Project Summary

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

Principal Investigator: Dr Firdausi Qadri

Research Protocol Title: A Randomized, Double-blind, Placebo-controlled Study evaluating the safety, tolerability, and immunogenicity of an oral inactivated ETEC Vaccine (ETVAX) alone and together with dmLT adjuvant in descending age groups in Bangladesh

Proposed start date: As soon as possible Estimated end date: Two years from starting

Background (brief):

1. Burden:

The global diarrheal disease burden remains high with approximately four billion cases estimated to occur annually in all age groups, with the highest incidence among infants and young children under five years of age. In this age group, enteric infections result in nearly 600,000 deaths each year and extracting an enormous physical and economic toll in low-resource countries. Diarrhea can also be a triggering event for death from other causes, particularly pneumonia. Recent GEMS data indicated that ETEC is among the top 4 causes of moderate to severe diarrhea among young children in developing counties and contribute to a higher risk of mortality and stunting.

The icddr,b Dhaka hospital treats more diarrheal patients from Mirpur than from any other part of Dhaka. It was observed that most of the isolated cases from the diarrheal patients are *V. cholerae* or ETEC and their general living and hygienic condition is poor with scarcity of safe drinking water and sharing of kitchen and toilet facilities. In a birth cohort study icddr,b has evaluated the natural history of ETEC infections and found that ETEC was the most common pathogen and was isolated in around 20% cases with an incidence of 0.5 episodes/child/year in 0-2 year old children. From the large feasibility study of cholera vaccine where they also conducted passive surveillance for enteric pathogens, it was observed that ETEC incidence rate varies from 1.1 to 1.47 /1000 /year and incidence rate is comparatively high in children less than 5 years old (4.5/1000/year) (Protocol# 12090). The 2% systemic surveillance data from icddr,b suggests that among the diarrheal specimens, ETEC isolation rate is 14% (1996-1998) and 11% (2007-2012) in all age groups. Recently it was observed that of the distribution, 52% of ETEC was isolated from those over five years of age and 48% in younger age groups, in which 14 to 20% are severely dehydrated.

2. Knowledge gap:

ETEC vaccine development remains a WHO priority. The information gained from experimental and clinical studies of the 1st generation vaccine, containing ETEC bacteria expressing common colonization factors (CFs) mixed with cholera toxin B subunit (CTB), suggested that a vaccine formulation containing increased amounts of CF antigens may induce stronger levels of anti-ETEC immunity and better protective efficacy in at-risk travelers and paediatric populations. Hence we have focused on developing a new generation ETEC vaccine that expresses increased levels of the different CFs in the previous formulations, and also contains CS6, which may be given at a relatively low dose of bacteria that is safe in infants and young children and still be highly immunogenic. Thus, the overall objective of this project is to develop an improved oral inactivated ETEC vaccine with increased immunogenicity for use in children living in endemic areas as well as in Western travelers going to ETEC-endemic areas. The recent completion of highly successful Phase I trials of safety and immunogenicity in Swedish adults (OEV-120 and OEV-121) supports the further evaluation of the safety and immunogenicity of this promising 2nd generation ETEC vaccine (ETVAX) among younger age-groups in an ETEC endemic area.

3. Relevance:

This Phase I/II trial will serve to assess whether ETVAX is safe and provides mucosal as well as systemic immune responses against the key protective antigens when tested in different age-groups in Bangladesh. This study provides an opportunity to test the safety profile of a mucosal adjuvant, double-mutant LT (dmLT), in adults and children, as well as provide the opportunity to potentially assess the ability of dmLT to further enhance the mucosal and systemic antibody responses to key antigens in the ETVAX vaccine among age groups in developing country sites, like Bangladesh, that have proved refractory to oral immunization with

enteric vaccines. In addition, this study also allows for the evaluation of the potential dose-sparing effect of dmLT when combined with a lower dose of vaccine. Finally, this clinical trial is considered an essential study along the critical path of the overall clinical development plan before determining whether the vaccine can be tested for protective efficacy in children in developing countries.

Hypothesis (if any):
Primary Hypothesis

Safety:
Orally administered ETVAX administered with or without dmLT adjuvant, will be safe and well-tolerated, in adults, toddlers, young children and infants in Bangladesh

Secondary Hypotheses
Immunogenicity:

1. The vaccine regimens under testing will elicit mucosal immune responses to at least two of the antigens in 50% or more participants in at least one of the dosing regimens in young children or infants
2. The responses induced by the vaccine regimens tested will be higher and/or more frequent than the responses in the placebo group.
3. In the event that dmLT is used, adjuvanted vaccine will induce stronger (increased magnitude and/or frequency and/or breadth of response) intestinal and/or intestine-derived and/or plasma anti-LTB and anti-CF/CS antibody responses than the ETVAX alone in young children and infants.

Objectives:
Primary Objectives:

Safety:
To evaluate the safety and tolerability of orally administered ETVAX, containing 4 different inactivated *E. coli* strains over-expressing CFA/I, CS3, CS5 and CS6 and a hybrid LCTB*A* protein, given alone and together with different dosages of dmLT adjuvant in descending age-groups in Bangladesh.

Secondary Objectives:
Immunogenicity

1. To assess vaccine induced IgA antibody responses in lymphocyte secretions by the ALS assay against LTB, CFA/I, CS3, CS6 and CS5 in descending age-groups in Bangladesh
2. To evaluate vaccine induced fecal secretory IgA (SIgA) antibody responses against CFA/I, CS3, CS5, CS6, O78 LPS and LTB in applicable age-groups in Bangladesh
3. To assess vaccine induced plasma IgA antibody responses against LTB, O78 LPS, CFA/I, CS3, CS6, and CS5 and IgG antibody responses against LTB and O78 LPS
4. To evaluate vaccine induced ALS IgA responses against O78 LPS (if sample volumes allow)
5. To assess adjuvant effect of dmLT on vaccine immune responses compared to responses when giving vaccine alone in descending age-groups in Bangladesh.

Methods:
This is a single site, Phase I/II, double-blind, randomized, placebo-controlled, dose-escalation, age-descending study that will first test the vaccine with and without dmLT in healthy adults, and then move sequentially into toddlers (24-59 months old), younger children (12-23 months old) and infants (6-11 months old). The study is designed as 4 parts, one for each of the four age-groups. All participants will receive two oral doses of ETVAX with or without dmLT adjuvant or placebo, with different vaccine dose levels assessed within each age-group. Before moving to higher dose levels, available safety data will be evaluated and reviewed by an Independent Protocol Safety Team (IPST) comprised of three independent safety monitors, who will review available safety data through Day 3 after the second immunization to make the recommendation to the Sponsor whether to proceed with the initiation of the next dose cohort in that same age group. Independent safety monitors will be individuals not associated with study operations and who have clinical trials and infectious disease experience. Before moving to the next lower age group, the icddr,b Data Safety Monitoring Board (DSMB) will review all

available safety data through Day 3 after the second immunization to make the recommendation to the Sponsor whether to proceed with initiation of the first dose cohort in an age group.

Outcome measures/variables:
Primary Safety Endpoints

1. Number of SAEs
2. Number of AEs
3. Number of vaccine induced reactogenicity events

Secondary Immunology Endpoints

1. Antibody response (≥two-fold increase in antibody titers between baseline and post-immunization), Geometric Mean Titer (GMT), and Geometric Mean Fold Rise (GMFR) between baseline and post-immunization to CFA/I, CS3, CS5, CS6 and LTB as measured by ALS IgA
2. Antibody response (≥two-fold increase in antibody titers between baseline and post-immunization), GMT, and GMFR to CFA/I, CS3, CS5, CS6 and LTB as measured by fecal SIgA.
3. Antibody response (≥two-fold increase in antibody titers between baseline and post-immunization), GMT, and GMFR to CFA/I, CS3, CS5, CS6 and LTB as measured by plasma IgA.
4. Antibody response (≥two-fold increase in antibody titers between baseline and post-immunization), GMT, and GMFR to LTB and O78 as measured by plasma IgG.
5. Antibody response (≥two-fold increase in antibody titers between baseline and post-immunization), GMT, and GMFR to O78 as measured by ALS IgA, fecal SIgA, and/or plasma IgA.

LIST OF ABBREVIATIONS AND ACRONYMS

ABBREVIATION/ ACRONYM	DEFINITION
Ab	Antibody
ASC	Antibody-secreting cells
AE	Adverse Event
AdvantageEDC℠	Emmes' internet-based Electronic Data Capture System
ALS	Antibody in lymphocyte secretion
CF	Colonization factor
CFR	Code of Federal Regulations
CFA/I	Colonization factor I
CRF/eCRF	Case Report Form/electronic Case Report Form
CS3	Coli Surface antigen 3
CS5	Coli Surface antigen 5
CS6	Coli surface antigen 6
CSR	Clinical Study Report
CRO	Contract Research Organization
CTB	Cholera Toxin B Subunit
rCTB	Recombinant Cholera Toxin B Subunit
dmLT	Double Mutant Heat Labile Toxin
DSMB	Data Safety Monitoring Board
EDC	Electronic Data Capture
ELISA	Enzyme-linked Immunosorbent Assay
ERC	Ethical Review Committee
ETEC	Enterotoxigenic *Escherichia coli*
FDA	(U.S.) Food and Drug Administration
GCP	Good Clinical Practice
GEMS	Global Enteric Multicenter Study
GLP	Good Laboratory Practice
GMT	Geometric Mean Titer
IB	Investigators' Brochure
Icddr,b	International Centre for Diarrheal Disease Research, Bangladesh
ICH	International Conference on Harmonization
ICF	Informed Consent Form
IPST	Independent Protocol Safety Team
IND	Investigational New Drug
IV	Intravenous
LCTB*A*	Hybrid protein between the B-subunit of the *E. coli* heat-labile enterotoxin (LTB) and the B-subunit of the cholera toxin (CTB)
LPS	Lipopolysaccharide
LT	*E. coli* heat-labile enterotoxin
LTB	B-subunit of the *E. coli* heat-labile enterotoxin
mLT	Single mutant heat labile toxin
MedDRA	Medical Dictionary for Regulatory Activities
MedDRA PT	Medical Dictionary for Regulatory Activities Preferred Term
MedDRA SOC	Medical Dictionary for Regulatory Activities System Organ Class
MSD	Moderate to severe diarrhea
NIH	(U.S.) National Institutes of Health
OHRP	(U.S.) Office of Human Research Protections
PE	Physical Examination
PI	Principal Investigator

ABBREVIATION/ ACRONYM	DEFINITION
PVS	PATH Vaccine Solutions
SAE	Serious Adverse Event
SBH	Scandinavian BioPharma
SDV	Source Data Verification
SOP	Standard Operating Procedure(s)
ST	*E. coli* heat-stable enterotoxin
TSS	Trained Study Staff
UG	University of Gothenburg
ULN	Upper Limit of Normal
VS	Vital Signs
WRAIR	Walter Reed Army Institute of Research
WBC	White Blood Cell
WHO	World Health Organization
WIRB	Western Institutional Review Board

PARTICIPATING INSTITUTIONS

Sponsor	PATH Vaccine Solutions (PVS) 2201 Westlake Avenue Suite 200 Seattle, WA 98121 USA
Clinical Trial Site	International Centre for Diarrheal Disease Research, Bangladesh GPO 128 Dhaka, Bangladesh
Research Laboratories	Immunology Unit, Enteric Vaccines, Centre for Vaccine Sciences, International Centre for Diarrheal Disease Research, Bangladesh (icddr,b) Department of Microbiology and Immunology The Sahlgrenska Academy of University of Gothenburg Box 435 40530 Gothenburg Sweden Scandinavian BioPharma Gunnar Asplunds Allé 16 SE-17163 Solna Sweden
Site Monitoring	GVK Biosciences Pvt Ltd 307-309, BPTP Park Centra Sector-30, Gurgaon-122001 Haryana, India
Medical Monitoring	The Emmes Corporation 401 N. Washington St., Suite 700 Rockville, MD 20850 USA
Data Center and Statistical Support	The Emmes Corporation 401 N. Washington St., Suite 700 Rockville, MD 20850 USA
Vaccine Developer	Scandinavian BioPharma (SBH) Sweden
Vaccine Manufacturers	**ETVAX:** Biovian Oy, Tykistökatu 6B, FI-20520 Turku, Finland **dmLT:** Walter Reed Army Institute of Research Pilot BioProduction Facility 501 Robert Grant Avenue Silver Spring, MD 20910 USA

Description of the Research Project

Hypothesis to be tested:

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

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

Primary Hypothesis:

Safety:

Orally administered ETVAX administered with and without dmLT adjuvant, will be safe and well-tolerated, in adults, toddlers, young children and infants in Bangladesh.

Secondary Hypotheses:

Immunogenicity

1. The vaccine regimens under testing will elicit mucosal immune responses to at least two of the antigens in 50% or more participants in at least one of the dosing regimens in young children or infants
2. The responses induced by the vaccine regimens tested will be higher and/or more frequent than the responses in the placebo group.
3. In the event that dmLT is used, adjuvanted vaccine will induce stronger (increased magnitude and/or breadth of response) intestinal and/or intestine-derived and/or plasma anti-LTB and anti-CF/CS antibody responses than the ETVAX alone in young children and infants.

Specific Objectives:

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

Primary Objectives

Safety:

To evaluate the safety and tolerability of orally administered ETVAX, containing 4 different inactivated *E. coli* strains over-expressing CFA/I, CS3, CS5 and CS6 and a hybrid LCTB*A* protein, given alone and together with different dosages of dmLT adjuvant in descending age-groups in Bangladesh.

Secondary Objectives

Immunogenicity:

1. To assess vaccine induced IgA antibody responses in lymphocyte secretions by the ALS assay against LTB, CFA/I, CS3, CS6 and CS5 in descending age-groups in Bangladesh
2. To evaluate vaccine induced fecal secretory IgA (SIgA) antibody responses against CFA/I, CS3, CS5, CS6, O78 LPS and LTB in applicable age-groups in Bangladesh
3. To assess vaccine induced plasma IgA antibody responses against LTB, O78 LPS, CFA/I, CS3, CS6 and CS5 and IgG antibody responses against LTB and O78 LPS
4. To evaluate vaccine induced ALS IgA responses against O78 LPS (if sample volume allows)
5. To assess adjuvant effect of dmLT on vaccine immune responses compared to responses when giving vaccine alone in descending age-groups in Bangladesh.

Exploratory Objectives

1. To assess the ability of ETVAX and/or ETVAX + dmLT to induce T cell responses (proliferation and/or cytokine responses as measured by flow cytometry) against the vaccine antigens
2. To measure the ability of ETVAX and/or ETVAX + dmLT to induce functional anti-toxin neutralizing antibodies to LTB To measure avidity of immune responses
3. To compare systems biology assessment of innate and adaptive immune response patterns in immunized participants and unimmunized controls using transcriptomics, proteomics and immune-profiling

Primary Endpoints

Safety and tolerability:

- Number of SAEs
- Number of AEs
- Number of vaccine induced reactogenicity events

Secondary Endpoints

Immunogenicity

- Antibody response (≥two-fold increase in antibody titers between baseline and post-immunization), Geometric Mean Titer (GMT), and Geometric Mean Fold Rise (GMFR) between baseline and post-immunization to CFA/I, CS3, CS5, CS6 and LTB as measured by ALS IgA
- Antibody response (≥two-fold increase in antibody titers between baseline and post-immunization), GMT, and GMFR to CFA/I, CS3, CS5, CS6 and LTB as measured by fecal SIgA.
- Antibody response (≥two-fold increase in antibody titers between baseline and post-immunization), GMT, and GMFR to CFA/I, CS3, CS5, CS6 and LTB as measured by plasma IgA.
- Antibody response (≥two-fold increase in antibody titers between baseline and post-immunization), GMT, and GMFR to LTB and O78 as measured by plasma IgG.
- Antibody response (≥two-fold increase in antibody titers between baseline and post-immunization), GMT, and GMFR to O78 as measured by ALS IgA, fecal SIgA, and/or plasma IgA.

Exploratory Endpoints

Immunogenicity

- T cell immune responses (proliferation and/or cytokines as measured by flow cytometry)
- Neutralizing antibody geometric mean titers (GMT)
- Avidity of immune responses
- Immunoproteomic profile of vaccine induced antibody responses in plasma or intestinally derived samples

Background of the Project including Preliminary Observations:

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

The global diarrheal disease burden remains high with approximately four billion cases estimated to occur annually in all age groups, with the highest incidence among infants and young children under five years of age [1-5]. In this age group, enteric infections result in nearly 600,000 deaths each year, comprising approximately 9 percent of global child deaths and extracting an enormous physical and economic toll in low-resource countries [6]. Indeed, diarrhea is second only to pneumonia among the leading causes of death due to disease in children under five years old. Diarrhea can also be a triggering event for death from other causes, particularly pneumonia [7].

Despite encouraging declines in overall diarrhea-associated mortality since 2000, incidence rates remain high and have changed very little even with incremental improvements in sanitation, water quality, and treatment for diarrhea [1, 2, 4]. Moreover, the mortality rate does not reveal the additional burden of diarrhea-associated morbidity, including malnutrition and failure of children to thrive both physically and cognitively [8-11]. Diarrhea is estimated to account for 306.5 million DALYs primarily among children less than five years old [10, 11]. The link between a high diarrheal disease burden and poor physical and cognitive development among infants and young children in developing countries is so strong that many experts are beginning to consider malnutrition itself as an enteric infectious disease. In addition, the stunting and IQ deficits that result from repeated diarrhea episodes are associated with poor school performance and reduced job-related productivity later in life. The economic impact of diarrhea-associated stunting is estimated to equate to about a 17 percent decrease in worker productivity and a cognitive impairment of approximately 10 IQ points [10].

Among older age groups (more than five years of age) in low-resource settings, diarrhea also remains an important cause of morbidity and mortality. Recent studies in these older age groups indicate that diarrhea morbidity rates have also stayed relatively constant since the 1980s and that more than 2.8 billion episodes of diarrhea are projected to occur annually in children older than five years of age, adolescents, and adults. Diarrhea-associated mortality rates in these older age groups

are also high, and the World Health Organization (WHO) estimates that the majority of these deaths occur primarily in Africa and Southeast Asia (1.15 million deaths annually) [2, 3].

A study comparing the global burden of diarrheal disease from 1992 to 2000 with earlier date ranges found that mortality has decreased significantly, but the number of episodes of diarrhea remained stable across the three survey periods and has actually changed very little over the last 20 years [12]. It remains a great public health concern that most of the diarrheal disease burden falls on the poorest children in the world. In addition, most diarrhea-related deaths have been shown to occur in the home or outside of primary or tertiary health care facilities [4], so it is likely that current projections may actually underestimate diarrhea mortality rates across all age groups. Although upgraded primary care, the increased use of oral rehydration therapy (ORT), and improved nutrition are undoubtedly responsible for the decrease in case-fatality rates, these interventions do not prevent diarrheal disease illness and its related sequelae. Also, the ever-increasing frequency of multiple antibiotic-resistant bacterial enteropathogens makes effective treatment of diarrheal illnesses like shigellosis, cholera, campylobacteriosis, and typhoid much more challenging and costly [13-15].

Among the primary infectious causes of diarrheal disease, ETEC is the most important pathogen for which there is no currently licensed vaccine. ETEC has been a long WHO target for vaccine development. In ETEC-endemic areas, infants and young children may experience two to five symptomatic diarrhea episodes due to this pathogen over their first three years of life, and illness due to this bacterium has been associated with an increased risk of stunting [1]. ETEC may be the first bacterial illness that a child experiences in life, and virtually all children living in endemic areas have had at least one symptomatic ETEC infection by the time they are four years of age, with many children experiencing more than that [16-18].

