economic development of Bangladesh that may have an impact on the epidemiology of *Shigella* spp and should guide future vaccine development strategies. While the Holt paper describes the evolution of *S. sonnei,* the hypothesis should apply to all *Shigella*. The conclusion is that vaccine development will be vital for containing the disease. Since all *S. sonnei* have the identical O-antigen, protection with a single vaccine is achievable. In contrast the control of *S. flexneri* may require coverage of 2 or more *S. flexneri* serotypes, such as 2a, 3a and 6.

3.2 WRSS1 as a prototype live attenuated S. sonnei vaccine for descending age study

Parents and public health authorities recognize dysentery as a threat to child health and development in LDCs. While shigellosis is common at any age in developing countries, it is in infants and young children where the consequences of infection are most severe. Early childhood diarrheas, such as shigellosis, are associated with substandard growth and physical fitness [18] and with impaired cognitive function[19] during the first decade of life. A vaccine against shigellosis would be in demand for childhood immunization programs in many LDCs [20].

Most experts agree that the optimal *Shigella* vaccine will provide protection against multiple *Shigella* serotypes. The likely candidates for a multivalent vaccine include *S. dysenteriae* type 1, *S. sonnei* and several *S. flexneri* serotypes. *S. sonnei* is considered a critical component because *S. sonnei* is the main serogroup found in industrialized countries [2,3]. *S. sonnei*, although only responsible for about 10% of episodes, remains an important cause of morbidity and mortality in less developed countries. Recent studies at icddr,b suggest a rapidly increasing trend for *S. sonnei* infections in Bangladesh (icddr,b Surveillance reports; unpublished data by Dr ASG Faruque; see section 3.1).

To accelerate the development of safe, effective, and affordable vaccines against *Shigella*, PATH Vaccines Solutions (PVS) is working with private- and public-sector partners throughout the world. PVS is pursuing a wide range of promising vaccine approaches and related research, in an effort to make *Shigella* vaccines available to children who live in endemic countries as quickly as possible. Live attenuated *Shigella* vaccines offer a promising option based on earlier field studies of streptomycin dependent strains and other vaccine candidates [21]. Since that time, advances in molecular biology techniques made it possible to create attenuated vaccines by the introduction of well-defined, stable deletions on the *Shigella* virulence plasmid. WRSS1, the Δ*virG* live attenuated *S. sonnei* vaccine developed by scientists at WRAIR [22], has proven so far to be a promising prototype candidate based on its history of safety, immunogenicity and stability. WRSS1 is intended to be an initial component of a trivalent *Shigella* vaccine, with similarly attenuated *S. flexneri* 2a and *S. flexneri* 3a vaccine candidates to be added over the course of the next few years. Currently, PVS is partnering with WRAIR and the icddr,b to evaluate prototype WRSS1 in a descending age study in Bangladesh, and will serve as the study sponsor.

We recently completed protocol PR# 12054 (VAC 008) at icddr,b. The adult cohort was completed first and then children 5-9 years old. In the adult cohort 30 adults received 1 or 3 doses of WRSS1 and 9 received matched placebo. In the childrens cohort 48 children received 1 or 3 doses of WRSS1 and 16 received matched placebo. WRSS1 was safe at dose levels ranging from 10^3 to 10^6 CFU in both adults and children. Most relevant, all of the symptoms in children were rated as mild. None of the children excreted the vaccine strain, however, *S. sonnei* specific immune responses were observed in most children in the two high dose groups (10^5 and 10^6 CFU) (see section 3.4.2.4). Because the vaccine is both safe and immunogenic, it is justified to test the vaccine in the target population for the vaccine i.e. children 12-24 months old.

3.3 Development of a ΔvirG S. sonnei Vaccine strain WRSS1

Live attenuated *S. sonnei* WRSS1 vaccine was constructed from parent strain Moseley by making a 212-bp deletion in *virG* (also known as *icsA*) [22]. The parent strain was isolated from a laboratory worker at WRAIR accidentally infected in 1975 with a laboratory *S. sonnei* strain. The person experienced a classic case of shigellosis with fever, severe intestinal cramps, diarrhea and finally dysentery. The worker was hospitalized for 3 days and recovered with IV fluids and ampicillin treatment. The isolated strain was lyophilized and stored in ampoules as *S. sonnei* Moseley strain in the laboratory of Dr Sam Formal. The Moseley strain was used as the parent strain primarily because it exhibited a stable Form 1 phenotype (>90% smooth colonies) when incubated on TSA plates overnight at 37°C [22]. More recently genomic sequencing of WRSS1 has revealed a chromosomal deletion of length 82 kb that occurred spontaneously during the construction of the strain. This deletion does not affect bacterial invasion of cultured cells, intracellular replication or colonization and immunogenicity in a rhesus monkey model of infection [23,24]. At 10^4 CFU, WRSS1 was safe and highly immunogenic in US and Israeli volunteers (see below).

S. sonnei WRSS1, like its parent strain Moseley, is invasive as indicated by the HeLa cell invasion assay. Unlike Moseley however, the vaccine is negative for the Sereny test and does not form plaques in tissue culture monolayers, indicating loss of ability to spread intracellularly and intercellularly. *S. sonnei* WRSS1

develops predominantly form I colonies (>90%) and agglutinates with *S. sonnei* O-specific antiserum, indicating retention and expression of the virulence plasmid that carries the O-antigen encoding genes.

A WRSS1 research seed from Hartman and Venkatesan [22] was manufactured as a lyophilized vaccine under current Good Manufacturing Practice (cGMP) at the WRAIR Pilot BioProduction Facility in Silver Spring, Maryland. Frozen Master Cell Bank seeds and Production cell Bank (PCB) seeds were manufactured in April 1997, and a frozen PCB seed was used for bulk vaccine production in June 1997. The bulk vaccine was grown in a 40-liter fermenter using Tryptone-Yeast Extract broth, and the resulting bacterial pellet was suspended in a cryopreservative composed of 7.5% Dextran T10, 2% sucrose, and 1.5% glycerol in Dulbecco's phosphate buffered saline (PBS), for lyophilization. All bovine animal products used in WRSS1 manufacture were derived from herds of the United States, Australia, or New Zealand. The final cGMP product (lot 0451) was lyophilized in 377 multi-dose vials and frozen at -80°C on 27 June 1997. Lot 0451 has consistently yielded 1-2 x 10^{10} CFU per vial when reconstituted with 5 mL deionized water. The cultured colonies are approximately 90% form l, indicating that the vaccine expresses a complete (smooth) LPS O-polysaccharide and is invasive. Lot 0451 passed the General Safety Test (i.p. injection of guinea pigs and mice) and the product did not cause keratoconjunctivitis in the guinea pig cornea (Sereny test). The cGMP vaccine also elicited protection against keratoconjunctivitis after two ocular immunizations of guinea pigs [22]. This protection was equivalent to that induced by the WRSS1 research seed harvested from trypticase soy agar (TSA) plates.

Reversion of the mutant vaccine to wild type: The bacterial vaccine strain WRSS1 was constructed from parent wild type strain Moseley by making a 212-bp deletion in *virG*, a plasmid coded virulence gene. A 212 bp gene deletion in bacteria is usually considered irreversible since the only way that it can revert is to recombine with a wild-type *virG* gene carried on the virulence plasmid. The virulence plasmid in *Shigella* is non-conjugative [25,26], meaning it cannot transfer itself from one bacteria to another without the help of a conjugative plasmid or helper plasmid. So, natural reversion of the *virG* deletion in WRSS1 cannot happen.

3.4 Previous Vaccine studies

3.4.1 Preclinical studies with *Shigella sonnei* live attenuated vaccine (WRSS1)

For efficacy studies in guinea pigs, ocular immunizations with 3 x 10^8 to 4 x 10^8 CFU of *S. sonnei* WRSS1 were performed on days 0 and 14, followed by challenge with 4 x 10^8 CFU of virulent strain *S. sonnei* 53G on Day 42 [22]. The *S. sonnei* 53G strain was isolated in 1954 from a 5 year old patient hospitalized with diarrhea in Tokyo, Japan and maintained at the CVD, Univ of MD. A Master Cell bank (Lot# 0593) was manufactured from a seed stock from the CVD at the WRAIR Pilot Bioproduction Facility. Glycerol stocks of 53G made from lot# 0593 were used for these animal studies. Guinea pigs immunized with WRSS1 and challenged with 53G were rated over a 5 day period as to time of development and severity of disease. Percentage of protection was defined as follows: full, percentage of eyes with a rating of 0; partial, percentage of eyes with a rating of 1. In animals immunized with *S. sonnei* WRSS1 grown on solid medium overnight, 11/16 eyes showed no signs of disease (69% complete protection) while 5 eyes showed mild conjunctivitis (31% partial protection). When reconstituted WRSS1 vaccine Lot # 0451 was used as the immunizing agent, 10/16 eyes did not develop disease (63% complete protection) while 4 eyes developed mild disease (25% partial protection). No significant difference was found in the levels of protection conferred by the two formulations. Immunogenicity of the vaccine was measured in guinea pigs by determining levels of serum immunoglobulin G (IgG) and IgA specific for *S. sonnei* O antigen. The geometric mean titers demonstrated that both formulations were immunogenic and produced comparable serum IgG and IgA titers against the O antigen [22].

3.4.2 Previous human use of WRSS1

3.4.2.1 Phase 1 Inpatient Study (Protocol No. 98-035)

Two double-blinded, placebo controlled trials were conducted with lot# 0451 under IND 8266 (Protocol No. 98-035) in which 27 healthy adult participants, aged 18-45, received WRSS1 at the Center for Vaccine Development (CVD), University of Maryland, Baltimore, MD. In the first trial, three vaccine inocula (3 X 10^3, 3 X 10^4, and 3 X 10^5 CFU) were evaluated. In the second trial (conducted under an amendment of Protocol 98-035) additional participants received 3 X 10^5 CFU or 3 X 10^6 CFU [27].

WRSS1 was well tolerated by the 27 participants who ingested doses ranging from approximately 3.8 X 10^3 to 3.6 X 10^6 CFU in that the only presumptive reactions characterized as "severe" were headaches reported by two vaccinees. Four participants, (one in the 3-log group and three in the 5-log group) evidenced transient fever of 37.8 C (100.0^{0}F) or greater). Although six participants (22%) developed objective criteria of illness (fever and diarrhea), these illnesses were not debilitating, were transient, and did not occur in a clear dose-related fashion. No volunteer had dysentery or fever (≥ 38.9°C (102°F)) or clinically significant laboratory abnormalities.

One adverse event that was considered probably related to vaccine administration involved arthralgia in one subject. This consisted of a complaint of arthralgia of the elbows and fingers on Day 1 post vaccination. The arthralgia was minor in severity and was accompanied by mild fever and loose stools. This event resolved by 24 hrs and did not require any treatment or action.

Table 2. Rate of Clinically Relevant Symptoms and Rate of Any Symptom Likely to be reported during an Inpatient Trial

Inoculum (CFU)	No. of participants	Loose stool	Fever (°F)	Head-ache	Cramps	Clinical symptoms[a]	Any symptom
None	7	2	0	0	0	0	2 (29%)
3x 10^3	7	3	1 (101.2)	3	2	1 (14%)	4 (57%)
3 x 10^4	4	0	0	1	0	0	1 (25%)
3 x 10^5	10	2	2 (100.7, 100.8)	1	2	3 (30%)	3 (30%)
3 x 10^6	6	2	0	1	1	2 (33%)	4 (66%)

[a] Two or more loose stools totalling at least 200 ml in 48 hrs, a single loose stool totalling at least 300 ml, dysentery (gross blood in stool) or fever of at least 100°F.

Excretion of WRSS1 by vaccinees in the phase 1 trials was indicated by a clear evidence of intestinal vaccine replication as demonstrated by recovery of 10^4 to 10^7 CFU of WRSS1 per gram of stool in 22 of 27 (82%) vaccinees. Vaccine was still being excreted by 66% of vaccinees when antibiotic treatment began (study day 7). WRSS1 was not detected in the stools of placebo controls who shared living quarters and toilet facilities with vaccinees. A single dose of WRSS1 induced a vigorous immune response including IgA anti-LPS ASC responses at levels which have been correlated with protection against shigellosis in participant challenge studies. These promising results suggested that WRSS1 should undergo further clinical development.

3.4.2.2 Phase 1 Outpatient Study (Protocol No. A-11538)

A second phase 1 dose-escalating, open label trial "Outpatient Study of Safety, Immunogenicity and Transmissibility of a Live-Attenuated *ΔvirG(icsA) Shigella sonnei* Vaccine (strain WRSS1), Given as a Single Dose of 10^3, 10^4 or 10^5 Colony Forming Units to Healthy Adult Israeli Volunteers" was conducted under IND 10254 in Israel (Protocol No. A-11538) [28]. Three groups of 15 participants were enrolled as outpatient vaccinees. Each group received one dose of approximately 10^3, 10^4 or 10^5 CFU of the live attenuated *S. sonnei* vaccine WRSS1 at Simbec-Tel-Aviv Sourasky Medical Center Clinical Research Center [24]. There was no control group.

In the two lower doses tested (5 x 10^3 CFU and 2 x 10^4 CFU) the vaccine was well tolerated with the most common complaint being mild abdominal pain followed by mild nausea. At the lower doses only one of 30 volunteers reported moderate diarrhea while 5 volunteers had mild diarrheal symptoms. Among the vaccinees receiving 10^5 CFU there was a tendency to develop more fever, headache and anorexia.

Table 3. Rate of Clinically Relevant Symptoms after Vaccination Protocol No. A-11538

Symptom	No. of volunteers/total no. (%)	
	10^3 and 10^4 CFU[a]	**10^5 CFU**
Any soft stool	17/30 (57)	10/15 (67)
Any liquid stool	6/30 (20)	4/15 (27)
Moderate diarrhea (>two liquid stools/24 h)	1/30 (3)	4/15 (27)[b]
Fever (≥100°F)	0/30 (0)	2/15 (13)
Total with moderate or severe gastrointestinal signs or symptoms[c]	5/30 (17)	5/15 (33)

a More signs and symptoms were reported by volunteers who ingested the 10^3-CFU dose than by volunteers who ingested the 10^4-CFU dose, and the data
for these two doses were combined for the safety analysis.
b The frequency of diarrhea significantly increased with the 10^5-CFU dose compared to the 10^3- and 10^4-CFU doses (*P*=0.036).
c Diarrhea, nausea, or abdominal pain.

More than 70% of the vaccinees excreted the vaccine for a period ranging between 1 and 23 days. However, there was no detection of transmission of the vaccine strain to close contacts. About 80% of the vaccinees receiving any of the doses showed a specific ASC response to the vaccine strain and there were no significant differences in the rate of the specific ASC response among the three groups receiving either 10^3 or 10^4 or 10^5 CFU. It was concluded that WRSS1 is a promising vaccine candidate when given to healthy adults in a range of 10^3 to 10^4 CFU. Further placebo-controlled studies are needed to establish a solid safety record and to evaluate further the immunologic potential of the vaccine.

3.4.2.3 Preliminary report on Phase 1b-2b Inpatient Study (DMID Protocol 09-0021) [29]

A phase 1 and phase 2b blinded, placebo-controlled inpatient trial entitled "Safety, Immunogenicity and Efficacy Studies of WRSS1, a Live Attenuated *Shigella sonnei* Vaccine Candidate in Healthy Thai Adults" was conducted under IND 10254 (DMID Protocol No. 09-0021). The study was conducted at the Vaccine Trial Centre, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Bangkok 10400 Thailand (Punnee Pitisuttithum, MD, P.I.). Coordination and laboratory support was provided by the Armed Forces Research Institute of Medical Sciences (AFRIMS), 315/6 Rajvithi Road, Bangkok 10400 Thailand (Ladaporn Bodhidatta, MD, co-P.I.). The study was part of a NIAID, NIH grant to AFRIMS (COL Carl J. Mason, PI.). Nineteen volunteers (20 - 40 years old) participated in a phase 1b trial that was initiated on 31 May 2010 [29]. Two months later, ten volunteers who had received the vaccine in phase 1b, in addition to 10 naïve controls, were recruited for the phase 2b trial where they were challenged with a virulent *S. sonnei* strain 53G [29]. All volunteers resided in the Mahidol University Vaccine Trial Centre during the inpatient phases of both studies. The volunteers were treated with ciprofloxacin for elimination of the vaccine strain on study day 8 in phase 1b and on study day 5 for the *S. sonnei* challenge strain in phase 2b.

In the phase 1b trial, all volunteers ingested a bicarbonate buffer solution (2 g of sodium bicarbonate in 150 ml of water) shortly before receiving the vaccine or placebo to neutralize gastric acidity. Thirteen volunteers ingested 1.49 x 10^4 CFU of WRSS1 lot 0451 suspended in 30 ml of water, and 6 placebo control volunteers ingested an equal volume of water without vaccine. All adverse events potentially related to ingestion of WRSS1 (or placebo) were mild, and there was no difference between the number of adverse events in vaccinees and placebo control groups (Table 4). WRSS1 was detected by culture in the stools of 3 vaccinees (23%) and in none of the controls. Nine of the 13 vaccinees (69%) had evidence of intestinal colonization with WRSS1 by culture or by PCR using primers that amplified a product which is unique to the vaccine. Antibody responses in 5 vaccinees (38%) were detected as IgA or IgG ELISA seroconversion against *S. sonnei* LPS. ASC and antibody lymphocyte supernatant assays (ALS) increased the total to 7 immune responders (54%) [29]. No placebo controls had an immune response against *S. sonnei* LPS.

Table 4. Adverse Events (clinically related symptoms) in phase 1b trial of 1.5 x 10^4 CFU WRSS1 in Thai adults.

Adverse Event	Vaccines (n=13)	Controls (n=6)
Diarrheal stool	2	2
Fever	0	0
Nausea	1	0
Abdominal pain	1	3
Tenesmus	0	1
Myalgia	1	0
Headache	1	1
Malaise	0	1
Urticaria	1	0
Eosinophilia	0	1

In the phase 2b challenge trial, 10 vaccinees and 10 controls received 1.7 X 10^3 CFU of virulent, wild-type *S. sonnei* 53G freshly harvested from TSA plates, diluted to the challenge dose and suspended in 30 ml of water after ingesting a bicarbonate buffer solution. Five volunteers in the vaccine group and 7 volunteers in the control group had ≥ 4-fold increases in *S. sonnei* LPS-specific serum IgA and IgG after challenge with 53G. *Shigella*-related illness was determined by blinded external review of clinical record summaries. Three vaccinees and 5 naïve controls experienced clinically relevant illness [29]. Although there was a trend toward vaccine protection in this phase 2b trial, there were insufficient clinical outcomes to achieve a statistically significant result.

Preliminary data from a phase 1b trial conducted in Thai adults showed that a 10^4 CFU dose of WRSS1 lot 0451 was well tolerated, but colonization and immune response data also suggested that this population was less responsive to the vaccine than were North American or Israeli volunteers. For example, ingestion of a 10^4 CFU dose of WRSS1 resulted in 23% fecal shedding in Thai volunteers versus 70% fecal shedding in North American and Israeli volunteers [27,28], and only half of Thai adults had a modest immune response against *S. sonnei* LPS while approximately 75 % of North American and Israeli volunteers had robust immune responses against WRSS1 [27,28]. These results probably reflect residual immunity against endemic *S. sonnei* in adult Thai volunteers. A similar result was seen earlier with SC602, a live attenuated *S. flexneri* 2a vaccine candidate, that was immunogenic for North American volunteers but did not colonize or induce immune responses in adults and older children in Bangladesh [30]. In previous studies, 10^5 CFU of WRSS1 was more immunogenic than 10^4 CFU in North American and Israeli volunteers [27,28]. Perhaps higher doses or multiple doses of WRSS1 will be needed to induce immunity in Thai or Bangladeshi populations.

Extensive clinical trials of WRSS1 in U.S., Israeli, and Thai adults suggest that this vaccine should undergo further clinical evaluation in less developed countries (*e.g.* Bangladesh), starting with a descending age study targeted toward 12-24 month-old infants who have yet to experience initial infections with *S. sonnei.*

3.4.2.4 Preliminary Blinded results of Current Study (VAC 008/PR-12054)

Per the current protocol, WRSS1 has been administered at the 10^4, 10^5, and 10^6 CFU dose levels in adults, ages 18-39 years and at 10^3, 10^4 10^5 and 10^6 CFU dose levels in children, ages 5-9 years old (Table 5). The vaccine has been safe thus far at all dose levels in both adults and children, with no vaccine-related serious adverse events reported to date. Overall there were fewer reactogenicity events reported in children as compared to adults and all reactogenicity events in children were reported as mild. Table 6 lists all solicited reactogenicity symptoms observed up to 6 days post-first vaccination and 3 days post-second and third vaccination for children (Note: the groups include both vaccinees and placebo recipients as the data remain blinded). It is important to note that all participants who experienced loose stool and/or diarrhea during the inpatient period tested negative for WRSS1 and other enteric pathogens.

Table 5. Number of participants receiving live, attenuated *S. sonnei* vaccine or placebo by WRSS1 dose

Cohort	WRSS1 dose (CFU)	No. of doses	Schedule (day)	Number of participants	
				Vaccine (N)	Placebo (N)
A1	3 x 10^4	1	0	10	3
A2	3 x 10^5	3	0, 28, 56	10	3
A3	3 x 10^6	3	0, 28, 56	10	3
B1	3 x 10^3	1	0	12	4
B2	3 x 10^4	3	0, 28, 56	12	4
B3	3 x 10^5	3	0, 28, 56	12	4
B4	3 x 10^6	3	0, 28, 56	12	4
Cohort A are adults 18-45 years old, cohort B are children 5-9 years old					

Table 6. Number (percent) solicited reactogenicity among children receiving WRSS1 or placebo

	WRSS1 dose cohort, WRSS1 dose/no. of participants			
	B1	B2	B3	B4
	$3x10^3$ CFU	$3x10^4$ CFU	$3x10^5$ CFU	$3x10^6$ CFU
Symptom†	n=16	n=16	n=16	n=16
Abdominal Pain	1 (6)	1 (6)	0	3 (19)
Abdominal Cramps	1 (6)	0	0	2 (13)
Headache	0	0	0	2 (13)
Light headedness	0	0	0	2 (13)
Myalgia	0	0	0	2 (13)
Fever	0	4 (25)	0	1 (6)
Loose stools (outpatient)	0	1 (6)	3 (19)	1 (6)
Nausea	1 (6)	0	0	1 (6)
Arthralgia	0	0	0	1 (6)
Vomiting	1 (6)	0	0	1 (6)
Loose stools (inpatient)	0	1 (6)	0	0
Diarrhea (inpatient)	0	1 (6)	0	0

† all symptoms were rated as mild on a scale of mild, moderate or severe
Note: no subject reported bloating, chills, constipation, diarrhea (outpatient), decreased appetite, dysentery, excess flatulence, malaise, reactive arthritis

[a] Graded from the Reactogenicity Record during the Inpatient Phase. During the **inpatient period**, diarrhea is defined as 1, grade 3-5 stool of >300 g; OR ≥3 grade 3-5 stools totaling ≥200 g during any 24-hour period within 72 hours after investigational product administration.
[b] During the **outpatient period** diarrhea is defined as ≥3, grade 3-5 stools in a 24-hour period.
[c] Severity determined on the basis of stool number, grading and stool weight during the **inpatient period**
[d] Severity determined on the basis of stool number and grading only during the **outpatient period**

None of the children excreted the vaccine even at a dose of 10^6 CFU, while 5 out of 10 adults (50%) excreted the vaccine strain at the 10^6 CFU dose.

Immune responses as defined as a 4-fold increase in antibody titer were observed at all dose levels (Table 7). The highest dose level appeared to provide the highest number of responders, particularly to Invaplex which is a combination of LPS and the IpaB and IpaD protein antigens. Adults demonstrated high-LPS specific IgA and IgG responses in ALS and serum, and moderate responses in stool at higher dose levels.

Table 7. Immune response to *Shigella sonnei* LPS and Invaplex antigen in children 5-9 years old after three doses (at day 63) of the live, attenuated S. sonnei vaccine or placebo by WRSS1 dose

		Cohort and WRSS1 dose (CFU)		
		B2	B3	B4
Immune measure	Type of specimen	10^4 n=14	10^5 n=16	10^6 n=15
LPS-specific IgA	ALS†	0	1 (7%)	3 (20%)
	Serum†	2 (15%)	4/ (27%)	4 (27%)
	Stool	4 (29%)	7 (44%)	4 (27%)
Invaplex-specific IgA	ALS	0	0	3 (20%)
	Serum	0	1 (7%)	5 (33%)
	Stool	5 (36%)	5 (31%)	4 (27%)
LPS-specific IgG	ALS	0	0	3 (20%)
	Serum	0	1 (7%)	2 (13%)
Invaplex-specific IgG	ALS	0	0	5 (33%)
	Serum	0	0	1 (7%)
(Note: presented groups include both vaccinees and placebo recipients as the data remain blinded).				
† Blood could not be obtained from one child each in B2 and B3 cohorts.				

In the cohort (n=15) that received the highest dose of 10^6 CFU (B4), 10 participants responded in either serum or ALS, 5 participants did not respond in either serum or ALS, and one subject was lost to follow up. Considering that 12 participants out of the 15 participants received the vaccine, a response rate in 10 (83%) would be quite good if all responders were in the vaccine group. Of note, only 2 of 6 participants who responded in ALS had a serum response and of 6 participants who responded in serum, only 2 of those responded in ALS. There were an additional 2 participants that had a 4-fold increase in immune response detected in the stool who did not respond in either serum or ALS (Table 8).

Table 8. Serum immune response to LPS or Invaplex after 3 doses of WRSS1 vaccine by dose cohort among children 5 to 9 years old, icddr,b (blinded)

Study design				No. of participants			No. of participants with ≥ 4-fold increase in titer		
Cohort	WRSS1 dose	No. of doses	Schedule (day)	Vaccine	Placebo	Missing serology	LPS	Inva-plex	Either antigen
B2	3 x 10^4	3	0, 28, 56	12	4	2	2	2	3
B3	3 x 10^5	3	0, 28, 56	12	4	1	6	6	8
B4	3 x 10^6	3	0, 28, 56	12	4	1	8	10	10

Note: IgA and IgG antibody to LPS and Invaplex antigens was measured by ALS and serum 7 days after each dose administration.

Table 9. Fold increase in titer from pre-vaccination (day 0) to post third dose (day 63) for cohort B4 (children receiving 10^6 CFU WRSS1 or placebo).

	ALS				Serum				Stool	
ID	IgA LPS	IgA INV	IgG LPS	IgG INV	IgA LPS	IgA INV	IgG LPS	IgG INV	IgA LPS	IgA INV
2120	6	6	7	20	3	2	0	1	26	9
2141	1	2	2	8	1	1	0	1	0	0
2142	2	2	1	10	2	2	0	1	1	1
2148	1	1	1	1	2	1	1	1	6	6
2149	1	1	1	2	1	1	1	2	8	5
2153	1	1	1	1	138	253	3	1	1	1
2072	1	1	1	1	293	160	5	3	3	3
2112	1	1	1	1	1	1	340	1	0	1
2160	1	1	1	2	1	159	3	8	0	0
2165	12	5	12	3	7	4	<4	<4	0	0
2166	1	1	1	4	1	1	2	2	1	1
2167	18	11	14	4	546	4	<4	1	17	16
Non responders										
2145	1	1	1	2	1	1	2	1	0	0
2147	1	1	1	2	0	0	1	1	0	1
2157	1	1	1	2	1	1	1	3	2	1
2179	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

In summary, blinded data from VAC 008 reveal that WRSS1 vaccine was safe and immunogenic in adults at the highest dose level and in children at the two highest dose levels ($3x10^5$ and 10^6 CFU). Immune response was observed to both LPS and invaplex antigens in both blood and stool. The safety and immunogenicity of the WRSS1 vaccine in 5 to 9 year old children at dose levels of up to 10^6 CFU, justifies moving to toddlers in this protocol.

3.5 Rationale for Current Study and Planned Future Development

Given the heterogeneous distribution of *Shigella* species and serotypes, any vaccination approach based on O-polysaccharide antigens must depend on multivalent or cross-protective strategies. *S. flexneri* 2a and *S. sonnei* antigens would be the basis of any multivalent *Shigella* vaccine because these two components should cover 40% to 75% of *Shigella* infections in LDCs and over 90% in developed countries [2,4,20]. As an initial step to achieving a multivalent *Shigella* vaccine for optimal coverage, we have started with WRSS1, a *S. sonnei* vaccine with a long history of safety and immunogenicity.

Previously, a *S. flexneri* 2a live vaccine candidate SC602 was tested in a randomized, double-blind, descending age inpatient and outpatient placebo-controlled trial in Bangladesh. The trial enrolled adults and children 8-10 years old [30]. SC602 showed 100% protection against shigellosis in US volunteers [31]. Bangladeshi volunteers were given a single oral dose of the vaccine (1×10^4, 1×10^5 or 1×10^6 CFU) and monitored for vaccine excretion and immunogenicity [30]. Adults were given 120 ml and children given 75 ml of bicarbonate solution prior to vaccine administration. None of the volunteers had diarrhea and no significant or severe symptoms were noted in adults or children. In the initial inpatient trial of 20 adult volunteers, SC602 was isolated from 1/5 participants in the $1x10^5$ dosing group, and 2/5 participants in the $1x10^6$dosing group. Two of 5 participants in the 6-log group also showed 4-fold increases in serum IgG anti-LPS immune responses, even though pre-vaccination antibody titers were high in this population [19]. None of the children excreted the vaccine strain and no immune responses were noted. Lessons learned from the SC602 trial includes the need to further modify vaccine dose and regimen for endemic populations, as what has worked in naïve volunteers may not be suitable for endemic populations. Some of the factors that affect colonization of live vaccines and immune responses in LDC's include, but are not limited to, the actual dose given and the number of doses, volumes of buffer and buffer composition for vaccine delivery, particularly in children, preexisting background immunity, differences in gut physiology and microbial composition, role of maternal antibodies in breast milk, and macro and micronutrient deficiencies. Some of these parameters can

be tested using vaccine candidates, such as WRSS1, that has demonstrated good safety and immunogenicity in immunologically naïve volunteers.