Recent studies in sub-Saharan Africa and South Asia conducted under the Gates Foundation-funded Global Enteric Multicenter Study (GEMS) project have reaffirmed the continuing importance of ETEC as an important cause of moderate-to-severe diarrhea (MSD) among children under five years of age, found among the top four causes of potentially life-threatening diarrheal illness in both regions. GEMS data also indicated that MSD among children living in these low-resource areas remains associated with a significant increase risk of mortality and stunting, and both agents continue to contribute to these poor health outcomes. In addition, the presence of ETEC, enteropathogenic *E. coli* (EPEC), or *Cryptosporidia* in the stools of diarrhea cases was also associated with an increased risk of mortality over the subsequent 60-day surveillance period [18].

Infections associated with ETEC have a negative impact on projected family productivity in that ETEC is frequently associated with hospitalization and lost work time for adult family members. For example, ETEC is associated with less dehydrating diarrhea (13 percent) than rotavirus (35 percent); however, because the incidence of multiple ETEC-associated diarrhea episodes is so much higher than rotavirus, the global number of cases of dehydration requiring ORT or intravenous (IV) fluid supplementation due to both agents are fairly close (i.e., they differ by approximately 30 percent).

Finally, ETEC is also a frequent cause of diarrhea among travelers to many parts of Africa, Asia, and Latin America, including military personnel deployed to these areas. ETEC is estimated to cause approximately 10 million episodes of travelers' diarrhea each year [19]. Illness due to ETEC infection has also been associated with the development of functional bowel disorders, like irritable bowel syndrome in travelers and military personnel during convalescence (10 to 14 percent of cases) [20, 21], thus further highlighting the importance of disease prevention and the potential benefit of having an effective vaccine.

ETEC causes disease by colonizing the small intestine by means of colonization factors (CFs) that usually are fimbriae and by the production of heat-labile (LT) or heat-stable toxins (ST) or both toxins [22]. LT is structurally, functionally and immunologically related to cholera toxin (CT); in particular the LTB and CTB subunits are closely related. Whereas LT is strongly immunogenic ST is not immunogenic unless coupled to a carrier protein; to date no safe ST toxoid is available for use in humans. More than 25 different CFs have been described; however, a few of them, e.g. CFA/I, CS3, CS5 and CS6 are more prevalent than others, accounting for 50-80 % of all CF-positive clinical ETEC isolates . In addition some CFs are immunologically related to these prevalent CFs, in particular to CFA/I and CS5.

Protection against ETEC is most likely mainly provided by antibodies against the different CFs and LT produced locally in the gut. Studies in experimental animals and human subjects strongly support that the CFs are key protective antigens against ETEC expressing homologous CFs. Thus, studies in a birth cohort in Bangladesh [23] have shown that reinfections with ETEC expressing homologous CFs are very rare, at variance with reinfections with ETEC expressing heterologous CFs, supporting the importance of CF immunity in an ETEC vaccine. Clinical trials both in travelers and in endemic countries have also shown that CTB may provide significant protection against LT disease However, it has been suggested that an LT-like toxoid may provide stronger protection against LT ETEC, at least in young, immunologically naïve children in comparison to CTB. Thus, we have previously shown that human subjects challenged with LT producing ETEC as well as Bangladeshi patients convalescing from *E. coli* LT diarrhea responded with significantly stronger local and serum antibody responses to LT than to cholera toxin.

Since ETEC infections are confined to the mucosal surfaces in the gut immune protection is most likely predominantly provided by locally produced secretory IgA antibodies against major protective antigens, i.e., CF and LT antigens. Hence, determination of immune responses against ETEC candidate vaccines should be focused on assessing intestinal or intestine-derived antibody responses.

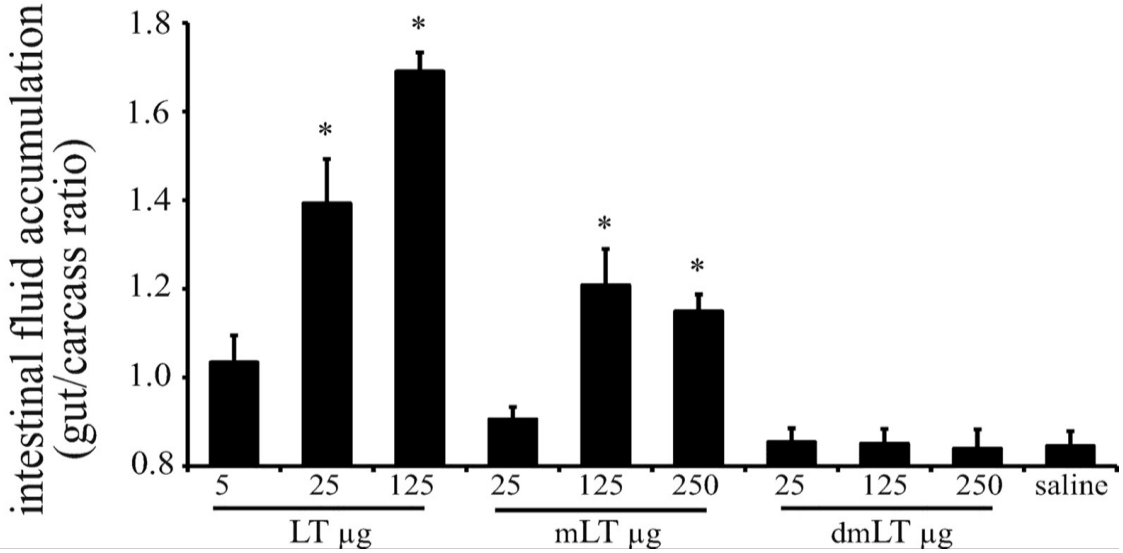
Mucosal Adjuvants

The LT toxin has been shown to have inherent mucosal adjuvant properties for co-administered antigens and thus has potential as a mucosal adjuvant for different co-administered vaccines [24, 25]. Several animal and human studies have evaluated LT as a mucosal adjuvant for co-administration with inactivated whole cell or purified subunit antigens derived from a wide range of bacterial and viral pathogens. Despite the significant potential of LT as a mucosal adjuvant, oral LT has considerable enterotoxicity for humans, thereby limiting its use as an adjuvant [24, 26]. Therefore, efforts have been made to construct LT molecules with decreased enterotoxicity, but with undiminished adjuvant properties. As described in more detail below, single mutant LT (mLT) was developed by Dr. John Clements and colleagues at Tulane University [28] but a high oral dose (100 μg) was still found to be reactogenic when given alone. Also, despite impressive adjuvant activity, mLT retained some level of gastrointestinal reactogenicity in Phase I trials when combined with bacterial vaccine antigens [27, 28]. Subsequently, double mutant LT (dmLT) was developed by the investigative team at Tulane as a 2nd generation mucosal adjuvant with further reduced enterotoxicity [25]; dmLT has been proven to be very safe in a Phase1 human trial at an oral dose as high as 100 μg [39] and to retain strong adjuvant properties for ETEC and other bacterial antigens in experimental animals.

Development and testing of double-mutant LT (dmLT)

*Escherichia coli*JM83(pLC403) codes for production of a genetically modified form of *E. coli* LT in which the arginine at amino acid position 192 has been replaced with glycine and the leucine at amino acid position 211 has been replaced with alanine in the A subunit portion of LT [25]. These two amino acid substitutions take place in known and putative proteolytic cleavage sites in the LT protein that are considered to be critical for activation of the secreted toxin molecules. The protein coded by this strain was designated LT(R192G/L211A), i.e. dmLT, and has been extensively evaluated for residual enterotoxicity *in vitro* and *in vivo* studies, and in pre-clinical animal studies for its ability to function as an adjuvant for co-administered antigens. Two oral doses of dmLT in rats were also found to lack local and systemic toxicity in a GLP toxicology study conducted by PATH and DMID, NIH. Figure 1 shown below highlights the lack of residual enterotoxicity in the dmLT protein compared to native LT (dmLT is at least 50-fold less toxic) and mLT (dmLT is at least 10-fold less toxic) in the patent mouse assay. Additional information regarding the retention of dmLT adjuvanticity using tetanus toxoid as a model antigen can be found in the dmLT Investigator's Brochure.

Figure 1. Comparative enterotoxicity of native LT, mLT, and dmLT (Lot No. 1575) in the Patent Mouse Model.



Gut-to-carcass ratios of 0.9 or above are considered indicative of enterotoxic activitiy.

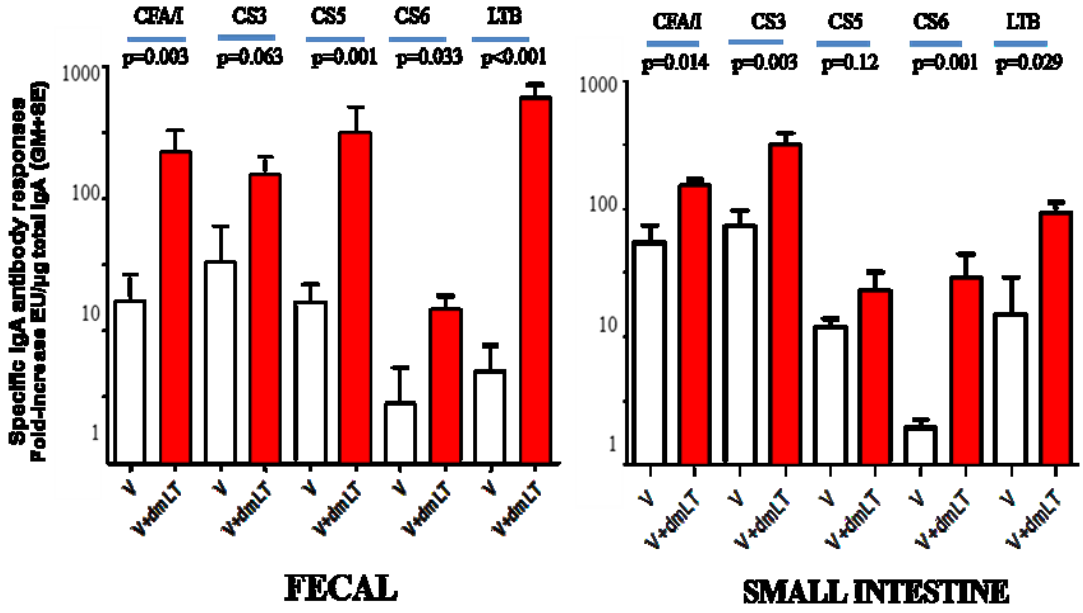
Nonclinical Studies

Immunogenicity and safety in mice of ETVAX with and without dmLT

ETVAX given alone or together with the dmLT adjuvant (see page 36-37 for product descriptions) was studied in mice after oral immunizations for its capacity to induce intestinal and serum antibody responses to the different vaccine components. Small intestine or fecal IgA responses showed 2 to 50-fold increase in specific antibodies to the different vaccine components following immunization. Immune responses were further increased when the vaccine was given in combination with the oral adjuvant (Fig 2).

A significant IgA immune response was also obtained in serum to each of the vaccine components and this was even further pronounced for IgG + IgM type antibodies. In most cases, the serum immune responses were further enhanced in the presence of the adjuvant [31]. In addition to demonstrating immune enhancement, this study also showed that when adding different dosages of dmLT when administered with vaccine (PV), the adjuvant effect of dmLT was especially strong for lower vaccine dosages [31]. This suggests the potential for vaccine dose sparing when an effective adjuvant is added, which could be especially valuable for use with ETVAX in younger age groups in which fractional doses of vaccine may be needed [32].

Figure 2. Intestinal IgA antibody responses in mice following oral immunization with ETVAX ETEC vaccine (V) with and without dmLT adjuvant.



cGLP Toxicology and Immunology Evaluation of ETVAX with and without dmLT

A repeated dose cGLP toxicity study was conducted in C57BL/6J mice, in which the main objective was to investigate whether ETVAX produced toxic reactions after repeated peroral administration in the animals. ETVAX was administered in two different doses, 5 x the human clinical dose and 25 x the human clinical dose, and administered with or without the addition of the dmLT adjuvant (lot 1575). PBS was used as the control item.

In comparison to PBS treated animals the vaccines in high doses affected the general health slightly and no major pathological, clinical chemistry or haematological observations were found. Acute gastritis was seen in some animals that were most probably the results of previous and passing injury on a microscopic level. These changes were found in the groups treated with ETEC Prototype vaccine (i.e. inactivated recombinant *E. coli* over-expressing CFA/I, 2.5x 10^{10} bacteria/ human dose)100 x clinical dose group (3/20 animals) or ETVAX vaccine (10^{11} bacteria/human dose) 25 x clinical human dose + dmLT adjuvant (2/20 animals). The gastritis was deemed to be transient and did not raise any safety concerns by the Swedish Medical Product Agency. Furthermore, symptoms consistent with acute gastritis have not been observed in two Phase 1 trials in which dmLT was co-administered with ETVAX and a live-attenuated ETEC vaccine in doses of up to 25 ug (Protocols OEV-121 and VAC 006). A strong anti-LCTB*A* response in serum was detected across all dosing groups (9-10 responders out of 10 mice/group); while the serum anti-CFA/I response was somewhat weaker and dependent on the dosing group (1-8 responders out of 10 mice/group).

In conclusion, the vaccine was shown to be safe and immunogenic when tested in mice; adjuvantation with dmLT increased the immune response without altering the safety profile.

Clinical Studies

Double Mutant heat Labile Toxin – dmLT (Protocol 09-0066)

A Phase I dose-escalation study evaluating the safety and tolerability of orally administered dmLT was completed at the Cincinnati Children's Hospital Medical Center and at the University of Maryland Center for Vaccine Development (DMID Protocol 09-0066). In this study, four groups were included to receive 5 µg (n = 6), 25 µg (n = 6), 50 µg (n = 12), or 100 µg (n = 12) dmLT. Volunteers were enrolled on Day -1 in the inpatient unit, dosed on Day 0, and observed for 72

hours for any adverse events prior to discharge on Day 3. Safety was assessed by solicited symptoms/memory aid and laboratory evaluations through Day 28, and long-term safety follow-up phone calls at Months 2 and 6 [39].

Across all groups, no moderate or severe solicited symptoms were reported in any volunteers during the initial 3 days of intense monitoring after dosing, nor up to 28 days of follow up, despite increasing dose escalations. No volunteers in any of the dosing groups met the protocol definition for diarrhea and no product-related SAE's were reported. In each group there were some occasional mild or moderate hematology and chemistry deviations from baseline values, but those were transient and showed no dose-related trends and were considered clinically non-significant. Table 1 summarizes the solicited reactogenicity symptoms reported at each dose level, including diarrhea, as well as the immunogenicity of ascending doses of dmLT.

Table 1: DMID 09-0066; Listing of Solicited Reactogenicity Symptoms in Groups 1–4 of oral dmLT in Phase 1 ascending dose trail (DMID protocol 09-0066). Numbers of responders and percent responders (%) are presented.

Response to vaccination	dmLT dose in µg and number of subjects (n)			
Symptoms	**5 µg** (N = 6)	**25 µg** (N = 6)	**50 µg** (N = 12)	**100 µg** (N = 12)
Reactogenicity Symptoms				
Abdominal pain	1 mild event (16.7%)	0	1 mild event (8.3%)	1 mild event (8.3%)
Anorexia	0	0	0	0
Diarrhea	0	0	0	0
Nausea	0	2 mild events (33.3%)	0	0
Vomiting	0	0	0	1 mild event (8.3%)

*Represents one missing sample.

Based on the positive safety profile of dmLT administered orally, another trial is currently ongoing in which dmLT is being evaluated sublingually (DMID Protocol 12-0023; www.clinicalTrials.gov) and a concept for testing dmLT intradermally has been approved by the NIH.

Previous ETEC vaccine candidates – first generation vaccine

Scientists at the University of Gothenburg (UG) and former SBL Vaccin AB (now Scandinavian BioPharma) have previously developed an oral inactivated ETEC vaccine [33]. This 1st generation ETEC vaccine (rCTB-CF), which contained a mixture of five clinically isolated inactivated ETEC strains expressing major colonization factors (CFs), in total $1x10^{11}$ bacteria + 1 mg recombinant CTB (rCTB) per dose and which was given in a two- or three-dose regimen, has been extensively evaluated for safety and immunogenicity in several Phase I/Phase II studies in children and adults living in ETEC endemic areas. The vaccine has also been evaluated in several Phase I/Phase II studies in Western travelers going to such areas and finally also in phase III studies in travelers and in one Phase III study in children in Egypt [33].

Studies in endemic areas

In Phase I/Phase II studies in Egypt, in which the rCTB-CF ETEC vaccine was evaluated in an age-descending manner starting with adults and subsequently in children, 6-12 years, 2-4 years, and 6-18 months of age, the vaccine was found to be safe and immunogenic in all age groups when given in two or three doses two weeks apart [29, 30]. However, when tested for protection in an active surveillance phase III study in 6-18 month old children the vaccine only provided a non-significant, 20%, protective efficacy against mild/moderate disease. No cases of severe (dehydrating) diarrhea occurred during the study period [33] .

When testing of the vaccine in parallel age-descending studies in Bangladesh, it was found to be well tolerated [29] except in the youngest age group (6-17 months) in which increased frequency of vomiting was observed [32]. The vaccine was immunogenic in all age groups, although responses in infants were lower than in older children. Based on the side-effects observed in the youngest age-group a dose-finding study was conducted in children 6-17 months of age who were given

full, half and quarter dose of vaccine; when given in a quarter dose the vaccine was still immunogenic and adverse events in the form of vomiting was only seen in 4% of the vaccinated children and in 2.5 % of the placebo recipients [32].

Studies in Adult Travelers

The 1st generation rCTB-CF ETEC vaccine has also been tested for efficacy in adult Western travelers and was well tolerated by these subjects [33, 35]. A protective efficacy against moderate/severe disease, i.e. diarrhea interfering with the traveler's daily activity, of 77% (p=0.039) against ETEC strains sharing antigen in common with the vaccine was observed in US adult travelers going to Mexico and Guatemala [35]. In a follow-up study, in which non-supervised vaccination was applied, a 60% protective efficacy (p=0.10) against moderate/severe disease ETEC with vaccine-shared antigens was observed [35].

In this rCTB-CF ETEC vaccine, the colonization factor CS6 was not represented. However, ST-CS6 ETEC was found to be the most common cause of Traveler's Diarrhea among travelers to Guatemala and Mexico [35] and has also been very prevalent in recent studies in Egypt. These results indicate that a new composition of ETEC vaccine should also include CS6.

Since the protective efficacy of the 1st generation oral ETEC vaccine was not significant in children in a poor ETEC endemic area, the development of an improved 2nd generation ETEC vaccine has been initiated with the goal of providing a broader level of protection in both young children living in ETEC endemic areas and travellers to these areas.

Second Generation Vaccine

Studies of a prototype vaccine (OEV-120)

The information gained from experimental and clinical studies of the 1st generation vaccine suggested that a vaccine formulation containing increased amounts of CF antigens may induce stronger levels of anti-ETEC immunity and better protective efficacy in at-risk travelers and paediatric populations. Hence we have focused on developing a new generation ETEC vaccine that expresses increased levels of the different CFs in the previous formulations, and also contains CS6 [33, 37, 38], which may be given at a relatively low dose of bacteria that is safe in infants and young children and still be highly immunogenic.

Thus, the overall objective of this project is to develop an improved oral inactivated ETEC vaccine with increased immunogenicity for use in children living in endemic areas as well as in Western travelers going to ETEC-endemic areas. New vaccine strains, expressing substantially increased levels of CF antigens as compared to the previous ETEC vaccine (and also including the previously missing CS6 antigen) have been developed [37, 38].

A prototype of this 2nd generation vaccine consisting of an inactivated recombinant *E. coli* strain (SBL 109) over-expressing one of the most prevalent CFs, i.e. CFA/I, together with a hybrid protein LCTBA was compared with the CFA/I expressing strain (SBL 101) of the 1st generation ETEC vaccine + rCTB with regard to safety and mucosal and systemic immunogenicity in a Phase 1 trial in Sweden (OEV-120). In this Phase 1 trial, the new vaccine components (SBL 109 and LCTBA), when given at both the 1x or 4x dose, were found to be safe, well tolerated, and immunogenic when compared to the previously tested dose of SBL101 + rCTB vaccine-toxoid combination (41). The new LCTBA toxoid in both dosages tested induced a stronger anti-LTB systemic antibody response than the rCTB toxoid given with SBL 101; while fecal anti-CFA/I IgA antibody responses were stronger in those subjects receiving the SBL 109 + LCTB*A* vaccine at the 4x dose [24].

Fully Formulated ETVAX Vaccine with and without dmLT (OEV-121)

The fully formulated ETVAX vaccine was evaluated in a Phase I, placebo-controlled, double-blind, safety and immunogenicity study in 129 healthy adult Swedish volunteers. The vaccine, consisting of four inactivated *E. coli* strains over-expressing the major colonization factors CFA/I, CS3, CS5, and CS6 and mixed with the LTB/CTB hybrid protein LCTB*A,* was administered alone or together with dmLT adjuvant. Study participants were randomized to receive two oral doses two weeks apart of either the vaccine alone, vaccine co-administered with dmLT adjuvant in a low dose (10 ug) or a higher dose (25 ug), or placebo (buffer solution). The vaccine was well tolerated by the study participants. Adverse events were few and generally mild with no differences observed between subjects receiving vaccine with or without dmLT or placebo. Altogether 89 solicited symptoms, deemed to be possibly or probably related to treatment, were recorded (Table

2). No significant changes of other clinical parameters, including serum chemistry and hematology, were observed in any of the volunteers. [43]

Table 2. Number of solicited AEs with possible or probable relationship[a] with treatment (safety analysis set)

	(A) Placebo		(B) Vaccine		(C) Vaccine +10 μg dmLT		(D) Vaccine + 25 μg dmLT	
	Dose 1 (n=34)	Dose 2 (n=34)	Dose 1 (n=35)	Dose 2 (n=34)	Dose 1 (n=30)	Dose 2 (n=30)	Dose 1 (n=30)	Dose 2 (n=28)
Nausea	3 [b] (9%) [c]	6 [1] [d] (18%)	6 (17%)	5 [2] (15%)	6 [1] (20%)	7 [4] (23%)	2 [1] (7%)	2 [1] (7%)
Vomiting	0	0	0	0	0	2 (7%)	0	1 (4%)
Diarrhea [e]	1 (3%)	0	0	0	1 (3%)	1 (3%)	0	0
Loose stools	2 (6%)	1 (3%)	3 (9%)	1 (3%)	2 (7%)	4 (13%)	5 (17%)	3 (11%)
Stomach ache	5 [1] (15%)	3 [1] (9%)	2 (6%)	4 (12%)	1 (3%)	1 [1] (3%)	7 (23%)	1 (4%)
Fever [f]	0	0	0	0	1 (3%)	0	0	0
Total	11 [1]	10 [2]	11	10 [2]	11 [1]	15 [5]	14 [1]	7 [1]

[a] Study physicians judged the relation to immunization (unlikely, possible, probably, or unclassifiable) based on experiences from previous ETEC vaccine studies, including a temporal relationship with vaccination, i.e. within 72 hours. [35]
[b] Numbers of AEs of any intensity; mild, i.e. no interference with normal activity, or moderate, i.e. partial interference. No severe AEs, i.e. preventing normal activity, which were deemed possibly/probably related to vaccination, were recorded.
[c] Frequencies of subjects experiencing AEs of any intensity.
[d] Numbers of AEs of moderate intensity are indicated in square brackets.
[e] Diarrhea was defined as three or more loose stools within 24 hours; the reported cases of diarrhea were all mild, consisting of 3-4 loose stools within 24 h.
[f] Fever was defined as >37.7°C orally or 38.0 °C rectally.
P>0.05 for all comparisons of vaccine groups (B, C and D) with the placebo group (A) and comparisons between the vaccine groups (Fisher´s exact test).

The intestinal (fecal) antibody and the intestine-derived antibody secreting cell responses (ALS) against the five primary antigens were significantly higher in both frequency and magnitude in all vaccine groups compared with the placebo group (Table 3). In total, 74 percent of all vaccine recipients and 83 percent of subjects receiving vaccine co-administered with the low-dose dmLT adjuvant showed a significant mucosal IgA antibody response to all five of the primary vaccine components (Table 4); a result that well exceeds the primary immunological endpoint pre-set for the study (at least 50 percent of the subjects responding to at least four of the five primary vaccine antigens.

Table 3. Frequencies of IgA responders[a] against the different primary vaccine antigens in ALS and fecal specimens (per protocol analysis sets).

	(A) Placebo	(B) Vaccine	(C) Vaccine + 10 µg dmLT	(D) Vaccine + 25 µg dmLT
ALS				
LTB	1/29 (3%, 0.1-18) [b]	26/29 *** (90%, 73-98)	28/29 *** (97%, 82-99)	22/26 *** (85%, 65-96)
CFA/I	1/24 (4%, 0.1-21)	15/27 *** (56%, 35-75)	20/28 *** (71%, 51-87)	17/24 *** (71%, 49-87)
CS3	2/24 (8%, 1-27)	24/27 *** (89%, 71-98)	23/28 *** (82%, 63-94)	21/24 *** (88%, 68-97)
CS5	1/24 (4%, 0.1-21)	15/27 *** (56%, 35-75)	19/28 *** (68%, 48-84)	14/24 *** (58%, 37-78)
CS6	3/24 (13%, 3-32)	15/27 *** (56%, 35-75)	20/28 *** (71%, 51-87)	15/24 *** (63%, 41-81)
Faeces				
LTB	2/28 (7%, 0.9-24)	21/29 *** (72%, 53-87)	21/25 *** (84%, 64-95)	16/24 *** (67%, 45-84)
CFA/I	0/26 (0%, 0-13)	20/30 *** (67%, 47-83)	14/24 *** (58%, 37-78)	12/24 *** (50%, 29-71)
CS3	2/26 (8%, 1-25)	21/30 *** (70%, 51-85)	12/24 *** [c] (50%, 29-71)	14/24 *** (58%, 37-78)
CS5	1/26 (4%, 0.1-20)	21/30 *** (70%, 51-85)	14/24 *** (58%, 37-78)	13/24 *** (54%, 33-74)
CS6	0/26 (0%, 0-13)	18/30 *** (60%, 41-77)	13/24 *** (54%, 33-74)	16/24 *** (67%, 45-84)

[a] Cumulative response rates after one or two immunizations. Fold rises ≥2 were considered as responses in all assays.
[b] Percentage of responders, 95% CI
[c] If only subjects with a day 0 specimen were included in the analysis, 10/16 subjects (63%) responded to CS3 in fecal specimens. For all other antigens and groups, comparable frequencies were recorded if all subjects or only subjects with a day 0 specimen were included.
*** P<0.001 for comparisons of all vaccine groups (B, C and D, respectively) with the placebo group (A) for all primary antigens (one-tailed Fisher´s exact test, with Holm´s correction). (42)

Table 4. Frequencies of IgA responders against different numbers of primary vaccine antigens in ALS and/or fecal specimens

Frequency of subjects[a] responding to:	(A) Placebo (n=20)	(B) Vaccine (n=23)	(C) Vaccine + 10 µg dmLT (n=23)	(D) Vaccine + 25 µg dmLT (n=20)
5 antigens [b]	0%	74%	83%	75%
4 antigens	0%	17%	4%	10%
3 antigens	5%	0%	4%	0%
2 antigens	10%	4%	4%	10%
1 antigen	5%	0%	4%	0%
0 antigen	80%	4%	0%	5%

[a] Only subjects from whom both fecal and ALS specimens were available
[b] LTB, CFA/I, CS3, CS5, CS6

Based on these highly encouraging results, OEV-122 is proposed to study the safety and immunogenicity of the vaccine when given to children living in areas endemic for ETEC.

Live Attenuated ACE527 Vaccine with and without dmLT – VAC 006

A double-blind, placebo-controlled trial Phase 1/2b study was conducted at the Johns Hopkins University Center for Immunization Research in which the safety, tolerability, and immunogenicity of a live attenuated ETEC vaccine (ACE527) [42] was evaluated alone and in combination with dmLT adjuvant. Subjects were randomized to receive $3x10^9$ cfu of each of three reconstituted ETEC strains (10^{10}cfu total dose) of ACE527 alone (N=24) or with 25 µg dmLT (N=24), or placebo (N=12) at Days 0, 28, and 56. Thirty-six of 60 subjects from the Phase 1 portion consented to participate in the Phase 2b challenge phase approximately six months after the last vaccination; an additional 21 unvaccinated controls were enrolled to boost statistical power; in total 57 subjects were challenged with ~10^7cfu ETEC strain H10407. Immunization with ACE527 administered with or without dmLT was safe and immunogenic. Adding dmLT to the vaccine formulation did not significant change it safety profile. ACE527+dmLT was highly protective against H10407 challenge, with only 3/13 subjects developing severe diarrhea (PE=65.9%, P=0.003 one-sided Barnard's test), while 7/13 (53.8%) subjects receiving ACE527 alone developed severe diarrhea (PE=20.5%; P=0.205). ACE527+dmLT was also 58.5% efficacious in protecting against diarrhea of any severity (P=0.016) and substantially reduced H10407 shedding post-challenge. While further investigation is needed on the contribution of dmLT as adjuvant to the enhanced ACE527 vaccine protection, these early challenge results make the case for its inclusion in this and other ETEC vaccine candidates.

Potential safety risks

Based on experiences from previous studies with 1st generation of inactivated ETEC vaccines and with the recent clinical trials of oral dmLT alone and ETVAX with and without dmLT (OEV-121), immunization with ETVAX and dmLT adjuvant is expected to give rise to only a low frequency of mild and transient symptoms, including loose stools, mild nausea or mild vomiting and abdominal bloating. Results from a clinical study of the dmLT adjuvant show that when administered alone in a single dose of up to 100 ug, also gives rise to very few, mild and transient adverse events [39]. In animal studies, no reproducible reactions to ETVAX administered alone or together with dmLT adjuvant have been observed using equivalent doses to those that will be administered to humans or 25 times higher doses. Furthermore, in the recent OEV-121 trial there was no significant difference in adverse events between participants receiving ETVAX ± dmLT and placebo. To ensure the safety of the volunteers participating in the study, dose escalation of the vaccine and inclusion if the adjuvant will proceed in a stepwise fashion and safety will be continuously monitored. Taken together, the risks for individuals participating in the study are assessed to be small.

Scientific Rationale

As indicated above, the first generation inactivated whole cell vaccine when tested in child populations in Egypt and Bangladesh was reasonably immunogenic but presented tolerability issues, especially with regard to vomiting, amongst the lower infant age-groups when high vaccine doses were given (~10^{11} bacterial cells). Encouragingly when fractional vaccine doses were given to infants in Bangladesh immune responses were noted with greatly improved tolerability. The 1stgeneration vaccine protected travelers against more severe forms of traveler's diarrhea [35]. However, in the one Phase 3 efficacy trial conducted in children, immunization of infants in Egypt did not result in significant protection. After the Phase 3 trials in travelers and infants were completed, a WHO review of the results resulted in recommendations which suggested that the vaccine could be improved by increasing the amount of CF antigen delivered with each dose as well as possibly giving the vaccine with a mucosal adjuvant [1]. The current formulation for the ETVAX vaccine addresses these recommendations and the recent completion of highly successful Phase I trials of safety and immunogenicity in Swedish adults supports the further evaluation of the safety and immunogenicity of this promising 2nd generation ETEC vaccine (ETVAX) among younger age-groups in an ETEC endemic area.

The icddr,b Dhaka hospital treats more diarrheal patients from Mirpur than from any other part of Dhaka. It was observed that most of the isolated cases from the diarrheal patients are *V. cholerae* or ETEC and their general living and hygienic condition is poor with scarcity of safe drinking water and sharing kitchen and toilet. In a birth cohort study in this community, icddr,b has evaluated the natural history of ETEC infections and found that ETEC was the most common pathogen and was isolated in around 20% cases with an incidence of 0.5 episodes/child/year in 0-2 years old children [16]. From the large feasibility study of cholera vaccine where they also conducted passive surveillance for enteric pathogens, it was observed that ETEC incidence rate varies from 1.1 to 1.47 /1000 /year and incidence rate is comparatively high in children less than 5 years old (4.5/1000/year)(Protocol# 12090). The 2% systemic surveillance data from icddr,b suggests that among the diarrheal specimens, ETEC isolation rate is 14% (1996-1998) and 11% (2007-2012) in all age groups. Recently it was observed that of the distribution, 52% ETEC was isolated from those over five years of age and 48% in younger age groups, in which 14 to 20% are severely dehydrated.

This Phase I/II trial will serve to assess whether ETVAX is safe and provides mucosal as well as systemic immune responses against the key protective antigens when tested in different age-groups in Bangladesh. This study provides an opportunity to test the safety profile of a mucosal adjuvant in adults and children, as well as provide the opportunity to potentially assess the ability of dmLT to further enhance the mucosal and systemic antibody responses to key antigens in the ETVAX vaccine among age groups in developing country sites, like Bangladesh, that have proved refractory to oral immunization with enteric vaccines. In addition, this study also allows for the evaluation of the potential dose-sparing effect of dmLT when combined with a lower dose of vaccine. Finally, protective efficacy results observed in the VAC 006 trial substantiate the rationale for dmLT inclusion in this vaccine candidate. This clinical trial is considered an essential study along the critical path of the overall clinical development plan before determining whether the vaccine can be tested for protective efficacy in children in developing countries.

Overall Clinical Development Strategy

The ultimate goal of the ETVAX vaccine clinical development plan is to demonstrate the safety and efficacy of this vaccine approach, with or without dmLT adjuvant, in a series of studies to gain sufficient information to achieve both local registration of the vaccine in the low income country(ies) where the pivotal trials will be conducted and prequalification by WHO to support product acquisition by GAVI, UNICEF or others for distribution, in general, to low income countries. Briefly, the overall clinical development plan consists of: 1) the first in human Phase I safety trial recently performed in Sweden; 2) the proposed Phase I/II descending age safety and immunogenicity trial in adults, children, toddlers and infants in Bangladesh; 3) an expanded phase II immunogenicity study in the target age group, as needed to further define the vaccine dose, inclusion of dmLT, schedule and age of administration, should the information obtained from the proposed trial be insufficient in that respect, especially should modifications be made to the current study product; and 4) Phase III efficacy trials to evaluate vaccine efficacy against moderate to severe ETEC diarrhea. Additional clinical trial evaluations could include: clinical assessment of lot-to-lot consistency (potentially could be combined with the efficacy trials); assessment of interference of the vaccine with the immune responses to routine EPI vaccinations; and assessment of vaccine safety and immunogenicity in other populations.

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, age-descending study that will start testing the vaccine in healthy adults, and move sequentially into toddlers, young children and infants. The study is designed as 4 parts. All participants will receive two doses of vaccine with or without dmLT adjuvant on an outpatient basis. Before moving to the next lower age group, available safety data will be evaluated and reviewed by the icddr,b Data Safety Monitoring Board (DSMB), after which they will make a recommendation whether to proceed to the next age group.

PART A: Adults (18-45 years, inclusive)

The first part (Part A) will enroll a total of 45 adult participants (30 vaccinees and 15 placebos) into one cohort. Approximately 45 participants will be randomized to receive two doses of ETVAX alone, (15 participants), ETVAX + 10 ug dmLT (15 participants), or placebo (15 participants) in a double blinded manner. ETVAX will be administered at full dose (10^{11} inactivated *E. coli* bacteria). The placebo preparation will be sodium bicarbonate buffer. Given a planned sample size of groups of 15 adults each receiving ETVAX with and without dmLT or placebo, Part A will have an approximately 90% chance of observing at least one serious adverse event or adverse event of special interest for events that occur at a rate of 15%. The number and allocation of participants in Part A are shown below.

PART A: Vaccine Dose and Participant Allocation By Cohort

Cohort	Vaccine	10 ug dmLT	(N)	Schedule
A1	Full dose	---	15	Day 0, 14 ± 2
	Full dose	YES	15	
	Placebo	---	15	
ICDDR,B DSMB SAFETY REVIEW OF PART A				

Upon accumulation of all available safety data through Day 3 after the second immunization, the Independent Protocol Safety Team (IPST), comprised of three independent safety monitors, will convene and make a recommendation to the icddr,b DSMB whether to proceed to Part B. Independent safety monitors will be individuals not associated with study operations and who have clinical trials and infectious disease experience. Subsequently, the icddr,b DSMB will convene to review the same dataset and make a recommendation to the Sponsor whether to proceed to Part B. If dmLT is not tolerated in this age-group, it will not be carried forward and ETVAX will be tested alone in Parts B, C and D. Note: Reactogenicity will be assessed through 7 days post dose 1 and dose 2; however, only data through Day 3 after the second dose is needed for all IPST and DSMB reviews.

PART B: Toddlers (2-4 years/24-59 months, inclusive)

The second part (Part B) will enroll a total of 150 toddlers (90 vaccinees and 60 placebos), age 2-4 years (24-59 months, inclusive) into six separate cohorts to be recruited step-wise. The first cohort (B1) will include 25 toddlers, randomized to receive two administrations of ETVAX at ¼ dose (15 children) or placebo (10 children). The second cohort (B2) will include 25 toddlers, randomized to receive two administrations of ETVAX alone at ½ dose (15 children) or placebo (10 children). The third cohort (B3) will include 25 toddlers, randomized to receive two administrations of ETVAX alone at full dose (15 children) or placebo (10 children). The fourth cohort (B4) will include 25 toddlers, randomized to receive two administrations of ETVAX at the highest safe dose (either the full dose, ½ dose, or ¼ dose) together with 2.5 ug dmLT (15 children) or placebo (10 children). The fifth cohort (B5) will include 25 toddlers, randomized to receive two administrations of ETVAX at the highest safe dose (either the full dose, ½ dose, or ¼ dose) together with 5 ug dmLT (15 children) or placebo (10 children). The sixth cohort (B6) will include 25 toddlers, randomized to receive two administrations of ETVAX at the highest safe dose (either the full dose, ½ dose, or ¼ dose) together with 10 ug dmLT

(15 children) or placebo (10 children). After each cohort, the IPST will review available safety data through Day 3 after the second immunization to make the recommendation to the Sponsor whether to proceed with the initiation of the next dose cohort. After Cohort B1, the DSMB will review available safety data through Day 3 after the second immunization to also make a recommendation to the Sponsor whether to proceed with the initiation of Cohort C1 (first dose cohort in 13-23 month old children). Given a planned sample size of groups of 15 toddlers each receiving varying doses of ETVAX with and without dmLT or placebo, Part B will have an approximately 90% chance of observing at least one serious adverse event or adverse event of special interest for events that occur at a rate of 15%. The number and allocation of participants in Part B are shown below.

PART B: Vaccine Dose and Participant Allocation By Cohort

Cohort	Vaccine	dmLT	(N)	Schedule
B1	¼ dose	---	15	Day 0, 14 ± 2
	Placebo	---	10	
Independent Protocol Safety Team (IPST) reviews data through Day 3 after the second administration and makes a recommendation to the Sponsor whether to proceed to the initiation of Cohort B2; DSMB reviews the same dataset and makes a recommendation to the Sponsor whether to proceed to the initiation of Cohort C1.				
B2	½ dose	---	15	Day 0, 14 ± 2
	Placebo	---	10	
IPST reviews data through Day 3 after the second administration and makes a recommendation to the Sponsor whether to proceed to the initiation of Cohort B3.				
B3	Full dose	---	15	Day 0, 14 ± 2
	Placebo	---	10	
IPST reviews data through Day 3 after the second administration and makes a recommendation to the Sponsor of the highest safe dose and whether to proceed with the highest safe dose plus 2.5 ug of dmLT (Cohort B4).				
B4	Highest safe dose	2.5 ug	15	Day 0, 14 ± 2
	Placebo	---	10	
IPST reviews data through Day 3 after the second administration and makes a recommendation to the Sponsor of the highest safe dose and whether to proceed with the highest safe dose plus 5 ug of dmLT (Cohort B5).				
B5	Highest safe dose	5 ug	15	Day 0, 14 ± 2
	Placebo	---	10	
IPST reviews data through Day 3 after the second administration and makes a recommendation to the Sponsor whether to proceed with the highest safe dose plus 10 ug of dmLT (Cohort B6).				
B6	Highest safe dose	10 ug	15	Day 0, 14 ± 2
	Placebo	---	10	
ICDDR,B DSMB SAFETY REVIEW OF PART B				

If dmLT is not tolerated in this age-group, ETVAX will be tested alone in Parts C and D (i.e., cohorts C3, C4, D4 and D5 will be dropped). Note: Reactogenicity will be assessed through 7 days post dose 1 and dose 2; however, only data through Day 3 after the second dose is needed for all IPST and DSMB reviews.