The proposed study builds upon successful preliminary observations with this vaccine in the US, Israel and Thailand and now in Bangladesh. While secondary objectives include studying the immunogenicity of the WRSS1 vaccine, the primary goal of the current trial is to establish a clear safety profile for the WRSS1 vaccine in toddlers 12-24 months. Blinded data from the current trial in Bangladeshi adults and 5-9 year old children (See Section 3.4.2.4) suggest that 10^6 CFU would be the minimal threshold dose in these two groups. It is thought that the target population in the current study would have a lower immunity to the organism than seen in these older groups, so 10^6 CFU could be effective in this group without achieving a dose excess that may have safety considerations. This protocol proposes to evaluate WRSS1 in toddlers 12-24 months old. The primary and secondary hypotheses will remain the same as the previous study. The toddler study seeks to examine critical variables to maximize immune responsiveness in young children, such as buffer volumes, dose number and dose levels for improved vaccine delivery and increased immunogenicity, using approaches compatible with routine immunization practices. WRSS1 vaccine is likely to have a more profound impact on the prevention of severe life threatening diarrhea in children than in adults, and therefore there is great urgency and interest in the prompt development of WRSS1 for use in infants and toddlers. The WRSS1 vaccine has been demonstrated to induce similar levels of immune responses to those observed during convalescence from natural infection.

We know from previous human trials of *virG*-deleted vaccines that this strategy is powerful and that the loss of *virG* significantly attenuates the strain. This knowledge has come primarily from repeated clinical trials of live oral *Shigella* vaccine candidates (WRSS1, SC602, WRSd1) [27,31,32]. We have tested the WRSS1 vaccine in an appropriate number of adults to ascertain maximally acceptable tolerability, followed by studies in 5-9 year old children. In this protocol we will proceed cautiously in toddlers by testing the lowest dose (10^3 CFU) before moving up to higher doses (up to 10^6 CFU). Furthermore, all participants in the study will receive the first dose of vaccine as in-patients with the dual purpose of obtaining more accurate clinical assessments as well as promptly addressing any potential safety issues. If the first dose is well tolerated, the second and third doses will be given in the CTU on an out-patient basis.

The overall long-term strategy for development of a live *Shigella* vaccine includes defining the formulation of buffers and vaccine vehicles that permit low-volume administration and maximum viability of the vaccine [33]. Ultimately, the number of needed doses and the identification of a maximally immunogenic, safe dose level of this WRSS1 prototype strain must be determined in the target population. Concurrent with this study, development and Phase 1 testing of a trivalent (*S. flexneri* 2a and 3a, *S. sonnei*) vaccine is being planned. Upon completion of this future US trial, the safety and immunogenicity of a novel complete, trivalent live attenuated vaccine in the target population will be evaluated for potential advancement to Phase 3 trials.

3.5.1 Rationale for exploratory objectives

A better understanding of the relationships between vaccination, immune response, and protection is of great interest to researchers. Most of the current vaccines work through antibodies in serum or on mucosal surfaces that prevent infection. Both concentration and functional characteristics of antibodies are important for this purpose. Vaccine-specific antibodies may correlate with protection or act synergistically with other immune functions. Cellular immune response kills or suppresses replication of intracellular pathogens and may also act in synergy with antibodies. For some vaccines, true correlates of protection are not known, but only surrogates (a useful measurement that is not functional in protection) are available. Though there are no definite immune correlates of protection for *Shigella* vaccines, it is generally believed that protection will require a humoral and a mucosal (as well as a memory) response since *Shigella* is a mucosal pathogen. The WRSS1 live, attenuated vaccine contains all the antigens of a wild type *Shigella sonnei* and induces a similar immune response. It is therefore important to characterize each of these arms of the immune response and explore for associations with the profile of the adaptive immunity in an attempt to derive correlates of protection.

Functional activity of serum/plasma antibodies after vaccination

Antibody-dependent, complement-mediated bactericidal killing confers protection against infectious microrganisms, and serum bactericidal antibody activity (SBA) has therefore been used as a test for the functional activity of serum antibodies and as a surrogate of protection for several vaccines. SBA assay has

been used to support meningococcal vaccine licensure [34]. Serum vibriocidal antibodies constitute the best correlate of protection against cholera and have been used extensively to monitor the immunogenicity of oral and subcutaneous cholera vaccines [35]. With the O-polysaccharide conjugate vaccine candidate, that has shown protection in Israeli field studies, protection is believed to be due to the high serum antibody titers to *Shigella* antigens that transfers over onto the mucosal epithelial layer in the gut and prevents the bacteria from invasion [36]. Whether it does so by simple binding, opsonophagocytosis and/or bactericidal activity is not clear. Therefore functionality of the serum immune response becomes important and the SBA assay is one measure of that parameter. Using a modified SBA assay for cholera, a serum shigellacidal activity for *S. flexneri* was previously established at icddr,b [37]. This assay will be appropriately modified to evaluate SBA activity in serum/plasma of toddlers immunized with WRSS1. The SBA activity will be correlated with the serum IgG and IgA titers to LPS and to Invaplex.

Role of cytokines

One of the key features of Shigella pathogenesis is the release of mature interleukin-1β (IL-1β) and interleukin-18 (IL-18) from the resident macrophages of the rectal mucosa, and subsequent production and secretion of a large array of proinflammatory cytokines and chemokines from the mucosal tissue [38]. The secretion of these cytokines is part of the activation of innate immunity that is triggered by the interaction of bacterial LPS with the TLR4/MD2/CD14+ receptor complex on macrophages and dendritic cells. In patients with acute *Shigella* infections, increased expression of proinflammatory cytokines in the rectal mucosa and stool and systemic dissemination in plasma correlated with the clinical severity of the disease [39,40]. Although specific antibodies participate in protection against natural *Shigella* infection, these observational studies suggest that cellular immunity also represents a dominant role that eventually leads to the elimination of *Shigellae* from the host.

The profile of cytokines in serum/plasma, lymphocyte supernatant and stool samples would add great value and information not only to the characteristics of the innate immune response achieved in toddlers after oral vaccination with a live vaccine but may also explain the levels of humoral and mucosal antibody responses to *Shigella* antigens.

3.6 Study Site

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

Recruitment, screening, and consenting will take place at the urban field icddr,b site in Mirpur, a suburb of Dhaka, Bangladesh. Each vaccine dose will be given in the Clinical Trials Unit (CTU) in the icddr,b campus (details of CTU is given under Facilities Available). All participants will gather in the Field Office, from where they will be brought to CTU by icddr,b transport.

In cases where the subject may require hospitalization, the participant can be shifted directly to the Dhaka hospital at the main icddr,b campus.

Vaccine preparation will take place at the INT Laboratory under BSL-2 conditions and transported to the CTU for dose administration within 2 hours of reconstitution according to the vaccine preparation SOP.

4.0 RESEARCH DESIGN AND METHODS

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

This is a single site, double-blind, randomized, placebo-controlled, dose-escalation study that will test the WRSS1 vaccine in healthy toddlers (12-24 months old). The study is designed as a dose escalation study comprising four cohorts. In each cohort, the first dose and immediate safety evaluation will be conducted at the icddr,b Inpatient Unit (Clinical Trials Unit, icddr,b main campus), where the participants will be admitted for observation for 24 hours post-vaccination. Follow-up visits for participants will take place on an outpatient basis at the Mirpur Field Office. Second and third vaccinations will take place on an outpatient

basis in the CTU. Before enrolling participants in subsequent cohorts to receive a higher vaccine dose, the safety data from the previous cohort(s) (through Study Day 7) will be evaluated and reviewed by the Internal Protocol Safety Team (IPST) comprised of the study physician, the Medical Monitor from CRO, the principal investigator, and the Medical Monitor from PVS.

Toddlers (12-24 months old)

This study will enroll a total of 64 toddler participants. 48 toddlers will be randomized to receive vaccine and 16 will be randomized to recieve placebo. The participants will be divided into four separate "dose" cohorts to be recruited step-wise. Each cohort will include 16 participants, randomized to receive three doses of WRSS1 vaccine (12 participants) or placebo (4 participants). Within each dose level, the study will initially enrol a sentinel group of 8 children (6 vaccinees and 2 placebos) while the remaining 8 (6 vaccinees and 2 placebos) will be enrolled after safety data through Day 7 is reviewed by the Internal Protocol Safety Team. The placebo preparation will be bicarbonate buffer. The number and allocation of participants are shown below.

Table 10. Vaccine Dose and Subject Allocation By Cohort

Dosing Group	Sub-groups	WRSS1 vaccine particles/mL	Dosing schedule	Number of participants	
				Vaccine group	Placebo group
C1	Group I	3×10^3	Day 0, 28, 56	6	2
	Group II	3×10^3	Day 0, 28, 56	6	2
C2	Group I	3×10^4	Day 0, 28, 56	6	2
	Group II	3×10^4	Day 0, 28, 56	6	2
C3	Group I	3×10^5	Day 0, 28, 56	6	2
	Group II	3×10^5	Day 0, 28, 56	6	2
C4	Group I	3×10^6	Day 0, 28, 56	6	2
	Group II	3×10^6	Day 0, 28, 56	6	2

Participants in all 4 cohorts are scheduled to receive three doses of WRSS1 at an increasing dose as described in the table or placebo at Days 0, 28 and 56. The first immunization will be given on an inpatient basis. If after review by the Internal Protocol Safety Team the first dose appears safe and well tolerated, all subsequent doses will be administered on an outpatient basis in the CTU.

For C1, C2 and C3, the Internal Protocol Safety Team will review the first dose data through Day 7 and decide to proceed to administration of next 2 doses as well as starting the next higher dose level. After the completion of C2, the icddr,b DSMB will review all available safety data and decide whether to proceed to cohorts C3 and C4. At the end of dosing of C4, the DSMB will review the safety and available immunogenicity data for all cohorts.

5.0 STUDY POPULATION

Participants will be recruited via word of mouth and home visits from Field Staff, and will be screened according to the following criteria:

5.1 Eligibility Criteria

Inclusion criteria

1. Male or female children aged between 12 and 24 months of age at the time of vaccination.
2. General good health as determined by the screening evaluation no greater than 30 days before admission.
3. Father or mother properly informed about the study, able to understand it and sign the informed consent form.
4. Normal bowel habits (< 3 grade 1 or2 stools each day; ≥ 1 grade 1 or 2 stools every 2 days).
5. Free of obvious health problems as established by medical history and clinical examination before entering into the study.

6. Parent or guardian available for the entire period of the study and reachable by study staff throughout the entire follow-up period.
7. Signed Informed Consent from the parent.

Exclusion criteria

1. Presence of a significant medical condition that in the opinion of the Investigator precludes participation in the study.
2. Infection with HIV.
3. Presence in the serum of HAV or HEV antibody or HBsAg.
4. History of congenital abdominal disorders, intussusceptions, abdominal surgery or any other congenital disorder.
5. Participation in research involving another investigational product (defined as receipt of investigational product) 30 days before planned date of first vaccination or concurrently participating in another clinical study, at any time during the study period, in which the child has been or will be exposed to an investigational or a non-investigational product.
6. Clinically significant abnormalities on physical examination.
7. Clinically significant abnormalities in screening hematology, serum chemistry as determined by the PI or the PI in consultation with the Study Physician.
8. History of febrile illness within 48 hours prior to vaccination.
9. Prior receipt of any *Shigella* vaccine.
10. Fever at the time of immunization. Fever is defined as a temperature ≥ 37.5°C (99.5°F) on axillary measurement.
11. History of known shigellosis, chronic diarrhea/dysentery in the past 2 months.
12. Current use of iron or zinc supplements within the past 7 days; current use of antacids (H2 blockers, omeprazol, OTC agents) or immunosuppressive drug.
13. Allergy to macrolid and penicillin classes of antibiotics
14. Clinical evidence of active gastrointestinal illness.
15. Prior receipt of a blood transfusion or blood products, including immunoglobulins.
16. Presence of any significant disorder (cardiovascular, pulmonary, hepatic including hepatitis C, renal, gastrointestinal, endocrine, immunological, dermatological, neurological, cancer or autoimmune disease) as determined by medical history and/or physical examination which would endanger the participant's health or is likely to result in non-conformance to the protocol.
17. History of any neurologic disorders or seizures or family history of rheumatologic disorders.
18. Acute disease at the time of enrolment.
19. Medically significant malnutrition, defined as moderate malnutrition (wt-for-age z-score between -3.0 and -2.0) and severe malnutrition (wt-for-age z-score <-3.0 or edema).
20. Any conditions which, in the opinion of the investigator, might jeopardize the safety of study participants or interfere with the evaluation of the study objectives.
21. Receipt of antimicrobial drugs for any reason or a fever ≥ 38°C within 7 days before vaccination.
22. History of diarrhea during the 7 days before vaccination.
23. Has any household member(s) who is immunocompromised or under the age of 1 year old.
24. Culture or PCR positive for any *Shigella* strain

6.0 RECRUITMENT, SCREENING, RANDOMIZATION, AND MASKING PROCEDURES

6.1 Recruitment

The icddr,b team will develop a plan in order to achieve enrollment of the specified number of children, and will track screened candidates along with enrolled participants. The team will review this plan periodically throughout recruitment in order to determine the effectiveness of the plan. Potential alternates will be recruited in order to ensure robust enrollment in the case a participant is found to be ineligible the day before vaccination.

Participants for this study will be recruited by word of mouth in the Mirpur field site. Upon identification of potential participants, field staff will visit the households in Mirpur surveillance area to have preliminary discussions with parents regarding potential eligibility and participation in the study. The ERC and WIRB-approved consent forms will be utilized by the field workers throughout the recruitment process to assist in

their interactions with parents of potential participants. Parents will be given the informed consent form (ICFs) to keep overnight for further discussion with their families. Interested individuals will be invited to the Mirpur field office for further discussions where they will receive and sign the ICFs upon their consent.

6.2 Screening

Parents of potential participants will be made aware that the screening process may take several visits to complete. Participants will be screened for eligibility based on a screening questionnaire listing the inclusion / exclusion criteria. A signed, dated informed consent document must be obtained by the PI or designee before initiating any study specific procedure. The study medical officers will discuss the informed consent document in detail with the parents. Group discussions may also take place to orient parent(s) to the study; however individual discussions will commence prior to obtaining consent signatures. Informed consent documents will be signed once the toddler's parent has willingly agreed to participate in the study and prior to initiation of any screening procedures and data collection. A literacy rate of 40% is expected in the Mirpur surveillance area with Bangla being the most commonly spoken language. An impartial witness will be present during the informed consent discussions with illiterate parent(s). A person who can only write his/her name and cannot read or write in Bangla will be considered as illiterate for the study purposes. The consent will outline study procedures, participant expectations, potential risks and benefits, as well as outline the compensation plan for their participation. Participants will be compensated based on the number of visits they are able to complete. Alternates will also be compensated accordingly.

According to protocol procedures, a medical history/exam and a series of clinical laboratory tests may be completed to rule out occult illness. These laboratory tests may include, but are not limited to hematology, serum chemistries, hepatitis A and E antibodies, HBsAg and HIV in serum. For detecting *Shigella* spp, stool will be cultured and bacterial DNA will be extracted from stool for *ipa* PCR.

During screening, participants will be assigned a study number which will be documented on that participant's source documents. If the participant qualifies for the study, he/she will be enrolled into the electronic data system with his/her study ID number. Only participants who have met all of the inclusion criteria and none of the exclusion criteria will be officially enrolled in the study and entered into the EDC database.

6.3 Randomization

Randomization will occur manually on the day participants are to receive their first study vaccination, after confirmation of eligibility and immediately prior to immunization. Subjects who replace those who vomit the first dose within 30 minutes after the administration of study vaccine/placebo will be administered the same vaccine/placebo assigned to the subjects being replaced.

Participants will be assigned to a coded treatment assignment provided by Emmes, and each participant's treatment assignment will be entered into the Emmes AdvantageEDC^{SM} system after product administration has occurred. The unblinded study pharmacist or member of the IP formulation team, who is not involved in other study activities (including assessment of safety), will be provided with the treatment assignment codes for preparation of the vaccine or placebo to be given to each participant. The unblinded study pharmacist (or designee) will maintain the treatment code list in a secure place.

6.4 Masking procedures

WRSS1 vaccine and placebo will be provided to participants by randomization. This process will be blinded to both the participants and the investigators and the study personnel who are involved in the clinical and laboratory evaluation of participants. Only study personnel who will not be involved in these evaluations will be assigned to prepare and distribute the vaccine or placebo to each participant according to the randomization code. These staff members will not reveal the randomization code to any other study staff member or participant. The vaccine preparation staff will disguise the appearance of the vaccine and placebo according to the vaccine preparation SOP. The sealed randomization code will be kept by the designated unblinded staff in a locked, secure location. In case of an SAE or any unexpected circumstance that requires breaking a code, the randomization code will be opened by the local DSMB for that particular participant to assess the clinical conditions and necessary steps.

7.0 STUDY PROCEDURES/EVALUATIONS

7.1 Clinical Definitions

Grading system for stools

Grade 1: hard (normal)
Grade 2: soft (normal)
Grade 3: thick liquid
Grade 4: opaque watery liquid
Grade 5: clear watery

Diarrhea:

- Diarrhea is defined as ≥ three grade 3-5 stools in a 24-hour period.
- If a subject meets the definition of diarrhea, the start of the diarrhea episode will be the time of the first grade 3-5 stool in the episode of diarrhea and the end of the diarrhea episode will be the time of the last grade 3-5 stool.
- Diarrhea severity will be determined on the basis of stool number and grading.

7.1.1 Grading system for diarrhea

Mild Diarrhea

- A case of diarrhea as defined above, which does not meet the case definition of moderate or severe diarrhea as defined below.

Moderate Diarrhea

- A case of diarrhea as defined above, with 4-5 grade 3-5 stools in 24 hours and/or moderate dehydration

Severe Diarrhea

- A case of diarrhea as defined above, with >6 grade 3-5 stools in 24 hours and/or severe dehydration

Dysentery: ≥ grade 3 stools with gross blood (confirmed by study personnel)

Shigellosis: Temperature ≥ 38.3°C (100.94°F), diarrhea and/or dysentery, and one or more severe intestinal symptom, and shedding of any *Shigella* spp.

Dehydration: Classification of the severity of dehydration will follow WHO guidance per icddr,b standard of care.

Grading the severity of child adverse events: toxicity grading scale for abnormal values will be based on appendices 2 and 3.

7.1.2 Inpatient Phase (All cohorts – Day 0 to Day 1)

Participants will be monitored daily during the inpatient phase of the study via examination by a study clinician, solicitation of daily progress reports, monitoring of gastrointestinal and systemic signs and symptoms, reviewing medical conditions, and noting AEs. Vital signs will be recorded two times daily, and more often as appropriate, based on the clinician's judgment. If a participant develops moderate or severe diarrhea, vital signs will be measured as necessary for clinical management according to the judgment of the clinician. In case of emergency children will be brought to the ICU of Dhaka Hospital.

All stools passed during the inpatient phase will be collected in disposable plastic containers for grading the stool consistency. Frequency of defecation on each day will also be recorded. At least one stool specimen from each participant will be used for culture and PCR for WRSS1. If no stool is passed during the day, 2 rectal swabs will be obtained (2 swabs in BGS for culture and PCR testing). Swabs will be discarded with other biohazardous waste. If a participant meets the protocol definition of diarrhea, those stool specimens will also be cultured. In addition, all stools passed by study participants while inpatients will be visually inspected for the presence of frank blood. Only stools with apparent frank blood will be tested using microscopy to confirm the presence of blood. Only those stools testing positive by microscopy will be recorded as positive for blood.

7.1.3 Outpatient Phase

On Day 28 and Day 56, all participants in Cohorts C1, C2, C3 and C4 will return to the CTU for the second and third dose of vaccine or placebo (assuming the initial dose is deemed safe). On Days 7, 35, 63, and 84, participants will return to the Field Office for follow-up visits as described in Section 8.6.

7.2 Concomitant Medications/Treatments

Current use of immunosuppressive drugs and antimicrobial agents is unacceptable for participation in this study as indicated in the exclusion criteria.

Oral rehydration solution (ORS) is not considered a concomitant medication. Parents will be encouraged to give ORS to participants with diarrhea; or other replacement fluids may be given when needed. Antimicrobial drugs for treatment of shigellosis and for long-term shedding of WRSS1 as per protocol Section 7.2.1, will also not be considered as concomitant medication. The medicine and treatment to be used in the study will be based on the judgment of the attending clinicians.

7.2.1 Antimicrobials

Pivmecillinam will be administered to participants who meet the criteria for early antibiotic treatment or who are still shedding vaccine at Day 84 or after. Standard of care followed in icddr,b's Hospital will be employed for treatment of all patients. Standard antibiotic treatment at the Dhaka Hospital of icddr,b for dysenteric patients is Pivmecellinam; patients with severe watery diarrhea will receive Azithromycin. Pivmecillinam will be administered orally at 15 mg/kg/dose 4 times a day for 5 days when necessary. Alternatively, Azithromycin will be given at 10 mg/kg once daily for 5 days.

These antibiotics are usually well tolerated, but side effects may occur such as diarrhea, nausea, vomiting, abdominal pain/discomfort, headache and dizziness. In rare cases, severe side effects may also happen such as nerve damage in arms or legs or allergic reactions that, if severe, can cause difficulty in breathing. There may be other risks or side effects which are currently unknown.

Criteria for early antibiotic treatment <u>for all participants</u>:

1. Participants meeting the criteria for shigellosis (temperature ≥ 38.3°C, and diarrhea and/or dysentery, and one or more severe reactogenicity symptoms, and shedding WRSS1)
2. Participants with >6 diarrheal stools per day, or >3 dysenteric stools per day, or require intravenous hydration, with or without fever
3. Any volunteer still shedding vaccine at day 84 will be treated with antibiotics.

7.2.2 Antipyretics

In the event that a participant develops a fever ≥38.0°C as confirmed by two independent axillary temperature measurements 10 minutes apart, 15 mg of acetaminophen per 1 kg child weight will be administered orally every 6 hours until the fever is resolved, not to exceed 90 mg/kg within a 24 hour period.

7.3 Laboratory Evaluations

7.3.1 Clinical Laboratory Evaluations

During screening, and at day 7, blood samples will be collected for clinical laboratory evaluations. Per icddr,b DSMB recommendation for protocol VAC 008/PR 12054, in those children who have a gradeable decrease in hemoglobin (Hb) level on day 7 after the 1st dose, a repeat measurement will be obtained on follow-up days, as appropriate, utilizing scheduled immunogenicity testing time points, if possible, to minimize venipuncture episodes. Samples will be packaged according to SOPs and picked up by laboratory staff. Standard clinical laboratory tests for the purpose of inclusion and exclusion of potential participants and for safety monitoring will be carried out by the Clinical Laboratory Services.

7.3.2 Research Laboratory Evaluations

For the first dose of each cohort and throughout the inpatient period, all research samples will be collected at the CTU and sent to the Immunobiology, Nutrition and Toxicology (INT) Laboratory for testing. For the second and third doses of each cohort, all specimens will be collected at the CTU; throughout all outpatient follow-up visits, all research samples will be collected at the urban field site in Mirpur, Dhaka. Samples will be packaged and transported via official transport to the INT Lab. Immunologic assays will be carried out at

the INT Laboratory, will be performed as per written SOPs, and will be a part of the study documentation. Culture and PCR detection of WRSS1 in stool will be done in Enteric Microbiology Laboratory. The assays will occur as outlined in the Study Event Schedule. For immunologic assay, samples will be stored so that all specimen time points can be tested together for each individual.

7.3.2.1 Systemic immune response to WRSS1

The systemic immune response to WRSS1 will be evaluated by assessing the IgA, IgG and IgM antibody responses to *S. sonnei* 2a LPS and Invaplex in serum/plasma samples at Days 0, 7, 35 and 63. Invaplex is a mixture of purified *S. sonnei* LPS and purified protein antigens IpaB and IpaC. Serotype-specific LPS from the Walter Reed Army Institute will be used to coat the ELISA plates. A ≥4-fold rise in serum/plasma antibody titers will be considered significant.

A serum bactericidal assay method can reliably measure functional activity of antibodies *in vitro*. The killing capacity of serum/plasma (or serum/plasma bactericidal antibody) as an immunological correlate for efficiency/ effectivity of the vaccine will be evaluated in toddlers at Days 0, 7, 35 and 63 using the method described previously[37]. The titer of the serum will be defined as the reciprocal of the last dilution in which no growth is evident by visual inspection. A ≥4-fold increase in antibody titers from baseline is defined as seroconversion.

Concentration of cytokines will be measured in serum/plasma using a multiplex platform that will allow simultaneous profiling of multiple cytokines and chemokines in a single sample. Serum/plasma samples from Days 0, 7, 35 and 63 will be used for this purpose.

7.3.2.2 Mucosal immune response to WRSS1 (ALS, Blood)

The mucosal immune response to WRSS1 will be evaluated by assessing specific IgA, IgG and IgM antibody responses to *S. sonnei* 2a LPS and Invaplex using the 'antibodies in lymphocyte supernatant' (ALS) assay on culture supernatants of cultured peripheral blood mononuclear cells from different study days (Days 0, 7, 35, and 63) before and after vaccination to determine LPS- and Invaplex-specific IgA, IgG and IgM responses from circulating lymphocytes. Concentration of cytokines will be measured in ALS supernatant using a multiplex platform

7.3.2.3 Mucosal immune response to WRSS1 (Fecal IgA and cytokines)

The mucosal immune response to the WRSS1 will be evaluated by assessing Fecal IgA antibody responses to *S. sonnei* 2a LPS and Invaplex in ELISA assays at Days 0, 7, 28, 35, 56, 63, and 84 according to the method described earlier[35]. Pro-inflammatory cytokines (IL-1β, IL-8, TNF-α, IFN-γ etc.) will be measured in stool extracts using commercially available kits.

7.3.2.4 Intestinal Colonization of WRSS1 (Stool Culture, PCR)

The shedding of WRSS1 vaccine in stool will be evaluated by stool culture and PCR at Days 0 (after vaccination), 1, 7, 28, 35, 56, 63 and 84.

8.0 STUDY SCHEDULE

8.1 Screening, Visit 01 (Days -30 to -9)

The following procedures will be completed during screening, between Days -30 and -9, to determine study eligibility and may occur over potential multiple screening visits. The study PI or designee must also obtain a signed study specific ICF from the participants prior to screening initiation. Additional screening visits may be scheduled for any follow-up as needed, but are not required. At the screening visit, the PI or her designee will provide parents of prospective participants with a detailed description of the study objectives and study participation requirements, as well as the potential health risks and benefits associated with study participation, including knowledge of diarrhea and *Shigella* bacteria. One or 2 additional participants will be recruited and screened for each group of 8 children in the case that another volunteer is found ineligible on admission / vaccination day (Visit 02, Day 0) or vomits in the 30 minute post-vaccination period. Alternates will be duly compensated for their time.

The following procedures will be conducted at the Screening Visit(s):

- Provision of written study description and ample time to read the ICF; signing the ICF
- Assessment for study eligibility via a review of inclusion and exclusion criteria
- Medical interview to collect relevant information on the participant's past and concomitant medical illness and medications
- Physical examination: Head/ears/eyes/nose/throat (HEENT), skin, lymph nodes, respiratory (lung), cardiovascular (heart), abdomen, neurological and musculoskeletal system. Weight and height will be measured at screening only for assessment of participant BMI
- Vital signs: heart rate, axillary temperature and respiratory rate
- Baseline hematology: Total and differential Cell Count and hemoglobin
- Baseline clinical chemistry: Creatinine, AST,ALT, bilirubin, γGT
- Screening serology: anti-HAV, anti-HEV and HBsAg
- HIV test in serum
- Stool culture and PCR for presence of any *Shigella* strain obtained between Day -30 to -9.
- For continued eligibility prior to vaccination on Day 0, another stool specimen will be obtained at days -7 to -5 for culture and PCR for presence of any *Shigella* strain. If a stool is not obtained between Day -7 and -5, rectal swab will be obtained on Day -5 in Buffered Glycerol Saline (BGS) for culture and PCR testing and the results back prior to vaccination on Day 0.

8.2 Day of First Vaccination (Visit 02)

Day 0 is the day of the first study product administration (WRSS1 or placebo). The following procedures will be conducted at Day 0 before vaccination in the Field Office:

- Assessment of continued eligibility by assessment of relevant inclusion/exclusion criteria
- Physical examination: HEENT, skin, lymph nodes, respiratory (lung), cardiovascular (heart), abdomen, neurological and musculoskeletal system
- Vital signs (heart rate, axillary temperature and respiratory rate); body weight will be measured
- Interim medical interview

Inpatient Admission

Eligible participants will be admitted to the CTU Inpatient Unit on the day of administration of WRSS1 or placebo. As stated above, alternates will be brought along to replace any participants who are determined to be ineligible on the day of vaccination or vomits in the 30 minutes post-vaccination period. Those alternates will be duly compensated for their extra time.