PART C: Younger children (12-23 months, inclusive)

The third part will enroll a total of 100 participants. The first cohort (C1) will include 25 children, randomized to receive two administrations of ETVAX alone at ¼ dose (15 children) or placebo (10 children). The second cohort (C2) will include 25 children, randomized to receive two administrations of ETVAX alone at ½ dose (15 children) or placebo (10 children). The third cohort (C3) will include 25 children randomized to receive two administrations of ETVAX at the highest safe dose (either ½ or ¼ dose) together with 2.5 ug dmLT (15 children) or placebo (10 children). The fourth cohort (C4) will include 25 children randomized to receive two administrations of ETVAX at the dose evaluated in C4 (either ½ or ¼ dose) together with 5 ug dmLT (15 children) or placebo (10 children). After each cohort, the IPST will review available safety data through Day 3 after the second immunization to make the recommendation to the Sponsor whether to proceed with the initiation of the next dose cohort. After Cohort C1, the DSMB will review available safety data through Day 3 after the second immunization to also make a recommendation to the Sponsor whether to proceed with

the initiation of Cohort D1 (first dose cohort in infants). Given a planned sample size of groups of 15 children each receiving varying doses of ETVAX with and without dmLT or placebo, Part C will have an approximately 90% chance of observing at least one serious adverse event or adverse event of special interest for events that occur at a rate of 15%. The number and allocation of participants in Part C are shown below.

PART C: Vaccine Dose and Participant Allocation By Cohort

Cohort	Vaccine	dmLT	(N)	Schedule
C1	¼ dose	---	15	Day 0, 14 ± 2
	Placebo	---	10	
IPST reviews data through Day 3 after the second administration and makes a recommendation to the Sponsor whether to proceed to the initiation of Cohort C2; DSMB reviews the same dataset and makes a recommendation to the Sponsor whether to proceed to the initiation of Cohort D1.				
C2	½ dose	---	15	Day 0, 14 ± 2
	Placebo	---	10	
IPST reviews data through Day 3 after the second administration and makes a recommendation to the Sponsor of the highest safe dose and whether to proceed with the highest safe dose plus 2.5 ug of dmLT (Cohort C3).				
C3	Highest safe dose	2.5 µg	15	Day 0, 14 ± 2
	Placebo	---	10	
IPST reviews data through Day 3 after the second administration and makes a recommendation to the Sponsor whether to proceed with the highest safe dose plus 5 ug of dmLT (Cohort C4).				
C4	Highest safe dose	5 µg	15	Day 0, 14 ± 2
	Placebo	---	10	
ICDDR,B DSMB SAFETY REVIEW OF PART C				

If dmLT is not tolerated in this age-group, ETVAX will be tested alone in Part D (i.e., cohorts D4 and D5 will be dropped). Note: Reactogenicity will be assessed through 7 days post dose 1 and dose 2; however, only data through Day 3 after the second dose is needed for all IPST and DSMB reviews.

PART D: Infants (6-11 months, inclusive)

The fourth part will enroll a total of 200 infants. The first cohort (D1) will include 40 infants, randomized to receive two administrations of ETVAX at 1/8 dose (30 infants) or placebo (10 infants). The second cohort (D2) will include 40 infants, randomized to receive two administrations of ETVAX at ¼ dose (30 infants) or placebo (10 infants). The third cohort (D3) will include 40 infants, randomized to receive two administrations of ETVAX at 1/2 dose (30 infants), or placebo (10 infants).The fourth cohort (D4) will include 30 infants, randomized to receive two administrations of the highest tolerable dose of ETVAX (either 1/8 dose, ¼ dose or ½ dose) together with 2.5 ug dmLT, and 10 placebo recipients. The fifth cohort (D5) will include 30 infants, randomized to receive two administrations of the highest tolerable dose of ETVAX as tested in D4 (either 1/8 dose, ¼ dose or ½ dose) together with 5 ug dmLT, and 10 placebo recipients. After each cohort, the IPST will review available safety data through Day 3 after the second immunization to make the recommendation to the Sponsor whether to proceed with the initiation of the next cohort. Given a planned sample size of 30 participants per dose group, the study will have an approximately 90% chance of observing at least one serious adverse event or adverse event of special interest for events that occur at a rate of 7.4%. The number and allocation of participants in Part D are shown below.

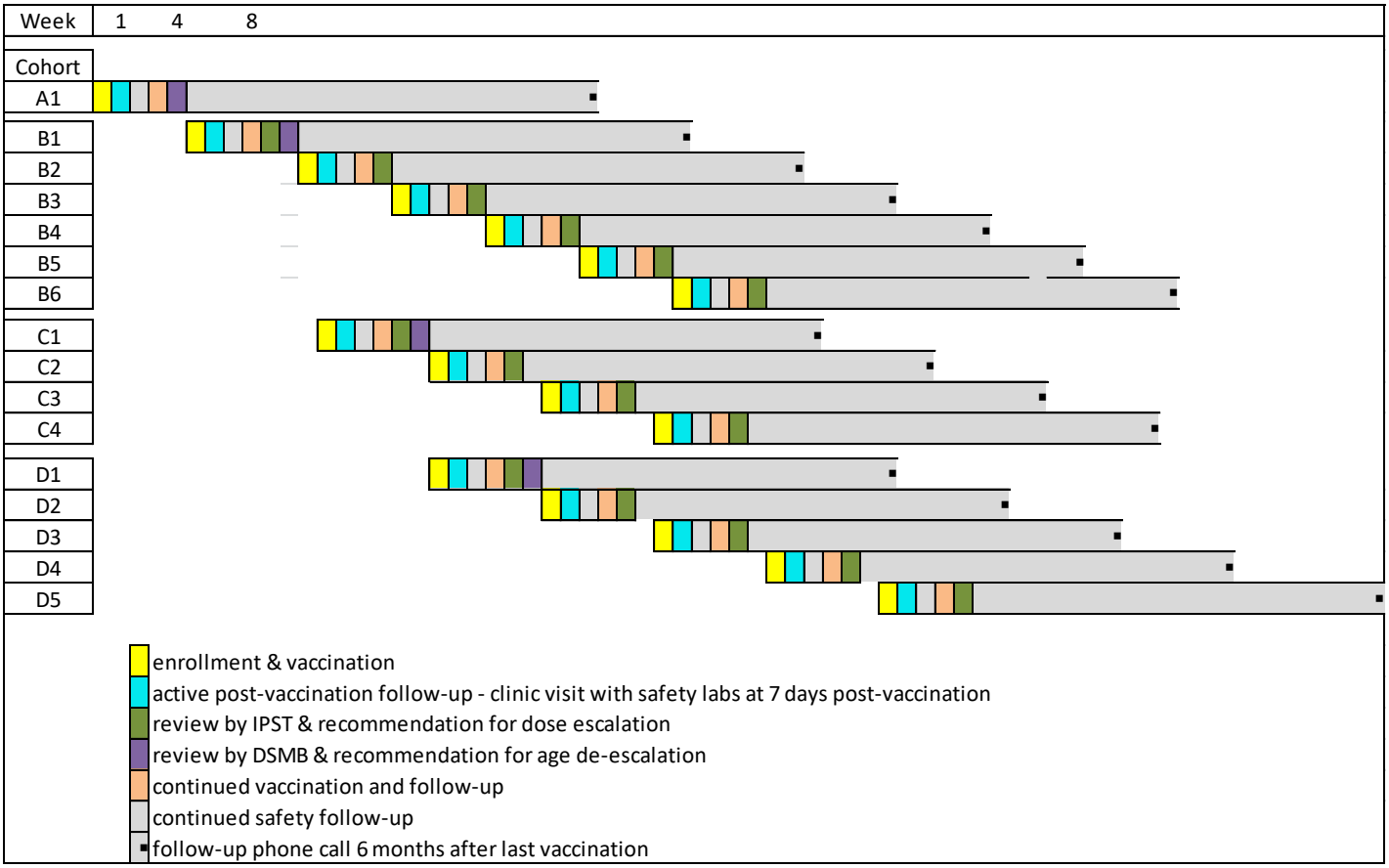
The number and allocation of participants in Part D are shown below.

PART D: Vaccine Dose and Participant Allocation By Cohort

Cohort	Vaccine	dmLT	(N)	Schedule
D1	1/8 dose	---	30	Day 0, 14 ± 2
	Placebo	---	10	

IPST reviews data through Day 3 after the second administration and makes a recommendation to the Sponsor whether to proceed to the initiation of Cohort D2.				
D2	¼ dose	---	30	Day 0, 14 ± 2
	Placebo	---	10	
IPST reviews data through Day 3 after the second administration and makes a recommendation to the Sponsor whether to proceed to the initiation of Cohort D3.				
D3	½ dose	---	30	Day 0, 14 ± 2
	Placebo	---	10	
IPST reviews data through Day 3 after the second administration and makes a recommendation to the Sponsor of the highest safe dose and whether to proceed with the highest safe dose plus 2.5 ug of dmLT (Cohort D4).				
D4	Highest safe dose	2.5 ug	30	Day 0, 14 ± 2
	Placebo	---	10	
IPST reviews data through Day 3 after the second administration and makes a recommendation to the Sponsor whether to proceed with the highest safe dose plus 5 ug of dmLT (Cohort D5).				
D5	Highest safe dose	5 ug	30	Day 0, 14 ± 2
	Placebo	---	10	
ICDDR,B DSMB REVIEW OF PART D				

After completion of all vaccinations, the DSMB will convene to review available safety data through Day 42 of the last dosing cohort to mark the last regularly scheduled DSMB meeting. The cohort dosing and dose escalation schedule is shown in the figure below.



Study Products

Oral ETVAX Vaccine (Batch BX1003574)

The monovalent bulks of the inactivated E. coli bacteria and the LCTBA hybrid protein have been produced by Unitech Biopharma, Matfors, Sweden, the final ETVAX vaccine was formulated, filled and released by Biovian Oy, Turku, Finland.

A vial of ETVAX vaccine represents a full dose and contains the following:

E. coli ETEX 21	1.3 mg CFA/I
E. coli ETEX 22	6.4 mg CS3
E. coli ETEX23	1.1 mg CS5
E. coli ETEX24	0.5 mg CS6
LCTB*A*:	1 mg
Phosphate buffered saline:	q.s. ad 14.0 ml

The vaccine should be stored at 2-8°C. Do not freeze.

The vaccine is given together with sodium bicarbonate effervescent granules (Recip), which is dissolved in water and mixed with the vaccine suspension prior to oral administration. The buffer is used to prevent degradation of LCTB*A* hybrid protein by the gastric acid.

Inactivated *E. coli* ETEX 21

The *E. coli* ETEX 21 strain was developed using a recombinant plasmid expressing the entire CFA/I operon under a tac promoter as described with the difference that the antibiotic selection marker (ampicillin) was replaced with the gene encoding thymidinylatesynthetase, (*thyA*) from *V. cholerae.*[35] The bacterial host for this construct is an *Escherichia coli*, CFA/I, O78, K^-. The strain was originally isolated from a patient in Dhaka, Bangladesh in 1985 suffering from diarrhea due to an ETEC infection. This strain was used as a component of the first generation ETEC vaccine and has been given to at least 2000 subjects. The plasmid(s) encoding the ST and CFA/I native genes were removed by natural selection. Further modification was done by knocking out the *thyA* gene on the chromosome by inserting a kanamycin resistance gene in the *thyA* gene. Furthermore, in a second round of chromosomal deletion the kanamycin gene was deleted in its first 200 nucleotides making it kanamycin sensitive. The combination of the *thyA* expressing plasmid and the *thyA* deficient host strain enables antibiotic-free selection of the recombinant ETEX 21 strain. Inactivation of the strain was performed by mild formalin treatment [37].

Inactivated *E. coli* ETEX 22

The *E. coli* ETEX 22 strain was developed using a recombinant plasmid expressing the entire CS3 operon under a*rns* promoter which in turn is under the *lac* operator. The selection system for this plasmid is also based on the *thyA*gene from *V. cholerae*. The bacterial host for this construct is the same *E. coli*, CFA/I, O78, K^- strain, as described for ETEX21. The combination of the *thyA* expressing plasmid and the *thyA* deficient host strain enables antibiotic-free selection of the recombinant ETEX 22 strain. Inactivation of the strain was performed by mild formalin treatment [37].

Inactivated *E. coli* ETEX 23

The *E. coli* ETEX 23 strain was developed using a recombinant plasmid expressing the entire CS5 operon under a under a tac promoter. The selection system for this plasmid is also based on the *thyA*gene from *V. cholerae*. The bacterial host for this construct is the same *E. coli*, CFA/I, O78, K^- strain, as described for ETEX 21 and ETEX 22. The combination of the *thyA* expressing plasmid and the *thyA* deficient host strain enables antibiotic-free selection of the recombinant ETEX 23 strain. Inactivation of the strain was performed by mild formalin treatment [37].

Inactivated *E. coli* ETEX 24

The *E. coli* ETEX 24 strain was developed using a recombinant plasmid expressing the entire CS6 operon under a tac promoter. The selection system for this plasmid is also based on the *thyA*gene from *V. cholerae*. The bacterial host for this construct is an *E. coli* K12 strain C600, previously used as a placebo in numerous Dukoral® and ETEC clinical trials. This strain was modified by knocking out the *thyA* gene on the chromosome by inserting a kanamycin resistance gene in the *thyA* gene. This strain was used as host for the SBL109 strain, studied in a previous clinical trial (EudraCT: 2009-015741-23, OEV-120) as a component of the "Prototype ETEC Vaccine No 2". In a second round of chromosomal deletion the kanamycin gene was deleted in its first 200 nucleotides making it kanamycin sensitive. The combination of the *thyA*

expressing plasmid and the *thyA* deficient host strain enables antibiotic-free selection of the recombinant ETEX 24 strain. Inactivation of the strain was performed by mild phenol treatment [37].

LCTB*A* protein

LCTB*A* is a hybrid protein between the B-subunit of the *E. coli* heat-labile enterotoxin (LTB) and the B-subunit of the cholera toxin (CTB). Seven amino acids in the CTB molecule have been replaced by amino acids at the corresponding positions of the LTB molecule [44]. The LCTB*A* encoding DNA was cloned on a plasmid under a *tac* promoter. The plasmid has the *thyA* gene from *E. coli* and is expressed in a *V. cholerae* strain that is deleted in its *thyA* gene, enabling antibiotic free selection of this plasmid. The *V. cholerae* strain that hosts the plasmid is a further development of the strain holding the rCTB213 encoding plasmid JS1569. The change is the deletion of the *thyA* gene, thus the genetic characteristics are Δ*ctxA,* Δ*thyA,* Δkan. The LCTBA protein was recently evaluated for safety and immunogenicity in 1 mg and 4 mg dosages together with inactivated CFA/I over-expressing SBL 109 bacteria in the OEV-120 clinical trial. This study revealed that the LCTB*A* protein is safe and strongly immunogenic at both dosage levels tested (40).

Oral dmLT Adjuvant

LT(R192G/L211A), or dmLT, is a derivative of wild-type enterotoxigenic *Escherichia coli* LT that has been genetically modified by replacing the arginine at amino acid position 192 with glycine and the leucine at amino acid position 211 with alanine (25). These two amino acid substitutions take place in proteolytic cleavage sites which are critical for activation of the secreted toxin molecules. The protein has been designated LT(R192G/L211A) and has been extensively evaluated in pre-clinical animal studies for its ability to adjuvant the immune responses for co-administered antigens.

Sodium bicarbonate protective buffer powder (Recip, Scandinavian BioPharma)

Protective buffer is used to neutralize gastric acidity upon ingestion of vaccine plus LCTBA toxoid. The buffer is also used as placebo in the study. The dried powder is supplied in moisture-proof sachets (5.6 g/sachet).

Sodium Bicarbonate buffer Powder:	
Sodium hydrogen carbonate	3 600 mg
Citric acid, anhydrous	1 450 mg
Sodium carbonate	400 mg
Raspberry flavor	70 mg
Sodium saccharin	30 mg

For use, the carbonate buffer is dissolved in 150 ml of potable water. The sodium hydrogen carbonate buffer has been produced by Recip AB in Sweden (Batch SH207A; expiration 28 February 2016).

Presentation and Formulation of Investigational Products

There are ten ETVAX vaccine vials in one carton and twenty-five buffer sachets in one carton. Each ETVAX vaccine vial contains one full vaccine dose (14 ml suspension).

dmLT is formulated as a freeze-dried (lyophilized), white to off-white cake, containing 700 µg of vaccine protein in a 3 mL, multi-dose, Wheaton Serum Vial. The product is lyophilized in a sodium phosphate buffer supplemented with 5% lactose to stabilize the product during the freezing and drying process. The vials are sealed by a West S-87-J 13 mm lyophilization stopper and a crimped metal collar. Although not shown on the label, each vial of dmLT Lot No. 1575 also has been assigned an individual vial number for product accountability purposes, which will appear in the upper right hand corner of the label.

Vials are labelled:

Recombinant double mutant Heat Labile Toxin LT(R192G/L211A) Expressed in *E. coli*	
BPR No.: BPR-928-00	Lot No. 1575
Contents: 0.7 mL (700 µg Lyoph.)	Storage: -20ºC ± 5°C
Caution: New drug limited by Federal Law to investigational use only.	
Date of Mfg: 20 Jul 09	
Manufactured By: WRAIR, Silver Spring, MD 20910	

Stability and Storage

ETVAX should be stored at 2-8°C until it is ready for use. Do not freeze. One year stability data for the lot of ETVAX to be used in VAC 014/OEV-122 are currently available.

The lyophilized vials of the dmLT adjuvant will be shipped from the pilot biologic production facility (PBF) at the Walter Reed Army Institute of Research (WRAIR) to icddr,b packaged on dry ice and maintained at a temperature of -10ºC to -90ºC. Upon receipt at icddr,b it must be stored at -20ºC ± 5ºC. While the storage temperature specified on the dmLT product label lot 1575 is -20ºC ± 5ºC, PATH Vaccine Solutions (PVS) acknowledges that this temperature range is too narrow. Therefore, PVS accepts -20ºC ± 10ºC as the temperature range for maintenance of vaccine potency during site storage of the vaccine. The expansion in the acceptable shipping and storage range for dmLT is documented in a memorandum from PVS that will be provided to the research pharmacy supporting the trial when the adjuvant is shipped from the pilot biologic production facility at the Walter Reed Army Institute of Research (WRAIR) in Forest Glenn, MD, USA. Temperature of the lyophilized vials of dmLT should also be maintained at -10ºC to -90ºC during transport to the Mipur field site.

This pilot lot has shown excellent stability and retention of adjuvant activity through 64 months of storage at both -20º C (lyophilized preparation) and at 2-10º C as a liquid suspension. In addition, the stability testing of this clinical lot of dmLT will continue over the course of the clinical phase of this trial.

Sodium bicarbonate protective buffer powder will be used for placebo and stored at room temperature.

Preparation and Administration

ETVAX and dmLT will be obtained from SBH and WRAIR, respectively, and maintained at the proper temperatures until the day of vaccination. The investigational products (ETVAX, dmLT, and placebo) will be prepared by the study pharmacist or a member of the IP formulation team on the day of vaccination (Day 0) according to written procedures. Participants will be required to fast for at least 60 minutes prior to the scheduled time of dosing. Participants will fast for an additional 60 minutes after ingestion of the investigational product or placebo. Infants should not breastfeed for 60 minutes before and after immunization.