- Admission to the CTU Inpatient Unit and overview of logistics during inpatient stay
- Inpatient unit orientation and review of requirements, expectations, and evacuation procedures
  Review of proper stool collection, hand-washing technique and hygiene procedures
- Vital signs (heart rate, axillary temperature and respiratory rate) prior to vaccination, 30 minutes post-vaccination, and once later in the day will be measured. Body weight will be measured.
- Serum sample collected for assessing IgG, IgA and IgM antibody titers to *S. Sonnei* LPS and invaplex.
- Whole blood sample collected for ALS
- Stools collected, graded, and visually inspected for blood. Microscopy will be performed if blood is suspected. Frequency of defecation will also be recorded.
- Stool sample for immunogenicity will be collected from Day -1 to Day 0 prior to vaccination.

Vaccination

Participants will be required to fast for 60 minutes before and after ingestion of the investigational product. To maintain the blind, staff who will not be involved in the clinical evaluation of the participants and laboratory evaluation of specimens will be responsible for randomization and labelling vaccine/placebo cups with treatment number.

If a participant vomits in the 30 minute post-vaccination period, the participant will remain in the study for safety follow-up evaluations required on Days 0 to 1 (inpatient period), 7, 28, 35, 56, 63, 84 and Day 224, and will not be re-dosed. If such an event occurs, an alternate participant may be dosed at the discretion of the IPST.

The following procedures will be conducted:

- Preparation of WRSS1 and placebo
- Participant randomization
- Dosing of WRSS1 or placebo
- Stool sample collected after vaccination will be assessed for shedding. In addition to routine culturing at study-defined time points, cultures will be performed on stools that meet the protocol-definition of diarrhea

8.3 Inpatient Period (Visit 03)

Participants will remain in the CTU Inpatient Unit overnight for post-vaccination observation. Vital signs will be measured at least 2 times in the day.

The following procedures will be conducted on Day 1:

- Physical examination including monitoring for intussusception and interim medical interview to collect any new AEs or assess ongoing AEs
- Vital signs twice a day: heart rate, axillary temperature and respiratory rate. If the participant is febrile, additional vital signs will be measured,if needed. Body weight will be measured.
- Stools collected, graded, and visually inspected for blood. Microscopy will be performed if blood is suspected. Frequency of defecation will be recorded on each day.
- Stool sample collected for shedding. If no stool is obtained during the day, 2 rectal swabs will be obtained in BGS (for culture and PCR testing). In addition to routine culturing at study-defined time points, cultures will be performed on stools that meet the protocol-definition of diarrhea.

Additional medically indicated clinical investigations will be performed based on study physician's judgment. A study physician and 2 nurses will be present 24 hours/day.

8.4 Discharge (Visit 03)

Routine discharge is scheduled for the day after dosing i.e. on Day 1 (24-hr after vaccination). Vital signs and physical assessment at discharge will be recorded in the source documents and in the electronic CRF. Guardians/mothers of participants will be educated about and given a simple Memory Aid to document frequency of defecation and stool grade each day up to the next 3 days for determination of diarrhea. Guardians/mothers will be informed that field staff will visit their home for 3 days to monitor their health, including taking their temperature, reviewing their Memory Aid, and recording any symptoms on a source document. The field staff will be responsible for that source document and bring it to and from the participant's home. Symptoms/signs solicited by the field staff and recorded on the source document will include the following: diarrhea, temperature, abdominal pain, nausea, vomiting, irritability, decreased activity, loss of appetite and excessive flatulence.

8.5 Outpatient Visits

On Day 7 after dosing, all participants will return to the Mirpur field office for follow-up visit as described in section 8.5.2. On Days 28 and 56, participants will return to the CTU for the second and third doses of WRSS1 or placebo (assuming the initial dose is deemed safe). The visits on Days 7, 35, 63, and 84 are for safety and immunogenicity assessment. The visit on Day 224 is for long term safety follow-up.

All mothers/guardians of study participants will be encouraged (but not required) to give them food approximately 70-80 minutes before dosing. Unscheduled visits may be completed at any time during the study for safety assessment and follow-up, based upon the clinical judgment.

8.5.1 Outpatient Vaccinations (Visit 05 and Visit 07)

The following procedures will be conducted on **Days 28 and 56**:

- Assessment of continued eligibility by review of relevant inclusion/exclusion criteria
- Interim medical interview to collect any new AEs or to assess ongoing AEs and concomitant medications review
- Physical examination prior to vaccination: HEENT, skin, lymph nodes, respiratory (lung), cardiovascular (heart), abdomen (including monitoring for intussusception), neurological and musculoskeletal system
- Vital signs: heart rate, axillary temperature and respiratory rate and body weight will be measured.
- Dosing of WRSS1 or placebo. If a participant vomits within 30 minutes after receiving his/her second dose, he/she will remain in the study and complete all follow-up safety assessments and receive the third dose at the discretion of the IPST
- Stool sample collected for immunogenicity and shedding. Study staff will visit participants at their home in the morning for specimen collection and transport back to the lab for fecal IgA processing, culture, and PCR. Stool specimens may be no more than 3 hours old. If no stool is available in the morning, the guardians may bring in the sample when they visit CTU for completion of their outpatient vaccination. If no stool is available before vaccination or the specimen is received outside of 3 hours from collection, 2 rectal swabs will be obtained in BGS (for culture and PCR testing)
- Guardians/mothers of participants will be educated about and given a Memory Aid to track stool frequency and grade each day up to the next 3 days for the determination of diarrhea
- Guardians/mothers will be informed that a field staff will visit their home for the next 3 days to monitor the health of the child, including taking their temperature, reviewing their Memory Aid and recording any symptoms on a source document. The field staff will be responsible for that source document and bring it to and from the participant's home and icddr,b (symptoms/signs collected daily for 3 days post-vaccination).

8.5.2 Outpatient Assessments (Visit 04, 06, 08, 09)

The following procedures will be conducted on **Day 7** for <u>all participants</u>:

- Physical examination : HEENT, skin, lymph nodes, respiratory (lung), cardiovascular (heart), abdomen (including monitoring for intussusception), neurological and musculoskeletal system
- Vital signs: heart rate, axillary temperature and respiratory rate and body weight will be measured
- Clinical Chemistry: Creatinine, AST/ALT, bilirubin, γGT
- Hematology: Total and differential Cell Count and hemoglobin
- Interim medical interview of guardians/mothers to collect any new AEs, to assess ongoing AEs, and to review concomitant medications
- Stool sample collected for immunogenicity and shedding. Study staff will visit participants at their home in the morning for specimen collection and transport back to the lab for fecal IgA processing, culture, and PCR. Stool specimens may be no more than 3 hours old. If no stool is available, the guardian may bring in the sample when they visit the Mirpur field site for completion of their child's outpatient follow-up visit. If no stool is available before the child returns home or the specimen is received outside of 3 hours from collection, 2 rectal swabs will be obtained in BGS (for culture and PCR testing).
- Whole blood sample collected for ALS; plasma from whole blood will be saved for ELISA

The following procedures will be conducted on **Day 35** (**Visit 06**) and **Day 63** (**Visit 08**)

- Physical examination : HEENT, skin, lymph nodes, respiratory (lung), cardiovascular (heart), abdomen (including monitoring for intussusception), neurological and musculoskeletal system
- Vital signs: heart rate, axillary temperature and respiratory rate and body weight will be measured
- Interim medical interview to collect new AEs, assess ongoing AEs and review concomitant medications
- Stool sample collected for immunogenicity and shedding. Study staff will visit participants at their home in the morning for specimen collection and transport back to the lab for fecal IgA processing, culture, and PCR. Stool specimens may be no more than 3 hours old. If no stool is available, the participant may bring in their sample when they visit the Mirpur field site for completion of their outpatient follow-up

visit. If no stool is available before the child returns home or the specimen is received outside of 3 hours from collection, 2 rectal swabs will be obtained in BGS (for culture and PCR testing).

- Whole blood sample collected for ALS; plasma from whole blood will be saved for ELISA.

The following procedures will be conducted on **Day 84 (Visit 09)**:

- Physical examination : HEENT, skin, lymph nodes, respiratory (lung), cardiovascular (heart), abdomen (including monitoring for intussusception), neurological and musculoskeletal system
- Vital signs: heart rate, axillary temperature and respiratory rate and body weight will be measured
- Interim medical interview to collect information about any new chronic health conditions or serious health events
- Stool sample collected for immunogenicity and shedding. Study staff will visit participants at their home in the morning for specimen collection and transport back to the lab for fecal IgA processing, culture, and PCR. Stool specimens may be no more than 3 hours old. If no stool is available, the guardians may bring in their sample when they visit the Mirpur field site for completion of their child's outpatient follow-up visit. If no stool is available before the child returns home or the specimen is received outside of 3 hours from collection, 2 rectal swabs will be obtained in BGS (for culture and PCR testing).

8.6 Early Termination

A participant may be withdrawn from the trial if any of the following reasons apply. However, any participant who has received WRSS1 vaccine will be encouraged to complete antibiotic treatment or ascertainment of clearance of infection and remain in the study for periodic safety evaluations for the duration of the study. An enrolled/vaccinated participant may be withdrawn from the study for any of these reasons.

a) Parent of participant withdraws consent.
b) Investigator or PVS Medical Officer decides that termination is necessary in the event of illness, for participant safety, or for social reasons.
c) If the participant needs treatment that is not allowed in this study.
d) Investigator or PVS Medical Officer decides that termination is necessary to protect the integrity of the study or achieve the objectives of the study.
e) Sponsor terminates the study.
f) If the child fails to follow instructions or miss study visits.

A volunteer may be withdrawn from receiving further vaccinations, but encouraged to complete safety-related follow-up. If the volunteer agrees, other study procedures (e.g., blood sampling for measuring levels of antibodies) may be continued. Withdrawal from further vaccination will occur for:

Safety-related reasons at the discretion of the PI, Study Physician, Emmes Medical Monitor, PVS Medical Officer, IPST, or the participant.

A participant may be discontinued from receiving further vaccinations, but encouraged to complete safety-related follow-up for the following reasons:

(a) A participant vomits in the 30 minute post-vaccination period during 1st vaccination
(b) Occurrence of a severe reactogenicity event.

An excessive number of withdrawals may affect the scientific validity of the study; therefore unnecessary withdrawal will be avoided whenever possible. Should withdrawals occur, efforts will be made to ensure participant safety and to continue and complete safety monitoring as thoroughly as possible. In case of participant withdrawal, for whatever reason, a final trial evaluation must be completed stating the reasons. Withdrawals due to non-attendance must be followed-up by the investigator to the extent possible to obtain the reason for non-attendance.

9.0 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

9.1 WRSS1

9.1.1 Biological Name and Structure

The vaccine will consist of reconstituted lyophilized ampoules of WRSS1 (lot# 0451) containing live attenuated *S. sonnei* bacteria. The vaccine was manufactured in June 1997 and stored at -80°C at the WRAIR Pilot Bioproduction Facility in Silver Spring, Maryland, under current good manufacturing practices. The viability and stability of WRSS1 has been tested periodically since the date of manufacture as shown in the table below. Viability results are averages of at least 2 vials. Stability results are from colony immunoblots using an IpaB monoclonal antibody. The presence of the engineered *virG* deletion allele in WRSS1 has also been confirmed via PCR.

Date of testing	Viability (CFU/vial)	Stability (% Form I CFU)
1997 Jul 1	1.8×10^{10}	92%
2002 Jun 4	3.2×10^{9}	ND
2003 Jan 29	1.6×10^{10}	ND
2004 Feb 6	1.6×10^{10}	ND
2004 Oct 7	1.3×10^{10}	ND
2006 Jan 5	5.0×10^{9}	ND
2009 Jun 23	1.7×10^{10}	96%
2009 Dec 30	1.4×10^{10}	97%
2010 Jun 2	2.4×10^{10}	97%
2012 Feb 7	3.0×10^{10}	95%
2013 Jun 24	1.4×10^{10}	93%
2014 Jun 23	6.0×10^{9}	94%
2015 Jul 15	2.7×10^{9}	93%

ND = not determined

So far, no changes in viability or stability of the vaccine has been noted since its manufacture. Prior to vaccination in Bangladesh, vaccine vials will be shipped on dry ice to the icddr,b INT Laboratory and maintained at -65°C to -85°C until use in the trial.

During the trial, the vaccine vial will be thawed and reconstituted with 5 mL Sterile Water for Injection (SWI) and dilution in saline to achieve the target concentration for dosing. 1 mL of vaccine in saline with the specific CFU dose will be added to the designated volume of saline. Table 11 outlines target volume and buffer volume for vaccine administration for toddlers.

Table 11: Vaccine and Placebo Volumes

	WRSS1 dose volume (ml)	Saline volume (ml)	$NaHCO_3$ buffer (ml)	Total volume
Placebo	N/A	5 ml	10 ml	15 ml
Vaccinee	1 ml	4 ml	10 ml	15 ml

9.1.2 Chemistry, Manufacturing, and Control

S. sonnei WRSS1 vaccine Lot No. 0451 was manufactured by Walter Reed Army Institute for Research (WRAIR) under cGMPs. A cryovial of PCB Lot No. 0433 was added to 1 liter of Luria Bertani (LB) medium (10 g tryptone, 5g yeast extract, 10 g NaCl) with 0.2% glucose and was shaken at 37 ^{0}C for 7.5 hrs; the one-liter culture was used to inoculate 30 liters of LB medium with glucose in a 40 liter production fermenter. When an OD_{600} of 1 - 2 (1.4×10^9 CFU/ml) was attained, the temperature was set to 4°C and the culture was harvested by continuous flow centrifugation. The cell pellet was resuspended in 2 liters of cryopreservative consisting of 7.5% Dextran T10, 2% sucrose, and 1.5% glycerol in Dulbecco's phosphate buffered saline (PBS) [final concentration 3.7×10^{10} CFU/ml].

9.1.3 Final container and composition

S. sonnei WRSS1 is formulated lyophilized in 50 ml serum vials with gray split rubber stoppers sealed with aluminum crimps. Each vial was filled with 5.0 g ±5% of *S. sonnei* WRSS1 vaccine prior to lyophilization. One ml, prior to lyophilization, contained 3.7 x 10^{10} CFU of *S. sonnei* WRSS1 in Dulbecco's PBS containing 7.5% Dextran T10, 2% sucrose, and 1.5% glycerol. Reconstituted vaccine in 5 ml SWI will yield 1-2 x 10^{10} CFU per vial or 2-3 x 10^9 CFU/ml.