Each ETVAX and dmLT vial is to be visually inspected to ensure there are no cracks in the vial and the screw top is fastened tightly. The vial should NOT be used if it fails visual inspection. If a vial fails inspection, the vial should be sealed in a zip-lock bag, marked “Do Not Use” and stored separately from the rest of the clinical material supply. The required volume of ETVAX, supplied as a liquid, is mixed with the required volume of sodium bicarbonate buffer solution on the day of preparation for use on dosing day. The time of investigational product preparation will be recorded on the appropriate Accountability Record. The vaccine dose in sodium bicarbonate buffer can be stored at room temperature for up to 90 minutes prior to dosing.

The lyophilized dmLT adjuvant will be reconstituted by adding 0.7 mL of water to vials containing 700 µg dmLT. The reconstituted adjuvant solution (1 mg dmLT per ml) will be further diluted 1:5 in phosphate-buffered saline (PBS) to final concentration 200 µg/ml. The diluted suspension may be stored for up to 6 hrs at +4 to +8°C, and is used to prepare the final dosages for volunteers receiving vaccine plus dmLT adjuvant.

Placebo recipients will be given buffer alone. The buffer ingredients are delivered as granules, which are dissolved in a glass of water and either given alone (placebo) or mixed with the vaccine and ingested.

On the first day of a new cohort, up to 5 participants will be dosed. In the absence of any safety signals, the remaining participants will be dosed on subsequent days.

PART A

Cohort A1:

- Participants randomized to treatment with placebo: A total of 150 ml of reconstituted bicarbonate buffer diluted with 14 ml water will be ingested
- Participants randomized to treatment with ETVAX vaccine only: 1 vial of vaccine will be mixed with 150 mL of reconstituted bicarbonate buffer and ingested.

Participants randomized to treatment with ETVAX vaccine + 10 µg dmLT: 1 vial of vaccine and 50 ul diluted dmLT will be mixed with 150 mL of reconstituted bicarbonate buffer and ingested. Vaccine and placebo volume allocation per cohort for Part A are shown below:

Cohort	Vaccine	10 ug dmLT	Reconstituted Volume for ingestion	N	Schedule
A1	Full Dose = 14 ml	---	150 ml (buffer) + 14 ml ETVAX = **164 ml**	15	Day 0, 14 ± 2
	Full Dose = 14 ml	YES	150 ml (buffer) + 14 ml ETVAX + 0.05ml dmLT = **164.05 ml**	15	
	Placebo	---	150 ml (buffer) + 14 ml water = **164 ml**	15	

PART B:

Cohort B1:

- Participants randomized to treatment with placebo: A total of 30 ml of reconstituted bicarbonate buffer diluted with 3.5 ml water will be ingested.
- Participants randomized to ETVAX vaccine only: ¼ vial of vaccine will be mixed with 30 mL of reconstituted bicarbonate buffer and ingested.

Cohort B2:

- Participants randomized to treatment with placebo: A total of 30 ml of reconstituted bicarbonate buffer diluted with 7 ml water will be ingested.
- Participants randomized to ETVAX vaccine only: ½ vial of vaccine will be mixed with 30 mL of reconstituted bicarbonate buffer and ingested.

Cohort B3:

- Participants randomized to treatment with placebo: A total of 30 ml of reconstituted bicarbonate buffer diluted with 14 ml water will be ingested.
- Participants randomized to treatment with ETVAX vaccine only: 1 vial of vaccine will be mixed with 30 mL of reconstituted bicarbonate buffer and ingested.

Cohort B4:

- Participants randomized to treatment with placebo: A total of 30 ml of reconstituted bicarbonate buffer diluted with the applicable volume of water* will be ingested.
- Participants randomized to treatment with ETVAX vaccine only: the applicable fractionated dose of vaccine will be mixed with 30 mL of reconstituted bicarbonate buffer and ingested.
- Participants randomized to treatment with ETVAX vaccine + 2.5 µg dmLT: the applicable fractionated dose of vaccine and 12.5 ul diluted dmLT will be mixed with 30 mL of reconstituted bicarbonate buffer and ingested.

Cohort B5:

- Participants randomized to treatment with placebo: A total of 30 ml of reconstituted bicarbonate buffer diluted with the applicable volume of water* will be ingested
- Participants randomized to treatment with ETVAX vaccine only: the applicable fractionated dose of vaccine will be mixed with 30 mL of reconstituted bicarbonate buffer and ingested.

- Participants randomized to treatment with ETVAX vaccine + 5 µg dmLT: the applicable fractionated dose of vaccine and 25 ul diluted dmLT will be mixed with 30 mL of reconstituted bicarbonate buffer and ingested.

Cohort B6:

- Participants randomized to treatment with placebo: A total of 30 ml of reconstituted bicarbonate buffer diluted with the applicable volume of water* will be ingested.
- Participants randomized to treatment with ETVAX vaccine only: the applicable fractionated dose of vaccine will be mixed with 30 mL of reconstituted bicarbonate buffer and ingested.
- Participants randomized to treatment with ETVAX vaccine + 10 µg dmLT: the applicable fractionated dose of vaccine and 50 ul diluted dmLT will be mixed with 30 mL of reconstituted bicarbonate buffer and ingested.

Vaccine and placebo volume allocation per cohort for Part B are shown below:

Cohort	Vaccine	dmLT	Reconstituted Volume for ingestion	(N)	Schedule
B1	¼ dose = 3.5ml	---	30 ml (buffer) + 3.5 ml ETVAX = **33.5 ml**	15	Day 0, 14 ± 2
	Placebo	---	30 ml (buffer) + 3.5 ml water = **33.5 ml**	10	
B2	½ dose = 7 ml	---	30 ml (buffer) + 7 ml ETVAX = **37 ml**	15	Day 0, 14 ± 2
	Placebo	---	30 ml (buffer) + 7 ml water = **37 ml**	10	
B3	Full dose = 14 ml	---	30 ml (buffer) + 14 ml ETVAX = **44 ml**	15	Day 0, 14 ± 2
	Placebo	---	30 ml (buffer) + 14 ml water = **44 ml**	10	
B4	Highest safe dose	2.5 ug = 0.0125 ml	TBD	15	Day 0, 14 ± 2
	Placebo	---	TBD	10	
B5	Highest safe dose	5 ug = 0.025 ml	TBD	15	Day 0, 14 ± 2
	Placebo	---	TBD	10	
B6	Highest safe dose	10 ug = 0.05 ml	TBD	15	Day 0, 14 ± 2
	Placebo	---	TBD	10	

PART C

Cohort C1:

- Participants randomized to treatment with placebo: A total of 15 ml of reconstituted bicarbonate buffer diluted with 3.5 ml water will be ingested
- Participants randomized to ETVAX vaccine only: ¼ vial of vaccine will be mixed with 15 mL of reconstituted bicarbonate buffer and ingested.

Cohort C2:

- Participants randomized to treatment with placebo: A total of 15 ml of reconstituted bicarbonate buffer diluted with 7 ml water will be ingested
- Participants randomized to ETVAX vaccine only: ½ vial of vaccine will be mixed with 15 mL of reconstituted bicarbonate buffer and ingested.

Cohort C3:

- Participants randomized to treatment with placebo: A total of 15 ml of reconstituted bicarbonate buffer diluted with the applicable volume of water* will be ingested

- Participants randomized to treatment with ETVAX vaccine + 2.5 µg dmLT: the applicable fractionated dose of vaccine and 12.5 ul diluted dmLT will be mixed with 15 mL of reconstituted bicarbonate buffer and ingested.

Cohort C4:

- Participants randomized to treatment with placebo: A total of 15 ml of reconstituted bicarbonate buffer diluted with the applicable volume of water* will be ingested
- Participants randomized to treatment with ETVAX vaccine only: the applicable fractionated dose of vaccine will be mixed with 30 mL of reconstituted bicarbonate buffer and ingested.

Participants randomized to treatment with ETVAX vaccine + 5 µg dmLT: the applicable fractionated dose of vaccine and 25 ul diluted dmLT will be mixed with 15 mL of reconstituted bicarbonate buffer and ingested. Vaccine and placebo volume allocation per cohort for Part C are shown below:

Cohort	Vaccine	dmLT	Reconstituted Volume for ingestion	(N)	Schedule
C1	¼ dose = 3.5 ml	---	15 ml (buffer) + 3.5 ml ETVAX = **18.5 ml**	15	Day 0, 14 ± 2
	Placebo	---	15 ml (buffer) + 3.5 ml water = **18.5 ml**	10	
C2	½ dose = 7 ml	---	15 ml (buffer) + 7 ml ETVAX = **22 ml**	15	Day 0, 14 ± 2
	Placebo	---	15 ml (buffer) + 7 ml water = **22 ml**	10	
C3	Highest safe dose	2.5 µg= 0.0125 ml	TBD	15	Day 0, 14 ± 2
	Placebo	---	TBD	10	
C4	Highest safe dose	5 µg = 0.025 ml	TBD	15	Day 0, 14 ± 2
	Placebo	---	TBD	10	

PART D

Cohort D1:

- Participants randomized to treatment with placebo: A total of 10 ml of reconstituted bicarbonate buffer diluted with 1.75 ml water will be ingested.
- Participants randomized to ETVAX vaccine only: **1/8** vial of vaccine will be mixed with 10 mL of reconstituted bicarbonate buffer and ingested.

Cohort D2:

- Participants randomized to treatment with placebo: A total of 10 ml of reconstituted bicarbonate buffer diluted with 3.5 ml water will be ingested.
- Participants randomized to ETVAX vaccine only: ¼ vial of vaccine will be mixed with 10 mL of reconstituted bicarbonate buffer and ingested.

Cohort D3:

- Participants randomized to treatment with placebo: A total of 10 ml of reconstituted bicarbonate buffer diluted with 7 ml water will be ingested.
- Participants randomized to ETVAX vaccine only: ½ vial of vaccine will be mixed with 10 mL of reconstituted bicarbonate buffer and ingested.

Cohort D4:

- Participants randomized to treatment with placebo: A total of 10 ml of reconstituted bicarbonate buffer diluted with the applicable volume of water* will be ingested

- Participants randomized to treatment with ETVAX vaccine + 2.5 µg dmLT: the applicable fractionated dose of vaccine and 12.5 ul diluted dmLT will be mixed with 10 mL of reconstituted bicarbonate buffer and ingested.

Cohort D5:

- Participants randomized to treatment with placebo: A total of 10 ml of reconstituted bicarbonate buffer diluted with the applicable volume of water* will be ingested.
- Participants randomized to treatment with ETVAX vaccine only: the applicable fractionated dose of vaccine will be mixed with 10 mL of reconstituted bicarbonate buffer and ingested.
- Participants randomized to treatment with ETVAX vaccine + 5 µg dmLT: the applicable fractionated dose of vaccine and 25 ul diluted dmLT will be mixed with 10 mL of reconstituted bicarbonate buffer and ingested. .

*So that the total volume equals that of the group receiving vaccine

Vaccine and placebo volume allocation per cohort for Part D are shown below:

Cohort	Vaccine	dmLT	Reconstituted Volume for ingestion	(N)	Schedule
D1	1/8 dose = 1.75 ml	---	10 ml (buffer) + 1.75 ml ETVAX= **11.75 ml**	30	Day 0, 14 ± 2
	Placebo	---	10 ml (buffer) + 1.75 ml water = **11.75 ml**	10	
D2	¼ dose = 3.5 ml	---	10 ml (buffer) + 3.5 ml ETVAX= **13.5 ml**	30	Day 0, 14 ± 2
	Placebo	---	10 ml (buffer) + 3.5 ml water = **13.5 ml**	10	
D3	½ dose = 7 ml	---	10 ml (buffer) + 7 ml ETVAX= **17 ml**	30	Day 0, 14 ± 2
	Placebo	---	10 ml (buffer) + 7 ml water = **17 ml**	10	
D4	Highest safe dose	2.5 ug = 0.0125 ml	TBD	30	Day 0, 14 ± 2
	Placebo	---	TBD	10	
D5	Highest safe dose	5 ug = 0.025 ml	TBD	30	Day 0, 14 ± 2
	Placebo	---	TBD	10	

Accountability and Disposal

The study pharmacist or a member of the IP formulation team will record all required mixing procedures on the appropriate buffer and vaccine preparation logs. A second unblinded party will verify all steps. The study pharmacist or a member of the IP formulation team will be responsible for maintaining accurate records of the shipment and dispensing of the investigational product. All efforts will be made to protect the integrity of the blind. The pharmacy records must be available for inspection by the Sponsor's representative and is subject to inspection by a regulatory agency (e.g., FDA) at any time. A Study Monitor will review the pharmacy records to assure proper accounting of study investigational products. Unused investigational product will be maintained until the clinical trial material accountability has been completed by the Study Monitor. Following completion of drug accountability, all unused product must be destroyed at icddr,b in accordance with site defined procedures and Sponsor instructions. A written explanation will be required for any product not disposed of in accordance with this Protocol.

To maintain blinding of the study, the test items (vaccine ± adjuvant) and placebo will be prepared by the unblinded study pharmacist or a member of the IP formulation team who has no contact with the study participants. The study pharmacist (or designee) will be responsible for ensuring that the treatment arms appear the same to participants and other blinded

staff. This will be done by utilizing the same dosing cups and labelling the cups to ensure that all other site staff except the study pharmacist and/or designee will be kept blinded as per written study-specific procedure (SSP). The randomization code will be generated by the Coordinating Center and implemented by the study pharmacist or a member of the IP formulation team. Randomization data are kept strictly confidential, accessible only to authorized persons, until the time of unblinding.

Once the dosing step is completed, the remaining dmLT vaccine dilutions should be aliquoted into one screw cap glass tube. The tube should be stored at 2-8°C. One tube from each dmLT dilution will be processed for dose verification maximally 7 days after preparation..

STUDY POPULATION

This study is a single-site clinical trial, to be performed at the Mirpur field site by the International Centre for Diarrheal Disease Research, Bangladesh (icddr,b). Mirpur is a densely populated part of the Dhaka metropolitan area with an estimated population of over 3.5 million. It consists of 16 wards with different low to middle income socio-economic groups of communities. The Mirpur site has been used extensively for many years with different vaccine trials and descriptive trials. The field office contains a participant waiting area, several examination rooms, a staff work and file room, a specimen processing area, archive room and a meeting room with 24 hours generator back-up. The area is well secured and has 24 hr security coverage and internet service. Socio- demographic information of the high risk population is available for this area. A geographic information system (GIS) is also available and is used for mapping of this area and identifying households and related information.

Study Population

The study population will consist of healthy adults (18-45 years, inclusive), toddlers (2-4 years/24-59 months, inclusive), children (12-23 months, inclusive) and infants (6-11 months, inclusive) from the communities in the vicinity of the study clinic. Children, toddlers and infants will only be enrolled if their parents are fully informed about the study and provide consent for their children's participation.

Eligibility

Participants will be in general good health. Final eligibility determination will depend on the results of the medical history, clinical examination, screening laboratory tests and fulfilment of all the inclusion and absence of any of the exclusion criteria, appropriate parental understanding of the study and completion of the consent process.

Investigators should always use good clinical judgment in considering a participant's overall fitness for inclusion in the trial. Some participants may not be appropriate for the study, even if they meet all inclusion/exclusion criteria. For instance, medical, occupational or other conditions present in the parents may make safety evaluations difficult or make toddlers and infants poor candidates for retention. All children, toddlers, and infants targeted for enrollment will need to have parents that can comprehend the purpose of the study and provide written or thumb print informed consent. In addition, the families should be resident in the area without plans to leave the study site during the course of the study.

PART A: Adult Eligibility Criteria

Inclusion criteria

1. Healthy male or female adults 18-45 years old, inclusive
2. General good health as determined by the screening evaluation no greater than 7days before enrollment and vaccination
3. Properly informed about the study, able to understand it and sign or thumb print the informed consent form
4. Available for the entire period of the study and reachable by study staff throughout the entire follow-up period
5. Females of childbearing potential who are willing to take a urine pregnancy test at screening and before the second vaccination. Pregnancy tests must be negative before each vaccination. Females of childbearing potential must agree to use an efficacious hormonal or barrier method of birth control during the study. Abstinence is also acceptable.
6. Informed Consent (signature or thumb print provided, with witness signature)

Exclusion criteria

1. Presence of any significant known systemic disorder (cardiovascular, pulmonary, hepatic, 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.
2. History of congenital abdominal disorders, intussusception, abdominal surgery or any other congenital disorder or presence of a significant medical condition that in the opinion of the Investigator precludes participation in the study. Known or suspected impairment of immunological function based on medical history and physical examination. Clinical evidence of active gastrointestinal illness and acute disease at the time of enrollment
3. Screening positive with hepatitis B antigen and/or hepatitis C antibodies
4. Participation in research involving another investigational product (defined as receipt of investigational product) during the 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 participant has been or will be exposed to an investigational or a non-investigational product
5. Clinically significant abnormalities in screening hematology or serum chemistry, as determined by the Study Physician
6. History of febrile illness within 48 hours prior to vaccination and fever at the time of immunization (fever is defined as a temperature ≥ 37.5°C (99.5°F) on axillary, oral, or tympanic measurement)
7. Prior receipt of any cholera (e.g., Dukarol, Shancol) or ETEC vaccine
8. Prior receipt of a blood transfusion or blood products, including immunoglobulins
9. Evidence of current illicit drug use or drug dependence
10. Current use of iron or zinc supplements within the past 7 days; current use of antacids (H2 blockers, omeprazole, OTC agents) or immunosuppressive drug
11. Any condition which, in the opinion of the investigator, might jeopardize the safety of study participants or interfere with the evaluation of the study objectives
12. Receipt of antimicrobial drugs for any reason within 14 days before vaccination
13. History of diarrhea during the 7 days before vaccination (see protocol definition of diarrhea)
14. Culture positive for ETEC, *Shigella*, *V. Cholerae or Salmonella* within 7 days before vaccination.
15. Acute disease at the time of enrollment or 3 days prior to enrollment
16. History of chronic administration (defined as more than 14 days) of immunosuppressant medications, including corticosteroids.

PARTS B, C, and D: Children, Toddler, and Infant Eligibility Criteria

Fulfilment of all of the following criteria is required to accept a participant for Parts B, C or D in the study:

Inclusion Criteria

1. Healthy male or female infants/toddlers/children ages:
    a. Part B: ≥24 and ≤59 months old at the time of enrollment
    b. Part C: ≥12 and <24 months old at the time of enrollment
    c. Part D: ≥6 and <12 months at the time of enrollment
2. General good health as determined by the screening evaluation no greater than 7 days before enrollment and vaccination
3. Parent properly informed about the study, able to understand it and sign or thumb print the informed consent form
4. Parent and child available for the entire study period of the study and reachable by study staff throughout the entire follow-up period
5. Informed Consent (signature or thumb of parent, with signature of witness, provided)

Exclusion Criteria

1. Presence of any significant known systemic disorder (cardiovascular, pulmonary, hepatic, 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.
2. History of congenital abdominal disorders, intussusception, abdominal surgery or any other congenital disorder or presence of a significant medical condition that in the opinion of the Investigator precludes participation in the study. Known or suspected impairment of immunological function based on medical history and physical examination. Clinical evidence of active gastrointestinal illness and acute disease at the time of enrollment
3. Screening positive with hepatitis B antigen and/or hepatitis C antibodies
4. Participation in research involving another investigational product (defined as receipt of investigational product) during the 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 participant has been or will be exposed to an investigational or a non-investigational product
5. Clinically significant abnormalities in screening hematology or serum chemistry, as determined by the Study Physician
6. History of febrile illness within 48 hours prior to vaccination and fever at the time of immunization (fever is defined as a temperature ≥ 37.5°C (99.5°F) on axillary, oral, or tympanic measurement)
7. Prior receipt of any cholera (e.g., Dukarol, Shancol) or ETEC vaccine
8. Prior receipt of a blood transfusion or blood products, including immunoglobulins
9. Current use of iron or zinc supplements within the past 7 days; current use of antacids (H2 blockers, omeprazole, OTC agents) or immunosuppressive drug
10. Any condition which, in the opinion of the investigator, might jeopardize the safety of study participants or interfere with the evaluation of the study objectives
11. Receipt of antimicrobial drugs for any reason within 14 days before vaccination
12. History of diarrhea during the 7 days before vaccination (see Protocol definition of diarrhea))
13. Culture positive for ETEC, *Shigella*, *V. cholerae, Salmonella* or Rotavirus (the latter for all children <5 years of age) within 7 days of vaccination
14. Acute disease at the time of enrollment or 3 days prior to enrollment
15. Known or suspected impairment of immunological function based on medical history and physical examination
16. Participant's parents/guardians not able, available or willing to accept active weekly follow-up by the study staff
17. History of chronic administration (defined as more than 14 days) of immunosuppressant medications, including corticosteroids. Infants on inhaled or topical steroids may be permitted to participate in the study
18. Any medical condition in the parentsinfant that, in the judgment of the investigator, would interfere with or serves as a contraindication to protocol adherence or a participant's parents' ability to give informed consent
19. Medically significant malnutrition, defined as moderate malnutrition (wt-for-ht z-score between -3.0 and -2.0) and severe malnutrition (wt-for-ht z-score <-3.0 or edema)

Continued Eligibility Criteria

Fulfilment of all of the following continuing eligibility criteria is required for all participants to receive their second vaccination (Day 14). If a participant does not meet the following continuing eligibility criteria prior to receiving their second immunization, vaccination can be deferred by up to two days. However, if a participant falls outside this deferred window, he/she will not receive the second vaccination but encouraged to continue all safety follow-up activities per protocol.

Inclusion Criteria

1. Participant available for study evaluations and reachable by study staff throughout the entire follow-up period
2. Continued informed consent

3. Negative urine pregnancy test for females of childbearing potential. Pregnancy tests must be negative before each vaccination. Females of childbearing potential must agree to continue use of an efficacious hormonal or barrier method of birth control during the study. Abstinence is also acceptable (PART A ONLY)

Exclusion Criteria

1. History of diarrhea during the 7 days before vaccination (see Protocol definition of diarrhea)
2. History of febrile illness within 48 hours prior to vaccination and fever at the time of immunization (fever is defined as a temperature ≥ 37.5°C (99.5°F) on axillary, oral, or tympanic measurement)
3. Current use of iron or zinc supplements within the past 7 days; current use of antacids (H2 blockers, omeprazole, OTC agents) or immunosuppressive drug
4. Receipt of antimicrobial drugs for any reason
5. Non-conformance to the protocol
6. Culture positive for ETEC, *Shigella*, *V. cholerae, Salmonella* or Rotavirus (the latter for all children <5 years of age) within 7 days of vaccination only if diarrhea was observed during post-dose 1 follow-up period
7. Acute disease at the time of vaccination visit or any time during the previous 3 days

SCREENING, RANDOMIZATION and masking PROCEDURES

Recruitment

The study population will be recruited, screened and qualified by the site staff, under the direction of the PI. Healthy participants will be recruited based on clinical history and physical examination from the Mirpur field site in urban Dhaka. The icddr,b team will develop a plan in order to achieve enrollment of the specified number of participants, 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 from the Mirpur field site. Upon identification of potential participants, Trained Study Staff (TSS) will visit the households in the Mirpur surveillance area to have preliminary discussions with potential participants regarding eligibility and participation in the study. The consent forms will be utilized by the field staff throughout the recruitment process. Potential participants/parents of potential participants 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. This will be prior to obtaining screening specimens.

Screening, Randomization, and Masking Procedures

Initial and Continuing Informed Consent

Participants/parents 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 or thumb print (with witness signature) 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 potential participant. Group discussions may also take place to orient participants to the study; however individual discussions will commence prior to obtaining consent signatures. Informed consent documents will be signed once the participant 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 participants. 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 form will outline study procedures, participant expectations, potential risks and benefits, as well as outline the compensation plan for their participation. Participants/parents will be reimbursed for their lost time and travel expenses.

Screening – Day -7 to Day -4

After the study investigator has obtained informed consent from the adult participants or, in case of children from the parents, the following procedures will be completed during screening to determine study eligibility and may occur over 2-3 screening visits. Additional screening visits may be scheduled for any follow up as needed, but are not required. At the screening visit(s), the designated study staff will provide participants and parents of prospective participants a detailed description of the study objectives and study participation requirements, as well as potential health risks and benefits associated with study participation. Baseline data are obtained during screening, which may occur over the course of several contacts/visits, between 7 days prior to and on Day 0, the day of first vaccination. All inclusion/exclusion criteria must be assessed from data obtained within that period, unless otherwise specified in the eligibility criteria. After study information has been provided and the appropriate informed consent has been obtained, the following procedures are performed before enrollment:

a) Written informed consent will be obtained and any remaining questions that the participant or the parent may have will be solicited/discussed
b) A participant ID number will be assigned once the study specific consent form has been signed.
c) Demographic information will be obtained.
d) Medical history will be obtained
e) Vaccination history will be obtained
f) History of medication use in the past 30 days will be obtained
g) Height/length & weight measurements will be obtained
h) A full physical exam will be performed. It will include: vital signs, HEENT, skin, lymph nodes, respiratory (lung), cardiovascular (heart), abdominal, neurological and musculoskeletal system.
i) Blood pressure will be obtained (Part A only).
j) Blood samples for screening laboratory testing will be collected: complete blood count (CBC) (including WBC with differential count, hemoglobin, platelet count), alanine transaminase (ALT), bilirubin, albumin and creatinine; Hep BsAg and C Ab.
k) Fecal sample for culture (ETEC, *Shigella*, *V. cholerae, Salmonella, Rotavirus for children <5 years old*) will be collected.
l) Blood samples for immunological testing will be collected
m) Fecal sample for measurement of baseline antibody responses will be collected
n) Urine pregnancy test for females of child bearing potential in Part A only will be obtained

Approximately 10 mL of blood will be collected from participants in Part A and 3.5 mL will be collected from participants in Parts B, C and D for the abovementioned assays during screening.

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.

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 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.

Masking procedures

The study pharmacist will not reveal the randomization code to any other study staff member, participant, or parent/guardian. Investigational study product will be prepared by a qualified unblinded study pharmacist or member of the IP formulation team and witnessed by another unblinded member of the IP formulation team.

Study procedures/evaluations

First Vaccination - Day 0

Prior to Vaccination

a) Interval medical history inclusion/exclusion criteria are reviewed to determine continuous qualification for enrollment, including medications;
b) Vital signs are obtained prior to dosing;
c) A brief physical examination will be performed;
d) Pregnancy status for females of child bearing potential will be verified as negative (Part A only);
e) Participants are randomized;
f) Participants will have fasted for one hour prior to vaccination.
g) A stool specimen will be collected for antibody analyses (backup sample to be used in case no screening sample is available or does not fulfil the SOP quality specifications)

Study Vaccination

a) The first dose of the study vaccine will be administered orally.

If a participant vomits within 30 minutes, the participant will remain in the study for safety follow-up evaluations required on Day 7, Day 14, Day 42 and Day 182 and will not be re-dosed. If such an event occurs, an alternate participant may be dosed at the discretion of the IPST.

Note: If a qualified participant selected for enrollment is disqualified prior to enrollment/randomization and dose administration (e.g., participant or parent withdraws consent or the PI reconsiders and disqualifies for a documented reason), another qualified participant will be selected for enrollment in place of the disqualified participant.

Post Dose – Observation Period

a) Participants will remain at the site for at least 60 minutes post-vaccination for direct observation, and potential adverse events will be recorded in the CRF.
b) The PI (or designee) may determine that a participant requires further on-site observation; additional site or clinical assessments may be completed as needed.
c) The participant or parent/guardian will be reminded that eating or drinking, including breastfeeding is prohibited for one hour post-vaccination.
d) Each participant will be provided with a symptom Memory Aid to record solicited symptoms on a daily basis through Day 7. Participants will be informed that a TSS will visit their home daily for 7 days to monitor their or their child's health, including taking temperature and recording any symptoms on a reactogenicity source document. The TSS will be responsible for that source document and bring it to and from the participant's home. TSS will review the information on the Memory Aid with the participant and interview the participant to elicit as much information as possible about any reported symptom(s). Based on information obtained from the participant, TSS should use their clinical judgment to assess the event and its severity and record the data on the reactogenicity source document. Participants will be instructed to bring the Memory Aid to the next scheduled clinic visit. Symptoms/signs solicited by the TSS and recorded on the source document will include the following: nausea (Part A only), abdominal pain/stomach ache (Parts A and B only), vomiting, diarrhea and fever.

Day 7 (to be defined by 7 ± 1 days after Day 0) – Clinic follow-up visit

On Day 7 (± 1 calendar day if necessary for participant compliance), the participant will return to the study clinic and the following procedures occur:

a) Any topics or new information considered by PI to be important to continued informed consent will be shared with the participant/parent/guardian.
b) Interim medical review, concomitant medications review, and AE review will occur.
c) A blood sample will be collected for hematology and clinical chemistry, as well as for immunology if sufficient blood is obtained (see Appendix I). Additional blood tests may be performed as necessary to follow-up and evaluate any abnormalities identified in monitoring labs.

d) Vital signs will be measured
e) A fecal sample will be collected for measurement of antibody responses.
f) Study staff will instruct the participant or parent/guardian regarding continued assessment of his/her or the child's health and need to contact study staff (a) for follow-up of specific AEs, (b) if systemic symptoms worsen or do not resolve, and (c) if other AEs occur.
g) The next visit for Day 14 ± 2 days is scheduled.

Approximately 10 mL of blood will be collected from participants in Part A and 3.5 mL will be collected from participants in Parts B, C and D for the abovementioned assays at this visit.

Day 14 ± 2 days (to be defined by 14 days after Day 0) – Second Vaccination

On Day 14 (plus or minus 2 calendar days if necessary for participant compliance), the participant will return to study clinic and the following procedures occur:

Prior to Vaccination

a) Interval medical history, including medications and AE review, is obtained and inclusion/exclusion criteria reviewed to determine continuous qualification for vaccination.
b) Any topics or new information considered by PI to be important to continued informed consent will be shared with the parent/guardian.
c) Vital signs are measured prior to dosing.
d) A targeted physical exam is performed, if indicated.
e) For females with child bearing potential in Part A only, a urine dipstick pregnancy test will be performed and verified as negative prior to vaccination.
f) Participants will have fasted for one hour prior to vaccination.

Study Vaccination

a) The second dose of the study vaccine will be administered orally.

If a participant vomits within 30 minutes, the participant will remain in the study for safety follow-up evaluations required on Day 42 and Day 182 and will not be re-dosed.

Post Dose – Observation Period

a) Participants will remain at the site for at least 60 minutes for children post-vaccination for direct observation, and potential adverse events will be recorded in the CRF.
b) The PI (or designee) may determine that a participant requires further on-site observation; additional site or clinical assessments may be completed as needed.
c) The participant will be reminded not to eat or drink anything for one hour post-vaccination.
d) Each participant will be provided with a symptom Memory Aid to record solicited symptoms on a <u>daily</u> basis through Day 21. Participants will be informed that a TSS will visit their home daily for 7 days to monitor their or their child's health, including taking temperature and recording any symptoms on a reactogenicity source document. The TSS will be responsible for that source document and bring it to and from the participant's home. TSS will review the information on the Memory Aid with the participant and interview the participant to elicit as much information as possible about any reported symptom(s). Based on information obtained from the participant, TSS should use their clinical judgment to assess the event and its severity and record the data on the reactogenicity source document. Participants will be instructed to bring the Memory Aid to the next scheduled clinic visit. Symptoms/signs solicited by the TSS and recorded on the source document will include the following: nausea (Part A only), vomiting, abdominal pain/stomach ache (Parts A and B only), diarrhea and fever.

Day 19-20 (to be defined as 5-6 days after the second dose) – Clinic follow-up

On Day 5 after the second vaccination (Day19 or Day 20 if necessary for participant compliance), the participant will return to study clinic and the following procedures occur:

a) Study staff will review and record the participant's interval health history, medication use and the assessment of the post-vaccination experience through personal interview
b) Interim medical review, concomitant medications review, and AE review will occur.
c) Vital signs will be measured.
d) A targeted physical exam will be performed, if indicated.
e) Blood samples will be obtained for immunological assays (see Appendix I).
f) A fecal sample will be collected for measurement of antibody responses.
g) Study staff will instruct the participant or parent/guardian regarding continued assessment of his/her or the child's health through Day 42
h) The next visit is scheduled.

Approximately 10 mL of blood will be collected from participants in Part A and 3.5 mL will be collected from participants in Parts B, C and D for the abovementioned assays at this visit.

Note: This Visit should occur 5-6 days after the second vaccination. If the second vaccination is administered outside Day 14 ± 2 days, this visit should still be scheduled 5-6 days subsequent to the second vaccination and the participant's follow-up schedule shifted accordingly to accommodate the remaining visits.

Day 28 ± 2 days (to be defined by 28 days post Day 0) – Home visit

On Day 28 (plus or minus 2 calendar days if necessary for participant compliance), TSS will visit the participant's home to collect a fecal sample for measurement of antibody responses, follow-up on specific AEs, solicit any new AEs, and to schedule the next visit.

Day 42 ± 4 (to be defined by 28 days post last vaccination) – Clinic follow-up

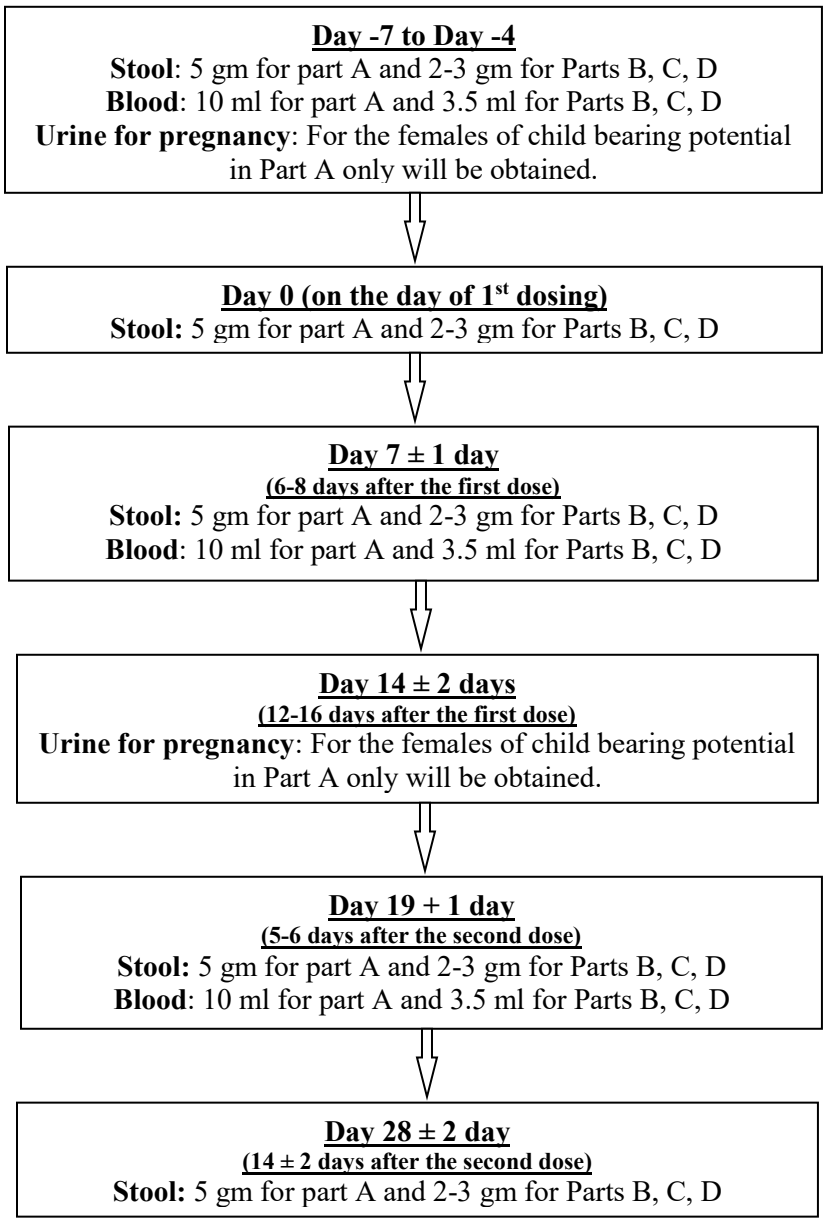
On Day 42 (plus or minus 4 calendar days if necessary for participant compliance), the participant will return to study clinic and the following procedures occur:

a) Any topics or new information considered by PI to be important to continued informed consent will be shared with the participant or parent/guardian.
b) Findings of the PI or study staff that suggest inaccuracy of reported self-assessments will be clearly documented.
c) Vital signs will be measured.
d) Interim medical review, concomitant medications review, and AE review will occur.
e) A targeted physical exam will be performed, if indicated.
f) The participant or participant's parent/guardian will be reminded to contact the study clinic with any new information about chronic illnesses, serious health events, and/or hospitalizations, and the Day 182 follow-up contact information will be provided.

Day 182 ± 14 days (to be defined by 168 days post last vaccination) – Final home visit

On Day 182 (plus or minus 14 calendar days if necessary for participant compliance), TSS will visit the participant's home to collect new information via questionnaire about new chronic diseases, serious health events, and/or hospitalizations since the last visit. If three attempts to reach the volunteer are unsuccessful, a registered letter will be mailed to the volunteers asking them to contact the study staff. These data 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.

A flow chart of sample collection is summarized below and can also be found in Appendix I: Schedule of Study Events



Interim or Unscheduled Visits

Interim contacts and visits (those between regularly scheduled follow up visits) may be performed at participants' or parents'/guardians' request, or as deemed necessary by the PI or designee, at any time during the study. All interim contacts and visits will be documented in participants' study records and on applicable case report forms.

In addition, study staff may request that participants make unscheduled visits to the study clinic when the PI or staff member considers it necessary for diagnosis and/or management of a finding or AE. These visits may include examinations and/or diagnostic tests, as indicated.

Early Termination

If a participant is withdrawn from the study for any reason prior to the planned final visit, every attempt is made to perform the following.

a) Review of AEs by PI (or designee);
b) Review of Memory Aid information to assess reactogenicity, if in use since the last visit;
c) Physical examination, including vital signs; and
d) Safety laboratory testing if withdrawal occurs prior to scheduled safety laboratory testing on Day 7.

Evaluation/Procedure Methods

Study procedures are performed and recorded in source documents as outlined in the Schedule of Events (Appendix I) and according to the following subsections.

Vital Signs

- Temperature in degrees Celsius (recorded to the nearest 0.1 degree) will be measured by axillary thermometer.
- Respiratory rate in breaths per minute will be measured by observation for a minute.
- Heart rate in beats per minute will be measured by automated device or manually.

Height/Length and Weight

- Height/Length is measured in cm and recorded to the nearest 0.1 cm.
- Weight is measured in kg and recorded to the nearest 0.1 kg.

Physical Examination

Full physical examination will include assessment of vital signs, head, eyes, ears, nose, oropharynx, neck, chest (auscultation), lymph nodes (neck, supraclavicular, axillary, inguinal), abdomen (auscultation and palpation), musculoskeletal, skin, and neurological.

Medical History

A comprehensive medical history will be collected including details of any previous vaccinations and reaction to vaccinations, birth (for Parts B, C and D), participation in clinical trials, surgery, previous hospitalization, allergy to food/drugs, current medication and history of any chronic or recurrent medical conditions.