Vials are labeled:

Shigella sonnei Strain WRSS1
BPR No.: BPR-228-00 Lot No.: 0451
Contents: 5.0 g ± 5% (lyophilized) Date of Mfg: 27 Jun 97
Caution: New Drug Limited by Federal Law for Investigational Use Only
Storage: -80 ± 10°C
Manufactured By: WRAIR, Washington D C. 20307

Labelled vials are sampled for QC release and stored at -80°C ± 10°C

9.2 Preparation, Administration and Dosage of Study Investigational Product, and Buffer

9.2.1 WRSS1

Lyophilized, frozen vials of WRSS1 Lot 0451 will be transported on dry ice to the INT Laboratory for storage at -80°C ± 10°C until the day of vaccination. On the day of vaccination the vaccine vials will be removed for preparation and placed on ice in a laminar flow hood and allowed to thaw for 30 minutes before adding 5 ml of sterile water for injection (SWI). The vaccine is allowed to rehydrate for another 15 minutes on ice, with intermittent swirling of the suspension to ensure homogeneous mixing of the vial contents. The reconstituted vaccine will then be diluted in sterile saline to arrive at the desired concentration and the diluted vaccine will be kept on ice until vaccination. Time of reconstitution will be noted. The dilutions will be titered and replica spread plate quantitative cultures will be made of the inoculum dilutions before and after vaccination to confirm viability and dose.
One ml of the vaccine solution in saline at the appropriate concentration will be placed into an additional 4 ml of sterile saline. Ten ml of a freshly prepared bicarbonate solution (2 g in 150 ml of SWI) will be placed in a second cup.
All participants will fast for 60 minutes prior to and after receiving the vaccine. Care must be taken to ensure that the time between reconstitution and administration of vaccine to the participants does not exceed 2 hours. Further details for vaccine preparation, administration, and dispensation are provided in the study-specific SOP.

9.2.2 Placebo

Placebo recipients will receive the designated volume of bicarbonate buffer plus the designated volume of sterile saline solution according to the Table 11.

9.2.3 Sodium Bicarbonate

Participants randomized to the vaccine and placebo group will receive the same amount of bicarbonate buffer as mentioned in table 11. Further details for buffer preparation, administration, and dispensation are provided in the study-specific SOP.

9.3 Accountability Procedures for the Investigational Product(s)

The PI will ensure that the investigational product supplies are stored as specified in the protocol and in a secured area, with access limited to authorized study personnel. The investigator will maintain accurate records of the receipt of all investigational products, including date received, manufacture or expiration date, amount received, and disposition. A record will be maintained that includes the dispensation date, amount of investigational product dispensed, initials, and identification number. The investigational product will be administered only at the specified institution. The investigator will keep an inventory of the study materials, will hold the amount of product needed, and will be responsible for adequate storage and dispensing of the

vaccines. Investigational product management will be delegated by the investigator to designated study staff at the INT Laboratory.

9.4 Assessment of Participant Compliance with Investigational Products

A study staff member or designee will witness the ingestion of WRSS1 or placebo.

10.0 ASSESSMENT OF SAFETY

10.1 Adverse Events

The investigator is responsible for accurate documentation of all AEs according to the detailed guidelines set out below. The guardians will be instructed to contact the investigator immediately should they observe any signs or symptoms in their child perceived as significant during the study period. Approximately 1 month after administration of the last dose of investigational product, all participants will be seen at the Mirpur Field Office for a final outpatient visit to close out any existing adverse events and identify and track any new chronic health conditions or SAEs.

10.1.1 Adverse Event Definitions (http://www.ecfr.gov/cgi-bin/text-idx?SID=e3eba258602082611eadeafcee788296&mc=true&node=pt21.5.312&rgn=div5#sp21.5.312.b)

Adverse event is any untoward medical occurrence in humans, whether or not considered drug related, that occurs during the conduct of a clinical trial. Any change in clinical status, ECGs, routine labs, x-rays, physical examinations, etc., that are considered clinically significant by the study investigator, is considered an AE.

This definition includes any noxious, pathological or unintended change in anatomical, physiological or metabolic functions, as indicated by physical signs, symptoms and/or clinically significant laboratory abnormalities occurring in any phase of the clinical study, whether associated with the study vaccine or placebo.

This definition also includes an exacerbation or worsening of pre-existing conditions or events, intercurrent illnesses, injuries, or vaccine or drug interaction, or worsening of abnormal clinical laboratory values.

Suspected adverse reaction is any adverse event for which there is a reasonable possibility that the drug caused the adverse event. A reasonable possibility implies that there is evidence that the drug caused the event.

Adverse reaction is any adverse event caused by the drug.

Serious Events (Serious Adverse Events, Serious Suspected Adverse Reactions Or Serious Adverse Reactions)

A Serious Adverse Event, including serious suspected adverse reaction or serious adverse reaction as determined by the Investigator or the sponsor, is any event that results in any of the following outcomes:

1. Inpatient hospitalization or prolongation of existing hospitalization
2. Life-threatening AE (Life-threatening means that the study participant was, in the opinion of the investigator or sponsor, at immediate risk of death from the reaction as it occurred.)
3. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
4. Congenital abnormality or birth defect
5. A medically important event that may jeopardize the participant or may require intervention to prevent one of the other outcomes listed above. Medical judgment should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalization, but may jeopardize the participant or may require intervention to prevent one of the other outcomes listed in the definition above, should be considered as serious. (Examples of such cases are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasia or convulsions that do not result in hospitalization; developments of drug dependency, overdose, or drug abuse.)
6. Death

Unexpected Adverse Event

An adverse event is "unexpected" when its nature (specificity) or severity is not consistent with applicable product information, such as safety information provided in the Investigator's Brochure or if not available, the investigational plan, or the protocol.

10.1.2 Reactogenicity

Reactogenicity events are AEs that are commonly seen with vaccines and/or known to occur for WRSS1 and similar enteric vaccines and will be collected in the source documents and eCRFs using a grading scale based on functional assessment or magnitude of reaction. Reactogenicity are solicited AEs that occur within 72 hour after WRSS1 vaccination and will be collected on the appropriate source document and recorded on a reactogenicity eCRF.

Based on prior studies of WRSS1 vaccine, it is anticipated that WRSS1 will be well-tolerated. However, there is the possibility that this vaccine may result in symptoms similar to infection with *Shigella*, including moderate to severe diarrhea (which, though unlikely, could lead to dehydration and the need for oral or intravenous rehydration). Other symptoms consistent with shigellosis that may occur are listed in the table below.

Table 12. Reactogenicity events to be solicited daily during the first 72-hours post-vaccination

Systemic symptoms	GI symptoms	
fever	abdominal pain	dysentery
decreased appetite	nausea	bloating
irritability	vomiting	excess flatulence
decreased activity	loose stool	constipation
	diarrhea	

The grading for reactogenicity events are given in Appendix 3.

Systemic and intestinal reactogenicity symptoms and severity grading are as follows:

Study Specific Definitions
Systemic symptoms – fever, decreased appetite, irritability, decreased activity
Intestinal symptoms – abdominal pain, nausea, vomiting, loose stool, diarrhea, dysentery, bloating, excess flatulence, constipation

10.1.3 Guidelines for Determining Causality of an Adverse Event

The Investigator should consider the following question when assessing causality of an AE to study drug:

Is there a reasonable possibility that the drug caused the event?

Reasonable possibility implies there is evidence that the study product caused the reported event. An affirmative answer designates the event as a suspected adverse reaction.

10.1.4 Follow-up of Adverse Events and Assessment of Outcomes

The Investigator should monitor for AEs until Day 224 for all participants. For reporting and follow-up of an SAE, see protocol section 10.1.8.

10.1.5 Treatment of Adverse Events

Treatment of any AE is at the sole discretion of the investigator and according to the best treatment currently available. The applied measures should be recorded in the CRF of the participant. The PI may also refer a participant for appropriate care, if applicable. PATH will provide payment for any harm caused by participation in the study.

10.1.6 Recording Adverse Events

Any research related injuries and adverse events will be followed periodically until the events are resolved or become stable. Follow-up process can be both telephone and/or asking the mothers/guardians to visit the study site with their children, which depends on their conveniences and/or the severity of the particular event per clinical judgment of the study physician and PI. Whenever possible, the investigators will record the diagnosis, if available. If no diagnosis is available, the investigators will record each sign/symptom as individual AEs. The nature of each event, date of onset, end date, outcome, severity and relationship (causality) to WRSS1 should be established. AEs already documented in the eCRF, i.e., at a previous assessment and not resolved, should be reviewed at subsequent follow-up assessments. If resolved, documentation in the eCRF should be completed. The entire duration of AEs will be recorded. AE severity will be recorded as the worst grade for the duration of each illness/symptom. Once the sign/symptom resolves for 24 hours or greater, the AE will be resolved. If the sign/symptom reoccurs after 24 hours, then it will be documented as a new event, unless clinician has judged these as constituent components of the same event.

10.1.7 Reporting Serious Adverse Events

All SAEs (irrespective of the causality) are to be reported to the DSMB of icddr,b Ethical Review Committee (ERC), Sponsor, medical monitors of Sponsor and Emmes within 24 hours of the study team becoming aware of the event.

All SAEs must be documented on an icddr,b **Serious Adverse Event Form** as well as entered into the electronic SAE Reporting System. A complete written report will follow the initial notification.

The PI will provide the SAE form to the Sponsor who will decide whether or not the SAE meets FDA and WIRB criteria for immediate reporting. The investigator will update the Sponsor, Sponsor MM, and Emmes MM with new information as available.

The Sponsor MM and Emmes MM are required to review all unanticipated events involving risk to participants or others, serious adverse events and all participant deaths associated with the protocol and will provide a written report. At a minimum, the Sponsor MM and Emmes MM must comment on the outcomes of the event or problem and, in case of a serious adverse event or death, comment on the relationship to participation in the study. The Sponsor MM and Emmes MM must also indicate whether he/she concurs with the details of the report provided by the principal investigator.

All unanticipated events involving risk to participants or others, serious adverse events related to participation in the study, and participant deaths related to participation in the study should be promptly reported by phone to the following Medical Monitors:

Tushar Tewari, MD PVS Medical Officer PATH Vaccine Solutions Phone: 91.11.2653.0080 Fax: 91.11.2653.0089 Email: ttewari@path.org	Robert Lindblad, MD Emmes Medical Monitor The Emmes Corporation Phone: 301-251-1161 Fax: 301-251-1355 Email: rlindblad@emmes.com

The Investigator is required to submit a completed SAE Report form via the electronic SAE reporting system within 24 hours of recognition of the event as an SAE. All additional follow-up information for the SAE must be entered or uploaded into the electronic SAE reporting system within 24 hours upon receipt of new information, and entered into the electronic SAE Reporting System.

Sites will be trained in reporting AEs and SAEs. Notification of reported events will be generated at the time of entry into the data system and all SAEs will be promptly reviewed by the medical monitor within the data system. Summary review of all reported AEs and SAEs will be compiled on a weekly basis to identify any safety trends. Review of safety lab results will also be conducted on a weekly basis. All SAEs that meet expedited reporting criteria (suspected adverse reactions that are serious and unexpected) will be submitted to FDA via phone/fax /e-mail within 7 days followed up with a written report within 15 days of the event, if life threatening or result in death or as a written report within 15 days for the remainder. Written reports will use the MedWatch 3500A form. Follow-up information on a Safety Report will be submitted as soon as the

information becomes available. Any series of events that as a group are considered "unexpected" would be submitted within 15 days of that determination being made by the sponsor. Annual reports would be submitted in accordance with IND regulations.

To the IRB

The Investigator is responsible for submitting the safety report (initial and follow up SAE reports) or other safety information (e.g. revised Investigator's Brochure) to the icddr,b ERC and DMSB according to icddr,b requirements, and for retaining a copy in the site's study file. The Investigator is also responsible for notifying the Western Institutional Review Board (WIRB), PVS' designated Independent Ethics Committee, according to WIRB guidelines outlined below:

WIRB Guidelines

All SAEs will be reported to WIRB according to WIRB guidelines and using the Unanticipated Problems that are Adverse Events form.

WIRB Phone: 800-562-4789, Fax: 360-252-2498.
An unanticipated problem is defined as any incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document, or the Investigator Brochure; and (b) the characteristics of the subject population being studied;
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the drugs, devices or procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Investigators are required to report adverse events that fit the above criteria *within 10 working days* of the time the investigator becomes aware of them.

10.1.8 Follow-up of Serious Adverse Events

All SAEs must be documented and followed up until the event resolves, subsides, or stabilizes or the study participant is lost to follow-up. Continued follow-up of SAEs is at the discretion of the PI according to good clinical management, or until the participant is referred to another institution for continued care. All SAEs will be reported to the DSMB of icddr,b; the PI will follow-up with the DSMB with any updates accordingly. WIRB expects reports of unanticipated problems to include a corrective action plan to address the issue, or written justification for why none is provided, on the Unanticipated Problems that are Adverse Events form.

10.2 Safety Oversight - Data Safety Monitoring Board (DSMB)

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

A Data and Safety Monitoring Board (DSMB) will be formed by the Ethical Review Committee to evaluate and assess the safety of study participants. Moreover, there will be an Internal Protocol Safety Team (IPST) comprising of the study physician, the Medical Monitor from Emmes, the principal investigator, and the Medical Monitor from PVS.

In each cohort, the first vaccine dose will be conducted at the icddr,b Inpatient Unit (Clinical Trials Unit, icddr,b main campus), for immediate safety evaluation where the participants will be admitted for observation for 24 hours post-vaccination. This will help in clinical assessments accurately and promptly, addressing any potential reactogenicities. Clinical signs and symptoms, including daily stool output and temperature will be monitored. Second and third vaccinations within each cohort will take place on an outpatient basis. Before enrolling participants in subsequent cohorts to receive a higher vaccine dose, the safety data from the previous cohort(s) (through Study Day 7) will be evaluated and reviewed by the Internal

Protocol Safety Team (IPST). This data include, but is not limited to, physical examinations, vital signs and solicited reactogenicity symptoms.

The DSMB will be composed of 3-5 members, two of which are nominated by the ERC chairperson. The PI also has the option of nominating 1-2 members with therapeutic expertise. The DSMB will convene three times during the study period, at the beginning before the trial is started, after completion of vaccination in cohorts 1 and 2 and at the end of the trial. IPST will review the safety of each cohort and have the option of convening a DSMB meeting if there is a safety issue that requires additional interpretation. The PI can also request a DSMB meeting depending upon the study complexity or in light of safety concerns. The DSMB will advise the PI of its findings and provide recommendations. The DSMB will review all unanticipated problems involving risk to the participants or others, serious adverse events, and all participant deaths associated with the protocol. The PI will inform the Emmes Medical Monitor and the PVS Medical Monitor in detail about the discussions of those meetings.

10.3 Stopping Rules

The following study halting rules refers to suspected adverse reactions and will automatically pause or halt further vaccinations within any given cohort:

1. One or more participants with a serious AE (SAE) occurring at any time following vaccination that is related to the WRSS1 vaccine
2. One or more participants experience systemic allergic reaction (i.e., bronchospasm, allergy-related edema/angioedema, hypotension or anaphylaxis) associated with administration of WRSS1 vaccine
3. One or more participants experience unexpectedly severe disease (i.e., hypotension (disproportionate to volume loss), renal dysfunction, or altered mental state (e.g., somnolence) after vaccine administration.
4. Severe diarrhea occurring in $\geq$20% of participants at a given dose level.
5. 20% or more of the participants present a vaccine related unanticipated moderate or greater AE, grade 3 fever or grade 3 laboratory abnormalities.