An interval medical history will consist of inquiring regarding changes (healthcare events, signs, symptoms and changes in use of prescription or nonprescription drugs or herbal preparations) since the last medical history discussion.

Clinical Definitions

Diarrhea

- Diarrhea is defined as ≥ 3 unformed or loose stools (mixed liquid and solid components) in a 24 hr period or at least one bloody loose or liquid stool or 1-2 liquid stool with at least some dehydration
- If a volunteer meets the definition of diarrhea, the start of the diarrhea episode will be the time of the first unformed stool which contributes to meeting the definition and the end of the diarrhea episode will be the time of the last unformed stool. If there is greater than 24 hours between loose or unformed stools then the illness will stop and a new AE will begin.

Mild Diarrhea

- At least 3 looser-than-normal stools without dehydration

Moderate Diarrhea

- Diarrhea with some dehydration (per IMNCI definition)

Severe Diarrhea
Diarrhea with severe dehydration (per IMNCI definition)

Dehydration: Classification of the severity of dehydration will follow WHO guidance as well as criteria used at the icddr,b hospital (WHO 1990).

Grading the severity of adverse events in adults and children: toxicity grading scale for abnormal values will be based on Appendix II and Appendix III, respectively.

Clinical Laboratory Testing

Laboratory evaluations for screening (hematology, clinical chemistry, and hepatitis B and C testing) and safety monitoring (hematology and chemistry) are outlined in Appendix I, Schedule of Events.

Withdrawal from Further Vaccination and Early Termination from Study

An enrolled/vaccinated participant may be terminated from the study for any of these reasons.

a) Participant or parent/guardian withdraws consent for any reason.
b) PI, EMMES medical monitor, IPST, icddr,b DSMB or PATH medical monitor decides that termination is necessary to protect the participant, the integrity of the study, or achieve the objectives of the study.
c) Interruption of study schedule makes the participant's data unusable according to protocol requirements.

Participants who are withdrawn from further vaccination will be encouraged to complete the safety-related participant follow-up. If the participant or parent agrees, other study procedures (e.g., blood sampling for measuring levels of antibodies) may be continued. Withdrawal from further vaccination may occur based on the local investigator decision at the time of an adverse event or based on the IPST review of these events on a cumulative basis if:

(a) Participant experiences reactogenicity of Grade 3 or higher.

(b) Participant experiences an event that contributes to study pause rules

In addition, participants may be withdrawn from further vaccination for any other safety-related reasons at the discretion of the PI, safety monitors, IPST, PATH medical officer, DSMB and the participant or the parent/guardian. The EMMES medical monitor and PATH medical officer will be informed of all issues related to withdrawal or termination of participant.

Participants or parents/guardians may withdraw themselves or their children, respectively, from study product administration due to what they perceive as an intolerable AE, or for any other reason. If withdrawal is requested, the participants or parents/guardians should be asked to have them or their child, respectively, continue (at least limited) scheduled evaluations, complete a study termination form, and be given appropriate care under medical supervision until the symptoms of any AE resolve or the condition becomes stable.

LABORATORY EVALUATIONS

Blood samples will be obtained to examine vaccine safety and immunogenicity.

Sample collection, distribution and storage

Samples to evaluate vaccine immunogenicity will be obtained and processed at the field study site and transported to icddr,b's Immunology Laboratory. Research specimens collected for the immunogenicity time points will be separated into aliquots by the icddr,b Immunology Laboratory per study specific procedures and processed or stored for immunological analysis and, where necessary, for potential shipment to UG and/or SBioPharma. Samples will be stored properly in controlled-temperature refrigerators/freezers. Backup generators are available for proper sample storage.

Stool samples will be obtained and processed at the field site and transported daily to the icddr,b Immunology Laboratory. Samples will be processed and tested according to icddr,b laboratory standard operating procedures (SOPs) for fecal specimens. Thereafter, residual specimens will be stored at -20° C.

Safety clinical laboratory assays

During screening and at Day 7, blood samples will be collected for safety clinical laboratory evaluations. Samples will be packaged and transported to the clinical laboratory according to SOPs and tested. Standard clinical laboratory tests for the purpose of inclusion and exclusion of potential participants and for safety monitoring will be carried out by the icddr,b Clinical Laboratory Services.

Immunological assays

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 Immunology Laboratory. Immunologic assays will be performed as per written SOPs and will be a part of the study documentation. Stools will be processed for total antigen specific fecal IgA in the Immunology Laboratory (stool cultures will be done when indicated in the Microbiology Laboratory). The assays will occur as outlined in the Study Event Schedule. Samples will be stored so that all specimen time points can be tested together for each participant.

The following prioritization scheme will be applied to the vaccine antigens according to analysis as specimen volumes allow:

- ALS IgA: LTB > CFA/I > CS3 > CS6 > CS5> O78LPS
- Fecal SIgA/total SIgA: LTB > CFA/I > CS3 > CS6 > CS5> O78LPS
- PlasmaIgA; LTB > 078 LPS > CFA/I > CS3 > CS6 > CS5
- PlasmaIgG: LTB > 078 LPS
- T cell and other immune responses: LTB > CFA/I > CS3 > CS6 > CS5

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

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

Sample Size Calculation:
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:

- Solicited gastrointestinal reactions
- Solicited systemic reactions
- Unsolicited Adverse events
- Adverse events where there is a reasonable possibility that the study product caused the event, i.e., are suspected adverse reactions
- Serious adverse events

The sample size for this study was selected to detect frequent adverse events. Given a planned sample size of groups of 15 adults, toddlers, and younger children each receiving one of varying dose levels of ETVAX with and without dmLT, the study will have an approximately 80% and 90% chance of observing at least one serious adverse event or adverse event of special interest for events that occur at a rate of 10.3% and 14.3%, respectively. Additionally, if no serious adverse events are observed in 15 participants, the upper bound of the one-sided 95% confidence interval on the rate of serious adverse event occurrence is approximately 18%.

For infant cohorts with 30 participants per dose group, the study will have an approximately 80% and 90% chance of observing at least one serious adverse event or adverse event of special interest for events that occur at a rate of 5.3% and 7.4%, respectively. If no serious adverse events are observed in 30 participants, the upper bound of the one-sided 95% confidence interval on the rate of serious adverse event occurrence is approximately 9.5%.

For infant cohorts, with 30 participants per group, this study is designed to provide approximately 73% power to detect as low as a 2-fold difference in geometric mean fold rise (post-vaccination / baseline) between comparisons of two groups

with varying doses of ETVAX and dmLT levels. This power calculation was based on the variability estimate, log10 standard deviation of 0.6, for CS6 obtained from a previous study of the vaccine in Sweden (VAC 003/OEV-121) and two-sample t-test using the 5% two-sided Type I error rate and 10% drop-out rate.

Outcome variables:

Primary Safety Endpoints

- Number of SAEs
- Number of AEs
- Number of vaccine induced reactogenicity events

Secondary Immunology Endpoints

- Antibody response (≥two-fold increase in antibody titers between baseline and post-immunization), Geometric Mean Titer (GMT), and Geometric Mean Fold Rise (GMFR) between baseline and post-immunization to CFA/I, CS3, CS5, CS6 and LTB as measured by ALS IgA
- Antibody response (≥two-fold increase in antibody titers between baseline and post-immunization), GMT, and GMFR to CFA/I, CS3, CS5, CS6 and LTB as measured by fecal SIgA.
- Antibody response (≥two-fold increase in antibody titers between baseline and post-immunization), GMT, and GMFR to CFA/I, CS3, CS5, CS6 and LTB as measured by plasma IgA.
- Antibody response (≥two-fold increase in antibody titers between baseline and post-immunization), GMT, and GMFR to CFA/I, CS3, CS5, CS6 and LTB as measured by plasma IgG.
- Antibody response (≥two-fold increase in antibody titers between baseline and post-immunization), GMT, and GMFR to O78 as measured by ALS IgA, fecal SIgA, and/or plasma IgA.

Exploratory Immunology Endpoints

- T cell immune responses
- Neutralizing antibody geometric mean titers (GMT) and
- Avidity of immune responses
- Immunoproteomic profile of vaccine induced antibody responses in plasma or intestinally derived samples

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.

Overview and general design

This study is a randomized, double-blind, dose-escalation, descending age clinical trial to assess the safety and immunogenicity of the inactivated whole cell ETVAX vaccine administered alone and with dmLT adjuvant as compared to a placebo (bicarbonate buffer). The primary objectives are to evaluate the safety and tolerability of the inactivated whole cell ETVAX vaccine with and without dmLT adjuvant in adults, toddlers, young children and infants.

The secondary objectives are:

1. To assess vaccine induced IgA antibody responses in lymphocyte secretions (ALS) against LTBD, CFA/I, CS3, CS6 and CS5 in descending age-groups in Bangladesh
2. To evaluate vaccine induced fecal secretory IgA (SIgA) antibody responses against CFA/I, CS3, CS5, CS6, O78 LPS and LTB in applicable age-groups in Bangladesh
3. To assess vaccine induced plasma IgA antibody responses against LTB, O78 LPS, CFA/I, CS3, CS6 and CS5 and IgG antibody responses against LTB and O78 LPS
4. To evaluate vaccine induced ALS IgA responses against O78 LPS (if sample volumes allow)
5. To assess adjuvant effect of dmLT on vaccine immune responses compared to responses when giving vaccine alone in descending age-groups in Bangladesh.

The secondary hypothesis of immunogenicity will be fulfilled if at least 50% or more of fully immunized young children and infants show significantly higher mucosal immune responses to at least two out of the five vaccine antigens tested (LTB, CFA/I, CS3, CS5, and CS6) by ALS IgA and or fecal SIgA/total SIgA assays compared to placebos.

A detailed statistical analysis plan for preparation of the final study report will be created and made final prior to database lock and unblinding for each age group.

Medical history and AEs will be coded using MedDRA® dictionary Version 16.1 or higher. The frequencies and percentages of participants with AEs will be summarized according to the coded terms of system, organ, class (SOC) and preferred term. Participant-wise data listings will be provided.

Randomization and blinding procedures

The randomization scheme will be generated and maintained by the Statistical and Data Coordinating Center (SDCC) at the Emmes Corporation, Rockville, MD. Participants will be randomized into the study cohorts manually using the assigned treatment code provided by Emmes. The assigned treatment information will be entered into the Emmes Corporation's AdvantageEDC^{SM} electronic data capture system for each participant, after product administration has occured.

The pharmacist (or designee) with primary responsibility for dispensing study products is responsible for maintaining security of the treatment assignments.

Analysis Population

Definitions of analysis populations to be analyzed are:

Enrolled Population
All screened participants who provide informed consent, regardless of the participant's randomization and treatment status in the trial.

Full Analysis (FA) population
All participants in the enrolled population who are randomized, receive a study vaccination, and have pre- and/or post-vaccination immunogenicity measurement(s). This population will serve as the supportive population for the immunogenicity objectives.

Per Protocol Populations
The per protocol populations will be specified per sample and antigen type. All participants in the FA population who receive both doses of the same study vaccine and have post second dose (all sample types) and pre-vaccination (stool and plasma) immunogenicity measurement(s), with no major protocol or laboratory test SOP violations that are determined to potentially interfere with the immunogenicity assessment of the study vaccine, will be included in the per protocol populations. These populations will serve as the primary analysis populations for the immunogenicity objectives.

The specific criteria for exclusion of subjects from the Per Protocol Populations will be established before breaking the blind and will be based on the blinded review of protocol and laboratory test SOP violations

The Safety Population
All participants in the enrolled population who receive a study vaccination and have safety data available. Treatment groups for safety analysis will be assigned according to the actual treatment received at Day 0. If a participant receives mixed doses (e.g. an ETVAX dose without dmLT at Dose 1 and the same ETVAX at Doses 2 with dmLT), the safety data collected after the start of the mixed dosing (e.g. post Dose 2) will be excluded from the analysis. All excluded data will be presented in the data listings. All safety analyses will be performed using this population.

Any additional exclusion from the Full Analysis population if warranted (e.g. a significant protocol deviation that is determined to potentially interfere with the vaccine induced immune responses) based on the blinded review of the data will be established and documented before breaking the blind.

Safety Analysis

The incidence of any adverse events (AEs) will be determined. Gastrointestinal and systemic AEs will be assessed post-vaccination using participant/parent/guardian interview (including memory aids), targeted physical examinations, vital signs and clinical laboratory tests and reactogenicity assessments to be completed following each vaccination. The solicited AEs of nausea (Part A only), abdominal pain/stomach ache (Parts A and B only), fever, vomiting and diarrhea will be evaluated daily by the TSS for 7 days post vaccination. Unsolicited AEs will be assessed through Day 42 and

serious adverse events (SAEs) will be assessed over the entire duration of the study. Proportions of subjects with events, overall, by grade and relationship with vaccination will be analyzed for each of the vaccine arms in comparison to the placebo arm. For the analysis of the solicited adverse events, the statistical comparisons using Fisher´s exact test will be performed using two-sided 5% Type I error rate without an adjustment for multiple comparisons. The primary purpose of statistical comparisons are to screen potential AEs that need further clinical evaluation. Therefore they are not considered formal statistical hypothesis testing and it is acknowledged that there will be an inflated Type I error rate (i.e. inflated false statistical significances) due to performing multiple testing without an adjustment.

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:

- Solicited gastrointestinal reactions
- Solicited systemic reactions
- Unsolicited adverse events
- Adverse events where there is a reasonable possibility that the study product caused the event, i.e., are suspected adverse reactions
- Serious adverse events

Immunogenicity Analysis

For all immunological analyses, the response rate and magnitude of antigen-specific responses will be analyzed after each immunization as specimen availability allows; as well as the cumulative response rate after two immunizations (i.e. response after either immunization). Response rates to the five primary antigens in the ETVAX vaccine (LTB, CFA/I, CS3, CS5, and CS6) will be further evaluated to address co-secondary immunogenicity hypothesis by analyzing the response rates to at least two of the five different antigens by ALS IgA and fecal SIgA and to at least one of the five different antigens by plasma IgA. In addition, other analyses summarizing response rates to multiple antigens may also be performed. These multiple antigen response rate analyses will be based on the results from antigen-specific responses, including different age cohorts. For example, only antigens with statistically significant and/or clinically meaningful vaccine induced responses may be included in the analysis of multiple common antigen analyses.

For descriptive analysis of the magnitude of responses, levels of antibodies against CFA/I, CS3, CS5, CS6 and LTB in ALS and fecal extracts as well as plasma will be determined for each individual during screening and in all post vaccination samples. The geometric mean (GM) of antibody levels and the GM of fold-rises (post vaccination / pre-vaccination) will be determined for each study group and comparisons made between pre- and post-vaccination levels. The response rate for ALS IgA, fecal SIgA, serum IgA and serum IgG will be determined as the proportion of participants having a ≥2-fold increase in antigen-specific antibody levels between pre- and post-vaccination samples. Additionally, other fold increases (e.g. 3-fold and 4-fold) will be explored. If insufficient amounts of pre-immune ALS specimens are obtained from young children and infants, to allow direct comparison between pre and post immunization specimens, antibody levels in post vaccination specimens will be compared with the geometric mean ALS antibody level against corresponding antigen in the same age group.

For each endpoint, two-sided 95% confidence Interval (CI) will be constructed using the Clopper-Pearson method for the response rate or log-normal distribution for GMT/GMFR. Primary statistical comparison of immune response frequencies and magnitudes in vaccine and placebo recipients will be carried out using standard parametric and non-parametric methods whichever is applicable (i.e. Fisher's exact test, Chi-square test, t-test, and Mann-Whitney U test for two group comparisons; and Chi square test, generalized linear model, and Friedman's test). For GMT and GMFR antigen specific endpoints, the log values will be used for the analyses. The log mean and the corresponding CI limits will then be exponentiated to obtain the GMT/GMFR and the corresponding CI. Statistical comparisons will be made between ETVAX dose levels and the dmLT levels, as well as comparison to the placebo group.

Due to the exploratory nature of all statistical comparisons for immunogenicity endpoints, all testing and estimations will be carried out using a 2-sided 5% Type I error rate without an adjustment for multiple comparisons. However within each

immunogenicity endpoint in the infants, Holm´s correction for multiple testing will be applied to control the multiple comparisons associated with multiple treatment groups.

Interim Analysis

Early final analysis of each Part when all cohorts within the Part have completed the primary safety and immunogenicity follow-up (Day 42, 28 days post last vaccination) may be performed to facilitate the decisions external to the study conduct (e.g. initiation of activities to prepare for another study). The unblinded analysis will be performed by an independent statistician who is not involved in the conduct of the study after all data have been cleaned and locked through Day 42 follow-up for all subjects in each Part. Analysis will be presented in group unblinded fashion and no individual listing will be generated. These analyses would not otherwise alter the course of the trial and blinded status of the study for investigators and study subjects until the completion of the study.

SAFETY ASSESSMENT AND REPORTING

This section defines the types of safety events that should be reported and outlines the procedures for appropriately collecting, grading, recording and reporting them.

Safety Events

All safety events observed under this protocol will be reported through the Advantage EDC data system throughout the study. Safety events related to vaccine reactogenicity are collected on study-specific forms. Reactogenicity data (solicited signs or symptoms) will be collected through visit Day 7 post-study vaccination for each administration; if a solicited sign or symptom has started during the 7 days post study vaccination and continues beyond Day 7 it will continue to be reported as a reactogenicity symptom. Any symptom starting after 7 days post any study vaccination will be recorded as an AE. Only when a solicited sign or symptom is considered an SAE, as defined below, will it be reported on an AE/SAE form set, in addition to the reactogenicity form. All other safety events that meet the definition of an AE or SAE that occur throughout the study are reported on the AE/ SAE form set. AE's will be collected through study visit Day 42. SAEs and new medical diagnoses, post-study chronic health conditions, serious health events, or hospitalizations will be solicited from Day 42 through Day 182 and will be reported on the AE/ SAE form set.

Reporting Period

Safety events are reported from the time of the first vaccination through completion of the study at 6 months after the final vaccination (as above). For participants who withdraw from the study, an attempt should be made to have the participant seen for an early termination visit 5-7 days after the last vaccination or study visit to elicit occurrence of AEs (serious and non-serious).

Definitions

Adverse Event (AE) or Medical Event

An adverse event is any untoward medical occurrence in humans, whether or not considered vaccine related, that occurs during the conduct of a clinical trial.

Suspected adverse vaccine reaction is any AE for which there is a reasonable possibility that the vaccine caused the AE. A reasonable possibility implies that there is evidence that the study product caused the event.

Adverse reaction is any AE caused by the vaccine.

Serious Adverse Events (Including Serious Adverse Events, Serious Suspected Adverse Reactions or Serious Adverse Reactions)

An SAE, including a serious suspected adverse reaction or serious adverse reaction as determined by the PI or the Sponsor, is any event that results in any of the following outcomes:

1. Death

2. Life-threatening AE (Life-threatening means that the study participant was, in the opinion of the PI or Sponsor, at immediate risk of death from the event as it occurred and required immediate intervention. It does not include an adverse event that, had it occurred in a more severe form, might have caused death.)
3. Inpatient hospitalization greater than 24 hours or prolongation of existing hospitalization
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. Congenital abnormality or birth defect
6. Important medical event that may not result in one of the above outcomes but may jeopardize the health of the study participant or require medical or surgical intervention to prevent one of the outcomes listed in the above definition of serious event

Unexpected Adverse Event

An AE is "unexpected" when its nature (specificity) or severity is not consistent with applicable product information, such as safety information provided in the Investigators' Brochure (IB), the investigational plan or the protocol.