The study PI, with the assistance of the IPST, DSMB and Data Management group at Emmes will be responsible for determining if the halting rules are met. During the safety data assessment period, which may require unblinding of safety data by the DSMB committee as deemed necessary, no new participants will be vaccinated. The DSMB will be responsible for recommending whether or not to re-start the study. An outline of the extent of the safety assessment conducted and justification for the decision taken will be documented in the study file. Ongoing participants will receive their subsequent vaccinations at the discretion of the PI and IPST. In the event that there is an acute safety issue, the Sponsor MM may suspend enrolment/treatment, if warranted, pending review by the DSMB.

10.4 Six Month Follow-up Safety Surveillance

At Day 224 ± 2 weeks, participants will be visited by study staff to inquire about post-study chronic health conditions, serious health events, or hospitalizations. If three attempts to reach the participant are unsuccessful, then no more visits are required and this fact will be documented in the volunteer's source documents and on the eCRF page. This long-term follow-up information will be summarized in the final clinical study report.

11.0 MONITORING BY SPONSOR'S REPRESENTATIVE

During the study, the investigators will maintain complete and accurate documentation for the study, including records detailing the progress of the study for each participant, laboratory reports, eCRFs, signed ICF for each study participant, drug disposition records, correspondence with the IRB, the study monitor and the sponsor, AE reports and information regarding participant discontinuation and completion of the study.

All required study data will be recorded clearly and accurately by authorized study personnel in the participant's source documents, and required information will be entered into appropriate eCRFs. Only designated study site personnel shall record or change data in the eCRFs. During the study, the PI will be responsible for ensuring the quality of data recorded in the eCRFs as per written eCRF completion guidelines. Training of study personnel on GCP conduct of the trial, including study procedures and data management responsibilities, will be provided by the PI and Emmes prior to study initiation and throughout the trial, as needed.

11.1 Study Monitoring

Sponsor monitoring responsibilities will be provided by Emmes as outlined in the Study Monitoring Plan. Monitoring will be conducted prior to beginning, at initiation, during, and at closeout of the study by the study monitor.

During the course of the study, the monitor will visit the clinical site at regular intervals to verify compliance to the protocol; completeness, accuracy, and consistency of the data; and adherence to local regulations and requirements, including GCP on the conduct of clinical research. The monitor should have access to participant medical records and other study-related records needed to verify the entries on the eCRFs.

The PI and the monitor must agree to cooperate to ensure that any problems detected in the course of these monitoring visits, including eCRF completion and query resolution, are resolved.

To ensure the quality of clinical data across all participants at the site, a clinical data management review will be performed on participant data received at Emmes. During this review, participant data will be checked for consistency, omissions, and any apparent discrepancies. In addition, the data will be reviewed for adherence to the protocol and GCP. To resolve any questions arising from the clinical data management review process, data queries and/or site notifications will be sent to the site for completion and return to Emmes.

Essential documents must be filed in the site study file on an ongoing basis. Monitoring visits will be performed according to Study Monitoring Plan.

11.2 Independent Auditing

PVS or PVS representatives may audit the study to ensure that study procedures and data collected comply with the protocol and applicable SOPs at icddr,b and Emmes, and that data are correct and complete. The PI will permit auditors (employees of the Sponsor or an external company designated by the Sponsor) to verify source data validation of the regularly monitored clinical study. The auditors will compare the entries in the eCRFs with the source data, and evaluate the study site for its adherence to the clinical study protocol and GCP guidelines and applicable regulatory requirements.

11.3 Regulatory Agency Auditing

The PI must be aware that representatives from regulatory authorities or the IRB may wish to inspect the eCRFs and associated study records. The PI will notify the Sponsor within 24 hours following contact by a regulatory agency. The PI and study coordinator must make the relevant records available for inspection and will be available to respond to reasonable requests and audit queries made by authorized representatives of regulatory agencies. The PI will provide the Sponsor with copies of all correspondence that may affect the review of the current study or his/her qualification as an investigator in clinical studies conducted by the Sponsor. The Sponsor will provide any needed assistance in responding to regulatory audits or correspondence.

12.0 SAMPLE SIZE CALCULATION AND OUTCOME PRIMARY AND SECONDARY VARIABLES

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

12.1 Introduction

This study is a double-blind, randomized, placebo-controlled, dose-escalation clinical trial to examine the safety, tolerability and immunogenicity of WRSS1. The primary objective of the study is to evaluate the safety and tolerability of the vaccine; the secondary objective is to evaluate vaccine immunogenicity.

Participants will be randomized separately for each cohort, at a vaccine: placebo ratio of 12:4. The randomization code will be generated and maintained by Emmes.

12.2 Sample Size

The chosen sample sizes (12 vaccinees and 4 placebo subjects/dose group) will not allow for detailed statistical comparisons across the dose groups and rigorous statements about the safety or immunogenicity of

the vaccines, it will provide preliminary safety information that may support testing the product in additional larger cohorts in the target population – children 12-24 months of age.

The goal of the safety evaluation is to identify safety concerns associated with product administration. The ability of the study to detect SAEs can be expressed by the true rate above which at least 1 SAE would likely be observed and the true event rate below which no events would likely be observed.

The active arms in which 48 participants will receive vaccine, will allow for the recognition of unacceptable toxicity rates (SAEs) occurring at a frequency of 10% or higher. For instance, the probability of observing one event among thirty participants is very high (close to 90%) if the true attack rate of that event is 10%.

12.3 Data Analysis

Describe plans for data analysis, including stratification by sex, gender and diversity. Indicate whether data will be analysed by the investigators themselves or by other professionals. Specify what statistical software packages will be used and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to determine further course of the study.

For Primary Safety Endpoints

All visits after the participants have been exposed to the study product will be included in the primary analysis of safety. To assess safety, the number and the percentages of participants experiencing at least one AE, and the number and percentage of participants experiencing each specific AE will be tabulated by study arm. Overall summaries by arm include: 1) any solicited reactogenicity; 2) any unsolicited AEs; 3) any SAEs, and 4) any unsolicited AEs and SAEs judged as having a reasonable possibility that the study product caused the event.

Proportions will be used to assess the tolerability to the study vaccine using only the participants randomized to the vaccine arm. Tolerability measures will be tabulated by study group along with their corresponding 95% confidence intervals.

For Secondary Immunology Endpoints:

Immunologic response will be presented for each parameter (e.g. IgA, IgG, and IgM antibody responses) and defined visits using descriptive statistics. Changes from baseline to each visit will be presented. Repeated measure analysis of titer values will be conducted to assess treated dose and temporal differences. The number and percentage of participants with a positive immune response will be presented by dose group and analyzed with repeated measures models using generalized estimating equations. Attempt will be made to recruit equal number of male and female participants in the study so that separate analysis for males and females can be conducted and gender-specificity in the immune responses to the vaccine can be analyzed. Review of immunogenicity data will not be done until after specimens are collected at Day 63.

Analysis of immunology data will include:

- Prevalence of 4-fold increases of anti-LPS and anti-invaplex IgG, IgA and IgM in ALS and serum/plasma over baseline (prevaccination) titers. Prevalence of 4-fold increase in LPS and invaplex-specific IgA in stool over baseline titers.

Analysis of vaccine shedding data will include:

- Prevalence and distribution of vaccine shedding as determined by quantitative culture and PCR

For exploratory Endpoints

- Prevalence of 4-fold increase in SBA antibody titers from baseline (seroconversion);
- The cytokine data will be analyzed by generalized estimating equation model between placebo and vaccine groups, (cytokine assessment at (baseline and post vaccination days) and the interaction between two groups).

12.3.1 General Analytic Plan Associated Assessment of Safety and Immunogenicity

When the use of descriptive statistics to assess group characteristics or differences is required, the following methods will be used: for categorical variables, the number and percent in each category; for continuous variables, mean (with standard deviation) or median (with quartiles), and range (minimum, maximum).

Within-arm assessment of the change from the baseline measurement to a follow-up measurement will be analyzed using McNemar's test (for categorical response variables), or the paired t-test or Wilcoxon signed-ranks test (for continuous variables) or generalized estimating equation model.

12.3.2 Final Analysis Plan

A thorough Statistical Analysis Plan will be developed before any data reports are produced. The analysis of safety will include inpatient and outpatient observations and interviews with participants through Day 84. Analysis of immunogenicity will include all participants who provide specimens through Day 84. The following specific outcomes will be analyzed:

- Rate of occurrence of reactogenic side effects and AEs. Rates and 95% CIs will be described.
- Rate of seroconversion to LPS and invaplex by IgG, IgA and IgM ELISA at multiple time points. A response for serum/plasma antibodies will be defined as a four-fold rise after immunization over baseline (prevaccination) titers.
- Prevalence of 4-fold increases of anti-LPS and anti-invaplex IgG, IgA and IgM in ALS over baseline titers.
- Rate of fecal response to LPS and invaplex by IgA ELISA at multiple time points. Fecal antibody responses will be defined as a four-fold rise for specific IgA or a four-fold rise of the ratio of specific over total IgA over baseline titers at any time point after immunization.
- Comparison of cytokine response at multiple time points between vaccine and placebo groups.
- Rate of SBA seroconversion at multiple time points.

13.0 DATA SAFETY MONITORING PLAN (DSMP) AND DATA MANAGEMENT

The PI is responsible for ensuring the accuracy, completeness, and timeliness of the data reported. Data collection is the responsibility of the clinical trial staff at the study site under the supervision of the study PI. Emmes is responsible for electronic data management, quality review, final safety analysis, and reporting of the study data.

13.1 Database Development

Case Report Form

The eCRFs will be developed by Emmes and approved by PATH Vaccine Solutions.

Completion of CRFs

Electronic Data Capture (EDC) will be the method of data collection in this study. Data for each participant will be recorded in the eCRF and verified by the Investigator. It is the Investigator's responsibility to ensure the accuracy, completeness, legibility, and timeliness of the data reported in the participant's eCRF and any supporting documentation.

Source documentation supporting the eCRF data should indicate the participant's participation in the study and should document the dates and details of study procedures, AEs and the participant's status. The Investigator at icddr,b will maintain all information in the eCRFs and all source documents that support the data collected from each participant.

The Investigator or designated representative should complete the eCRFs as soon as possible after information is collected. Completed eCRFs must be submitted for each screened participant who signs the study specific ICF and is vaccinated. The PI/Investigator will retain all essential documents and a CDROM copy of the eCRF data.

Provider of Data Management

Data Management activities will be provided by Emmes and according to SOPs.

Details of Data Management

A Data Management Plan (DMP) will be written by Emmes and contain all study-specific requirements.

Coding

Coding of the AE and concomitant medication data will be done by Emmes. The most recent versions of the coding dictionaries will be used: MedDRA for coding of the AEs and WHO Drug for the coding of the concomitant medications. Once all coding has been completed, listings with the coded data will be provided to the Sponsor for review and approval by the Sponsor Medical Monitor.

13.2 Data Validation

Emmes will confirm that all data entered into the database are correct and consistent with CRFs.

13.2.1 Source Data Verification

For source data verification (SDV), the monitor (on behalf of the study sponsor) must have direct access to source documents that support the data recorded, e.g. medical records, original laboratory records and ICFs. If source data are electronic, these data must be printed, signed and dated by Investigator and stored in the Investigator study file. Clinical laboratory data will remain in study participant records. Essential documents, including ICFs, must be filed and kept in the Investigational Study File (ISF) on an ongoing basis.

13.2.2 Definition of Source Data

Source data are all information in original records or certified copies of original records of clinical findings, observations, or other activities in a clinical study. Source data are contained in source documents.

13.2.3 Definition of Source Document

Original source documents, data and records, e.g., hospital records, medical notes, laboratory notes, evaluation checklists, pharmacy dispensing records, records kept at the pharmacy and at the laboratory, documentation of shipments etc.

13.3 Database Locking Procedures

- *A final database lock,* for the primary analysis will occur after all participants have completed all follow-up visits and a case by case review of the severity of any AEs has been performed and finalized.
- Remaining immunology data will be maintained in a separate immunology database.

13.4 Record and Specimen Archival

The Investigator is responsible for retaining all essential documents listed in the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) Guideline. Study documents will be retained for a minimum of 2-years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. No records will be destroyed without the written consent of the IND sponsor, PVS. It is the responsibility of the Sponsor to inform the investigator when these documents no longer need to be retained.

13.5 Screen Failures

If a participant signs the study specific ICF but is not randomized because of non-eligibility (a screen failure), the reason for his/her non-eligibility should be entered in the medical records/notes/charts. Also, a screening log must be kept. Screen failure data will not be recorded in the eCRFs.

13.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or manual of procedures requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly. Major deviations from the protocol, as defined as deviations that impact participant safety or endpoints, will be promptly reported to the Sponsor, WIRB, and DSMB. Minor deviations from the protocol will be recorded on a source document and corresponding eCRF, and included in the annual report. Examples of minor deviations are events outside the control of the investigators (e.g., participant missed visit window or study parameters that are not part of the primary endpoint was not performed at a visit).

14.0 OBLIGATIONS AND ROLES OF THE SPONSOR, INVESTIGATOR AND STUDY PERSONNEL

This study will be conducted according to Good Clinical Practices and in accordance with all federal regulations regarding the protection of human participants in research including US 21 CFR Part 50 and US 21 CFR Part 312. The investigators agree to perform the research in strict accordance with this protocol, the International Conference on Harmonization (ICH) Guideline for Good Clinical Practice (E6).

In addition, the investigator must follow local and institutional requirements including, but not limited to, investigational product, clinical research, informed consent and Investigational Review Board (IRB) regulations. The Sponsor will provide notification to the investigator of protocol and amendment approvals by regulatory authorities when applicable.

The MM will be responsible for reviewing all serious and unexpected adverse events and providing an unbiased written report of the event.

15.0 ETHICS/PROTECTION OF HUMAN PARTICIPANTS

Describe the justifications for conducting this research in human participants. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how participants' rights will be protected, and if there would be benefit or risk to each participants of the study. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Discuss procedures safeguarding participants from injuries resulting from study procedures and/or interventions, whether physical, financial or social in nature. [Please see Guidelines]

15.1 Clinical Development Plan

The proposed study builds upon successful preliminary observations with this vaccine in the US, Israel and Thailand (see WRSS1 Investigator's Brochure) and in older endemic populations (adults and children in Bangladesh). Upon establishing a safety profile in the first two age groups (adults and children), and with concurrence with the DSMB, a separate protocol has now been developed to evaluate the WRSS1 vaccine in toddler group (12-24 months old). The new protocol seeks to examine critical variables to maximize immune responsiveness in young children, using approaches compatible with routine immunization practices.

The overall long term strategy for development of a live *Shigella* vaccine includes defining the formulation of buffers and vaccine vehicles that permit low-volume administration and maximum viability of the vaccine, establishing the number of needed doses, and identifying a maximally immunogenic, safe dose level of this WRSS1 prototype strain in the target population. WRAIR is currently developing the *S. flexneri* vaccine strains to produce a broadly protective multivalent oral shigella vaccine.

15.2 Ethical Conduct of the Study

This study will be conducted using Good Clinical Practice (GCP) and in accordance with the World Medical Association (WMA) Declaration of Helsinki and all US federal regulations regarding the protection of human participants in research, including The Nuremberg Code, The Belmont Report, US 21 CFR Part 50 and US 21 CFR Part 312 - Protection of Human Participants and 32 CFR 219 (The Common Rule).

The investigator agrees to conduct the research in strict accordance with this protocol, the ICH Guideline for GCP, as well as in conformity with any US federal and Bangladeshi provincial or local regulations regarding the conduct of clinical studies. The sponsor and investigator must comply with all applicable regulations. In addition, the investigator must follow local and institutional requirements including, but not limited to Investigational Medicinal Product (IMP), clinical research, Informed Consent Forms (ICF) and Institutional Review Board (IRB) regulations.

The Sponsor will provide notification to the investigator of protocol and amendment approvals by regulatory authorities. Except where the investigator's signature is specifically required, it is understood that the term "investigator" as used in this protocol and on the electronic Case Report Forms (eCRFs) refers to the investigator or appropriate study personnel that the investigator designates to perform a certain duty. Nevertheless, the investigator alone is ultimately responsible for the conduct of all aspects of the study. Sub-investigators or other appropriate study personnel are eligible to sign for the investigator on designated eCRFs.

15.3 Institutional Review Board

The icddr,b Research Review Committee (RRC); and icddr,b Ethics Review Committee (ERC) and Western Institutional Review Board (WIRB) will review the protocol.

15.4 Informed Consent Process

The study staff will discuss the informed consent document in detail with the parents of potential participant. Group discussions may also take place to orient participant to the study; however individual discussions will commence prior to obtaining consent signatures. Before any study-related activities and in agreement with applicable regulatory requirements, the Investigator must ensure that the parents of participants are fully informed about the aims, procedures, potential risks, and potential benefits of the study. The parents will be given the written, IRB approved ICF, allowed ample time to read the consent, encouraged to ask questions about the study, have the questions answered and then be given time to decide if they would allow their children to participate in the study. It will be emphasized that participation is voluntary, and that the parents of the participant has the right to withdraw their children from the study at any time without prejudice.

The Investigator must obtain voluntary, personally signed and dated informed consent from parents before any study-related procedure. The original, signed ICF must be kept in the site study file.

15.5 Risk/Benefit

The WRSS1 vaccine has been administered previously to participants at doses equal to or higher than the doses to be used in this study. Participants may experience fever and/or diarrhea post-vaccination, and possibly dehydration requiring oral or intravenous rehydration. Nausea, abdominal cramps and malaise are also possible post-vaccination. There is a theoretical risk of an oral vaccine causing intussusceptions which requires emergency management. There is also a rare risk that an oral live attenuated vaccine may cause seizures or reactive arthritis. Good phlebotomy practices will be performed during blood draws, to minimize the risk to the participant. Participants and staff will be trained in proper hand washing techniques.

The results of this study will provide additional information for obtaining potential immune responses in endemic populations in an effort to overcome mucosal administration issues such as enteropathy. This information will contribute to the scientific enteric vaccine field overall and potentially inform public health professionals for establishing immunization schedules and policies for at risk populations.

16.0 USE OF ANIMALS

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

No animals will be used in this study.

17.0 PUBLICATION POLICY

PATH Vaccine Solution (PVS) has registered this trial on www.ClinicalTrials.gov accordingly to the trials registration policy (NCT01813071). Upon abstract and/or manuscript development by the investigators from icddr,b, PVS must review and approve any abstract and/or manuscript prior to publication as stated in the clinical trial agreement. Sub studies, if any, will have to be concurred by icddr,b and PVS.

18.0 FACILITIES AVAILABLE

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

The urban field site in Mirpur, a suburb of Dhaka in Bangladesh will be used for recruitment, screening, and consenting, outpatient dosing and/or assessment. A team of field staff will be involved in initial selection of participants and visit participants' home. One/two physicians and 1/2 nurses will be present during screening, and consenting, outpatient vaccination and/or assessment. The data entry of outpatient dosing and/or assessment will be done in level 8 of the main icddr,b building. The Mirpur site has been used for intensive studies of cohorts of children including epidemiology of amoebiasis using molecular techniques; Phase1/2 studies of new vaccines for enterotoxigenic *E. coli* and rotavirus, and community-based interventions for

malnutrition. Children will be brought to the ICU of Dhaka Hospital in case of emergency when beds in the ICU are available.

For each cohort, the first dose of vaccine and subsequent inpatient follow-up period will take place at the icddr,b Clinical Trials Unit (CTU) located on the icddr,b main campus and is about 150 yds from the main building. One full-time physician and 2 nurses will be available at the CTU during the entire period of inpatient phase. Another 3-4 physicians and nurses can be called from the main hospital during the inpatient days in CTU. icddr,b can provide the services of security guards, attendants, cook etc. The CTU has 18 beds (9 for females and 9 for males), patient exam room for vital signs recording and observation, dining area, recreation area, kitchen and wash rooms for the participants on the same floor. The sample processing room, and storage of study medication (refrigerator and ambient storage space available), document storage cabinets, store room, and data entry room are also located inside the CTU. The data entry for the Inpatient duration will be done at the CTU. The participants are provided with individual lockers for storing their belongings during the admission to the facility (total 18 lockers available).The participants are provided with dress gowns at the time of admission to the facility and are not allowed to go out of the facility during the inpatient phase. There is a separate recreation room for the volunteers with facilities of TV, magazine and indoor games. The CTU also has a separate dining area for the volunteers, which is close to the kitchen. Fire extinguishers are available to deal with any fire emergencies. A list of all emergency contact numbers are available in the patient exam room. A physician and 2 nurses will be available at the CTU during the entire period of inpatient phase. The study volunteer, in case of an emergency, will be directly transferred to the Dhaka hospital located on the ground floor of the icddr,b's main building on campus. An emergency trolley (Crash cart) will be available throughout the inpatient period, equipped with an ambu bag, laryngoscope, intubation tubes, emergency medicines, ECG machine, oxygen cylinders and oxygen masks, nebulizer, infusion equipment, and disposable syringes.

Laboratory analysis of specimens for screening and safety will be carried out in the Clinical Hematology and Microscopy Lab, the Clinical Biochemistry Lab, and the Clinical Microbiology and Immunology Lab under the Clinical Laboratory Services. Analysis of immunology specimens, and stool culture/PCR will take place at the icddr,b INT Laboratory and Enteric Microbiology Laboratory, respectively, under the direction of the PI, Dr. Rubhana Raqib.

Vaccine preparation will take place at the INT Laboratory under BSL-2 conditions and transported to the CTU for dose administration within 2 hours of reconstitution according to the vaccine preparation SOP.

19.0 Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however, exercise judgment in assessing the "standard" length.

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APPENDIX 1: Time and Event Schedule

	Screen	**Admission and 1st Dose Inpatient**	**Discharge**	**F/U Visit**	**2nd Dose**	**F/U Visit**	**3rd Dose**	**F/U Visit**	**F/U Visit**	**F/U Visit**
Visit Number	**Visit 01**	**Visit 02**	**Visit 03**	**Visit 04**	**Visit 05**	**Visit 06**	**Visit 07**	**Visit 08**	**Visit 09**	**Visit 10**
Study Day	Day -30 to -9	Day 0	Day 1	Day 7	Day 28	Day 35	Day 56	Day 63	Day 84	Day 224
Visit Window	-30 to -9 days	0 to 1 days	0	Visit 02 + 6-8 days	Visit 02 + 27-29 days	Visit 05 + 6-8 days	Visit 05 + 27-29 days	Visit 07 + 6-8 days	Visit 07 + 27-29 days	Visit 09 + 126-154 days
Informed Consent	X									
Inclusion/Exclusion Criteria	X	X			X		X			
Medical History	X									
Physical Exam	X	X	X	X	X	X	X	X	X	
Vital Signs[1]	X	X	X	X	X	X	X	X	X	
Screening serology[2]	X									
Hematology	X			X						
Clinical Chemistry	X			X						
Admission and familiarization		X								
Randomization		X								
WRSS1 Vaccine Dosing		X			X		X			
Discharge from Inpatient Unit			X							
Interim Medical Interview, Concomitant Medications review, and AE Review		X	X	X	X	X	X	X	X	
Reactogenicity Assessment		X	X		X		X			
Field staff Visits	X		X		X		X			X
Stool Culture[3]	X	X	X	X	X	X	X	X	X	
Volunteer Memory Aid			X		X		X			
Stool for IgA (ELISA)		X		X	X	X	X	X	X	
Stool Grading, frequency of defecation		X	X		X		X			
Stool for Shedding[4]		X	X	X	X	X	X	X	X	
Antibiotic Treatment									X	
Serum/plasma Sample for Serology (ELISA)		X		X		X		X		
Whole Blood for ALS		X		X		X		X		
SAE/AE follow-up		X	X	X	X	X	X	X	X	X
Total Blood Volumes (mL)[5]	3.5	5		5		5		5		

Purple shading indicates stool specimen collection Blue shading indicates vaccination time points Orange shading indicates blood specimen collection

[1] If a vital sign needs to be repeated it should be obtained within approximately 30 minutes of the original reading. Only the vital sign that needs to be repeated will be repeated. Both the original and repeat measurements will be recorded in the paper source documents. The second measurement will be input on the eCRF in the database.
[2] Screening serology will include anti-HAV, anti-HEV and HBsAg
[3] Stool culture for presence of any *Shigella* strain should also be obtained between Days -7 and -5 for continued eligibility prior to vaccination on Day 0
[4] Any participant found to still be shedding any vaccine strain at V84 will be treated with antibiotic therapy.
[5] Blood draw volumes for participants will not exceed 5 ml/kg per draw or 10 ml/kg in any 8 week period

APPENDIX 2: Clinical Toxicity Grading – CHILDREN (12-24 months)

During screening, participants will have blood drawn to determine if any clinical laboratory abnormalities exist that would preclude study participation. Participants that have 2 mild (grade 1) abnormalities may be included if the Study Physician and Principal Investigator determines that their participation will not present undue risk to the participant. (Mild decreases in both hemoglobin and hematocrit will be counted as 1 abnormality). Participants with more than 2 mild abnormalities may be included in the study only with the consensus of the principal investigator and either the medical monitor of CRO or the study sponsor. This consensus will be documented with a note in the participant's chart by the PI or designee.

Solicited Lab AEs	Grade 1 – Mild	Grade 2 – Moderate	Grade 3 - Severe	Grade 4 – Potentially Life Threatening
Hemoglobin (gm/dl)	9.5 - 10.9	8.0 - 9.4	6.5 – 7.9	< 6.5
Leukocytes (cells/mm^3)				
Decreased	2,000-2,500	1,500-1,999	1,000-1,499	< 1,000
Increased	13,501-15,000	15,001-20,000	20,001-25,000	
Neutrophils (cells/mm^3)*	1,500-1,999	1,000-1,499	500-999	<500
Eosinophil (cells/mm^3) – absolute values	0.6 to 1.5 x 10^9/L	1.5 to 5 x 10^9/L	> 5 x 10^9/L	>100.0 x 10^9/L
Lymphocytes (cells/mm^3)	750-1,000	500-749	250-499	<250
Platelets/mm^3	100,000-125,000	50,000-99,900	25,000-49,999	< 25,000
Creatinine (mg/dl)	1.1 – 1.3 x ULN	1.4 – 1.8 x ULN	1.9 – 3.4 x ULN	≥ 3.5 x ULN
ALT (SGPT)	1.25 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10.0 x ULN	> 10.0 x ULN
AST (SGOT)	1.25 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10.0 x ULN	> 10.0 x ULN
Bilirubin (Total)	1.1 – 1.5 x ULN	1.6 – 2.5 x ULN	2.6 – 5.0 x ULN	> 5.0 x ULN
Gamma GT	>ULN – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 20.0 x ULN	>20.00 ULN
* Neutrophils include band cell (stab form) at all ages & a small number of metamyelocytes and myelocytes in the first few days of life. In healthy adults, 1-5% bands are normally seen				

Source:

1. Wintrobe's Clinical Hematology 12th Edition.
2. Dacie and Lewis Practical Hematology 10th Edition.
3. McPherson RA, Matthew R. Pincus MR. Henry's Clinical Diagnosis and Management by Laboratory Methods. 22nd ed. Philadelphia: Elsevier Saunders; 2011. 254-5.
4. Common Terminology Criteria for Adverse Events according to the National Cancer Institute, National Institutes of Health, U.S. Department of Health and Human Services.

APPENDIX 3: Grading for Reactogenicity events – CHILDREN (<36 months)

Event	Grade 1 – Mild	Grade 2 – Moderate	Grade 3 - Severe	Grade 4 – Potentially Life Threatening
General Severity Grading	**Event that is easily tolerated**	**Event that interferes with daily activity, requires medication/therapy, or a change in medication/therapy**	**Event that prevents daily activity and requires medical intervention**	**Event that requires emergency room (ER) visit or hospitalization**
Systemic Reactogenicity	Grade1 – Mild	Grade 2 – Moderate	Grade 3 - Severe	Grade 4 – Potentially Life Threatening
Fever (Axillary)	≥100.4°F and ≤ 101.1°F (38.0-38.4°C)	>101.1°F and ≤ 102.0°F (38.5-38.9°C)	> 102.0°F – 104°F (39.0°C - 40°C)	> 104.0°F (40°C)
Loose stool	Transient or intermittent episodes of unformed stools OR Increase of ≤3 stools over baseline per 24-hour period	Persistent episodes of unformed to watery stools OR Increase of 4 to 6 stools over baseline per 24-hour period	Increase of ≥7 stools per 24-hour period OR IV fluid replacement indicated	Life-threatening consequences (e.g.,hypotensive shock))
Abdominal pain	Mild abdominal pain, No interference with activity	Moderate abdominal pain, Some interference with activity	Severe abdominal pain, prevents daily activity	
Nausea/Vomiting	No interference with activity of 1-2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Anorexia	Loss of appetite without alteration in eating habits	Oral intake altered without significant weight loss or malnutrition; oral nutritional supplements indicated	Associated with significant weight loss or malnutrition (e.g., inadequate oral caloric and/or fluid intake); IV fluids, tube feedings or TPN indicated	
Bloating/abdominal	Asymptomatic	Symptomatic, but not	Symptomatic, interfering	

Distension/ excess flatulence		interfering with GI function	with GI function	
Constipation	Occasional or intermittent symptoms; occasional use of stool softeners, laxatives, dietary modification, or enema	Persistent symptoms with regular use of laxatives or enemas indicated	Symptoms interfering with daily activities; obstipation with manual evacuation indicated	
Irritability	Easily consolable	Requiring increased attention	Inconsolable	
Decreased activity	Symptom present, but activity level unchanged	Activity reduced, but still able to function	Activity markedly reduced, bed ridden	

Reference. Terminology Criteria for Adverse Events (TCAE), In Trials of Adult Pancreatic Islet Transplantation, Version 5.0 (03 August 2011)