Toxicity Grading

The PI/PI designee assigns toxicity grades to indicate the severity of adverse experiences and toxicities. The toxicity grading criteria provided in Appendix X grade AEs from Mild (grade 1) to Life Threatening (grade 4). All AEs leading to death are Grade 5 events. AEs are graded with the worst severity grade during the illness/symptoms. AE severity will be graded using the attached grading scale (Appendix X), which has been developed on the basis of the *Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events*, 2009, of the US National Institutes of Health, with modifications to reflect site norms. For laboratory values not included in Appendix II, grading will be based on the *Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events*, 2009, available at webpage: http://rsc.tech-res.com/Document/safetyandpharmacovigilance/Table_for_Grading_Severity_of_Adult_Pediatric_Adverse_Events.pdf

Guidelines for Determining Causality of an Adverse Event

Every effort should be made by the investigator to explain each AE and assess its causal relationship to administration of investigational product. The degree of certainty with which an AE can be considered possibly, probably or definitely related to the vaccine (or alternative causes, e.g., natural history of the underlying diseases, concomitant therapy, etc.) will be determined by how well the event can be understood in terms of one or more of the following:

- Reaction of similar nature previously observed with this vaccine strain.
- Reported in literature for similar types of vaccine strains.
- Temporal association with vaccine administration.

Causality of all AEs should be assessed by the investigator using the following method:

Unrelated: There is no suspicion that there is a relationship between test article and AE, there are other more likely causes and administration of the test article is not suspected to have contributed to the AE.

Possible: AE occurs within a reasonable time after the administration of the test article but can also be reasonably explained by other factors (as mentioned above).

Probable: AE occurs within a reasonable time after the administration of the test article and cannot be reasonably explained by other factors (i.e., clinical condition, environmental or toxic factors, or other treatments).

Definite: The AE can be explained only by receipt of the test articles.

Adverse Event Identification, Resolution and Reporting

To improve the quality and precision of acquired AE data, the PI should observe the following guidelines:

- Whenever possible, use recognized medical terms when recording AEs on the AE CRF. Do not use colloquialisms and/or abbreviations.

- If known, record the diagnosis (i.e., disease or syndrome) rather than component signs, symptoms and laboratory values on the AE CRF (e.g., record congestive heart failure rather than dyspnea, rales, and cyanosis). However, signs and symptoms that are considered unrelated to an encountered syndrome or disease should be recorded as individual AEs on the CRF (e.g., if congestive heart failure and severe headache are observed at the same time, each event should be recorded as an individual AE).
- AEs occurring secondary to other events (e.g., sequelae) should be identified by the primary cause. A "primary" AE, if clearly identifiable, generally represents the most accurate clinical term to record on the AE CRF. If a primary serious AE (SAE) is recorded on an SAE CRF, events occurring secondary to the primary event should be described in the narrative description of the case.
- Grade 2 or higher abnormal laboratory test results will be entered as AEs with the toxicity grade associated with the abnormal laboratory test and attribution of the abnormality relative to the study product. Grade 1 abnormal laboratory test results will be entered as AEs if the PI determines them to be clinically significant.
- Death is an outcome of an event. The event that resulted in the death should be recorded and reported on the SAE CRF.
- For hospitalizations for surgical or diagnostic procedures, the illness leading to the surgical or diagnostic procedure should be recorded as the SAE, not the procedure itself. The procedure should be captured in the case narrative as part of the action taken in response to the illness.

AEs may be discovered through any of these methods.

- Observing the participant.
- Questioning the participant or participant's parent/guardian, which should be done in an objective manner.
- Receiving an unsolicited complaint from the participant or the participant's parent/guardian.
- Review of medical records/source documents.

Each participant will have a scheduled observation following each vaccination, including a symptom-directed physical examination, if indicated. Participants will be monitored in the clinic for at least 60 minutes after each vaccination. On Days 0 to 7 after each vaccination, TSS will visit participants' home for 7 days to monitor their health, including taking temperature and recording any symptoms on a reactogenicity source document. Symptoms/signs solicited by the FRA and recorded on the source document will include the following: nausea (Part A only), abdominal pain/stomach ache (Parts A and B only), vomiting, diarrhea and fever.

Follow-up visits will be conducted on post-vaccination Days 7, 19, 28, and 42 ± 4, including verification of reactogenicity records and directed assessment. On Day 7, blood will be drawn for monitoring hematology and chemistry tests. Participants and parents/guardians will be instructed to call the study team if they observe significant systemic signs or symptoms, and then to continue monitoring and reporting his/her or their children's condition. All participants will be followed for safety for six months after the last vaccination, with a scheduled home visit.

AE Resolution

All reported AEs should be followed until resolution or stabilization, or until completion of the study. Participants who have an ongoing study product-related SAE at study completion or at discontinuation from the study will be followed by the PI or designee until the event is resolved or determined to be irreversible, chronic, or stable by the PI.

General Recording and Reporting Procedures

A multi-page AE/SAE form set will be used, allowing all AEs to be submitted through a single reporting mechanism. SAEs will require additional information reported on additional pages within the Internet data entry system. As appropriate or per request of the EMMES medical monitor or PATH medical officer, source documents (e.g., hospitalization discharge summaries) may be uploaded to the AE/SAE form set, as well. The PI will treat or refer, as appropriate, participants experiencing AEs and observe them at suitable intervals until their symptoms resolve or their status stabilizes.

SAE Recording and Reporting Procedures

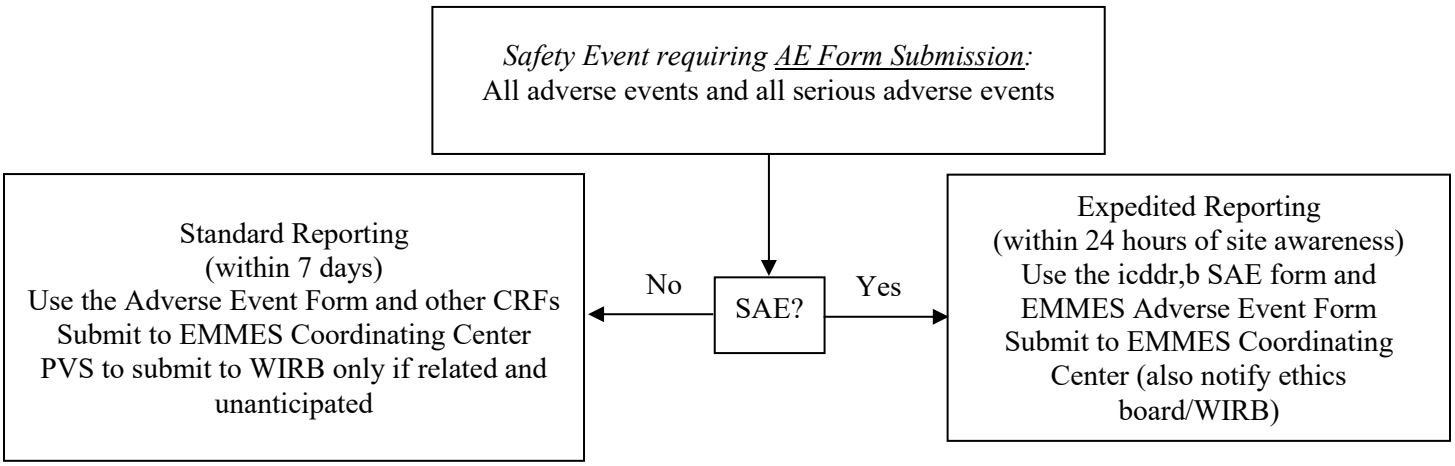
SAEs will be recorded on the standard icddr,b Serious Adverse Event Form and the EMMES generated AE case report form (CRF). The site is obligated to report SAEs to the EMMES Coordinating Center within 24 hours of the site's knowledge of the event. The following attributes will be assigned by the PI or assignment by designee will undergo documented review by the PI:

- Description
- Date of onset and resolution (if known when reported)
- Severity
- Assessment of relatedness to test article
- Action taken

The PI will apply clinical judgment to determine whether an AE is of sufficient severity to require discontinuation of administration of study vaccination for that participant. If necessary, the PI will suspend any trial procedures and institute the necessary medical therapy to protect a participant from any immediate danger.

Site Reporting of Events

Figure 9: Reporting Decisions for Adverse Events



- Notify the PI.
- Complete the standard icddr,b SAE form as well as the EMMES generated AE CRF, and transmit an AE Form through the Internet data entry system. Information regarding a SAE report must be recorded in the participant's medical chart and entered in the Internet data entry system.
- SAE follow-up reports should include hospital admittance notes, hospital discharge summary, clinical notes, resolution date, treatment, and any other pertinent information regarding the event. If not immediately available, reporting should not be delayed to provide these documents.
- In the event of a death, the SAE Form must be completed and transmitted along with other supporting data (e.g., death certificate, medical notes).
- PVS will submit the AE/SAE to WIRB only if it is considered unanticipated and related to study product according to WIRB defined procedures.

Serious Adverse Event Notification

Notifications and Review

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

In addition, the PI will provide the EMMES Coordinating Center with data for all SAEs, as defined per the protocol, on an ongoing basis by entering the information in AdvantageEDC^{SM}. The EMMES Coordinating Center is responsible for notifying the Sponsor and the CRO performing site monitoring and will do so simultaneously with the reporting to the clinical database. Notification of reported events will be generated at the time of entry into the data system, and all SAEs will be reviewed promptly by the EMMES medical monitor within the data system. As noted above, this notification should be within 24 hours of site awareness of the event. Site personnel will be trained in reporting AEs and SAEs.

The EMMES medical monitor will review all unanticipated events involving risk to participants or others, SAEs and all participant deaths associated with the protocol and will provide a written report. At a minimum, the EMMES medical monitor must comment on the outcomes of the event or problem and, in case of an SAE or death, comment on the relationship to study product. The EMMES medical monitor must also indicate whether he/she concurs with the details of the report provided by the PI.

Summary review of all reported AEs and SAEs will be compiled on a weekly basis to identify any potential safety trends. Review of reactogenicity and safety monitoring laboratory tests will also be conducted on a weekly basis during the periods such monitoring is being performed.

Expedited Reporting

The EMMES Coordinating Center will be responsible for expedited Safety Reports and IND Annual reports to the US Food and Drug Administration (FDA). The PI will be responsible for reporting to the icddr,b DSMB and Ethical Review Committee (ERC). PVS will be responsible for reporting to WIRB according to procedures outlined below.

FDA Reporting

When expedited reports as defined below are required, the cover memorandum, MedWatch Form FDA 3500A, and any pertinent attachments will be processed by the EMMES medical monitor and a copy of the completed report will be submitted by fax or courier delivery before the regulatory reporting deadline, to the following persons:

- FDA medical officer as appropriate (submitted as an amendment to the applicable IND)
- icddr,b PI (who is responsible for forwarding the report to the local/central IRB)
- PATH medical officer
- IPST
- icddr,b DSMB

If relevant follow-up information becomes available, the EMMES medical monitor will be responsible for obtaining and reviewing details from the site. A follow-up MedWatch form will be completed and forwarded to all parties that received the earlier SAE report. A copy of the safety sections for annual FDA reports will be forwarded to PVS.

Suspected adverse reactions that are serious and unexpected will be reported to the FDA within 15 days, or for deaths and life threatening events that are both suspected adverse reactions and unexpected, within 7 days (per 21 CFR 312.32).

Subsequent review by the FDA, the IPST, DSMB/ERC, or the Sponsor may suspend further study product administration or procedures. The study Sponsor, FDA, IPST, and DSMB/ERC retain the authority to suspend additional enrollment and treatments for the entire study as applicable.

Notifying the Independent Protocol Safety Team (IPST) Committee

The EMMES Coordinating Center will provide the IPST, the PATH Medical officer and the EMMES Medical monitor with listings of all SAEs on an ongoing basis. Furthermore, they will be informed of expedited reports of SAEs.

Notifying the Ethics Committee/Institutional Review Board

The PI will ensure the timely dissemination of required SAE information, including expedited reports, to the icddr,b Ethics Review Committee (ERC) and DSMB in compliance with applicable local regulations and guidelines. The PI is

responsible for submitting the safety report (initial and follow up SAE reports) or other safety information (e.g., revised IB) to the ERC and DSMB per request and for retaining a copy in the site's study file.

WIRB Guidelines

All SAEs will be reported to WIRB according to WIRB guidelines and using the WIRB Ten Day Adverse Event Form.

WIRB Phone: 800-562-4789, Fax: 360-252-2498.

PVS is required to report SAEs that fit the following criteria within 10 working days of the time of becoming aware of them:

- Event is UNANTICIPATED (an unanticipated event is any AE for which the nature, severity or frequency is not identified in the IB nor described in the protocol. Events that are already cited in the IB or protocol are not unanticipated and do not have to be reported to WIRB), and
- Event is a SUSPECTED ADVERSE REACTION to the study procedures or product. If the SAE is clearly not related to the study product or procedures, it would not represent a risk to other participants in the research **and, therefore, does not have to be reported to WIRB.**

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. The EMMES Corporation is responsible for data management activities, including quality review, analysis, and reporting of the study data according to SOPs.

Case Report Form Development and Completion

Electronic Data Capture (EDC) will be the method of data collection in this study. The eCRFs will be developed by The EMMES Corporation and approved by the icddr,b and PVS. Clinical data (including AEs, concomitant medications, and reactogenicity data) and clinical laboratory data will be entered into the 21 CFR Part 11-compliant EDC system provided by The EMMES Corporation. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

Data for each participant will be recorded in the eCRF. It is the PI's responsibility to ensure the accuracy, completeness, legibility, and timeliness of the data reported in the participant's eCRF and any supporting documentation. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Source documentation supporting the eCRF data should document the dates and details of study procedures, AEs and participant status. The PI/institution will maintain all information in the eCRFs and all source documents that support the data collected from each participant.

The PI 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. The PI will retain all essential documents and a CD-ROM copy of the eCRF data (after the study is closed and unblinded and the Clinical Study Report is completed or at such time that the site no longer has access to the electronic data system).

Details of Data Management

A Data Management Handbook (DMH) will be written by The EMMES Corporation and contain all study-specific requirements.

Coding

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. The EMMES Corporation is responsible for data management activities, including quality review, analysis, and reporting of the study data according to SOPs.

Data Validation

The EMMES Corporation will inspect the data entered into the database for completeness and consistency.

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 the PI and stored in the participant's study file. Clinical laboratory data will remain in study participant records. Essential documents, including ICFs, must be filed and kept in the study files on an ongoing basis.

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.

Definition of Source Document

Original source documents include 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. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained.

Database Locking Procedures

- A final database lock for the primary analysis will occur after all participants have completed all follow-up visits, including the 6 month safety follow-up call, a case-by-case review of the severity of any AEs has been performed and finalized, all data anomalies have been resolved and monitoring is complete.
- Remaining immunology data will be maintained in a separate immunology database.

Record Archival

The PI is responsible for retaining study records for a period of 2 years following the date that a marketing application is approved for the product or, if no application is to be filed or, if a file application is not approved, until 2 years after the investigation is discontinued and the FDA is notified. The Sponsor will be responsible for providing the site with date of vaccine approval or IND withdrawal.

These records are also to be maintained in compliance with icddr,b and local authority medical records retention requirements, whichever is longest. Storage of all trial-related documents will be such that confidentiality will be strictly maintained to the extent provided by US and local law.

Screen Failures

If a participant or participant's parent signs the ICF but the participant is not randomized because of ineligibility (a screen failure), the reason for his/her ineligibility should be entered in the medical records/notes/charts. Also, a screening log must be kept. Data from participants who fail screening will not be recorded in the eCRF, with the exception of demographic data and the reason for screen fail.

Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or site SOP requirements. The noncompliance may be either on the part of the participant, the PI, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

- Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3
- Quality Assurance and Quality Control, Section 5.1.1
- Noncompliance, Sections 5.20.1 and 5.20.2.

It is the responsibility of the site to exercise continuous vigilance to identify and report deviations within 5 working days after identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations must be promptly reported via the appropriate eCRF within the electronic data system.

All deviations from the protocol must be addressed in study participant source documents. A completed copy of WIRB reportable Protocol Deviation forms must be maintained in the regulatory file, as well as in the participant's source document. Protocol deviations must be sent to the icddr,b ERC per its guidelines. The site PI/study staff is responsible for knowing and adhering to icddr,b ERC requirements.

STUDY MONITORING

Sponsor monitoring responsibilities will be provided through a CRO experienced in monitoring clinical site activity. A site initiation visit will be conducted prior to beginning the study, and monitoring will be conducted at initiation, during, and at closeout of the study by the study monitor or designee.

During the course of the study, the monitor will visit the clinical site at intervals to verify compliance to the protocol; completeness, accuracy, and consistency of the data and study product accountability; adherence to US CFR regulations, and any additional regulations and requirements, including GCP, of the conduct of clinical research. The monitor should have access to participant medical records, study product accountability 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 in a predefined timeframe.

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 The EMMES Corporation. 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 resolution as soon as possible; all queries must be resolved prior to database lock.

Essential documents must be filed in the site study file on an ongoing basis and available for review by the Sponsor's contracted site monitor. Monitoring visits will be performed according to the Clinical Monitoring Plan.

Independent Auditing

PVS representatives may audit the study to ensure that study procedures and data collected comply with the protocol and applicable SOPs at the clinical site and The EMMES Corporation, 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.

Regulatory Agency Auditing

The PI must be aware that representatives from US/local regulatory authorities or WIRB 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 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.

OBLIGATIONS AND ROLES OF THE SPONSOR, PI AND STUDY PERSONNEL

This study will be conducted according to GCP and in accordance with all US federal regulations regarding the protection of human subjects in research including US 21 CFR Part 50 and US 21 CFR Part 312, as well in accordance with Bangladeshi regulations.

The Sponsor will assure the trial is conducted in compliance with the protocol, GCP, and regulatory authority requirements. The Sponsor will provide the investigators with the funding and information needed to conduct the trial properly, ensuring proper monitoring of trial activities, ensuring that the trial is conducted in accordance with the general investigational plan and protocols contained in the submissions to the regulatory authorities. The Sponsor will ensure that the FDA and the investigators are promptly informed of significant new adverse effects or risks with respect to the study vaccine.

The PI agrees to perform the research in strict accordance with this protocol, the International Conference on Harmonisation (ICH) Guideline for Good Clinical Practice (E6), as well as in conformity with any US or local regulations regarding the conduct of clinical studies.

In addition, the PI must follow local and institutional requirements including, but not limited to, investigational product, clinical research, informed consent and icddr,b regulations. The Sponsor will provide notification to the PI of protocol and amendment approvals by regulatory authorities when applicable. Any modifications to the research protocol, the ICF, and/or the questionnaires or change in PI must be submitted to WIRB and icddr,b ERC for review and approval prior to implementation. The PI may deviate from the protocol without prior approval only when the deviation is necessary to eliminate an apparent immediate hazard to the participant.

The PI will notify the icddr,b ERC and DSMB of SAEs and protocol deviations according to the icddr,b ERC requirements. Any deviation to the protocol that may have an effect on the safety or rights of the participant, or the integrity of the study, must be reported to the Sponsor by the PI as soon as the deviation is identified. In that event, the PI will notify the Sponsor immediately by email, notify the icddr,b ERC, enter the deviation into the appropriate eCRF, and confirm notification to the Sponsor in writing within 10 working days after the change is implemented. All deviations will be noted in the annual report to the Sponsor, and in the final study report for the FDA.

Except where the PI'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 PI or appropriate study personnel that the PI designates to perform a certain duty. The PI 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 PI on designated eCRFs.

The EMMES medical monitor will be responsible for reviewing all serious and unexpected AEs and providing an unbiased written report of the event.

Data Safety Monitoring Plan (DSMP)

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

SAFETY MONITORING

Extensive safety monitoring will be provided for this protocol. The PI and/or designated site staff will be responsible for continuous close safety monitoring of all study participants and for alerting the Sponsor if unexpected concerns arise or stopping criteria are met.

Safety Oversight

A Data and Safety Monitoring Board (DSMB) will be formed by the Ethical Review Committee to evaluate and assess the safety of study participants. In addition, an Independent Protocol Safety Team (IPST) formed for the study will carry out the safety and clinical evaluation at the icddr,b. Moreover, there will be a team comprising of the study physician, the Medical Monitor from EMMES, the principal investigator, and the Medical Officer from PVS for evaluation of the study at different intervals in the study.

Before enrolling participants in subsequent cohorts within an age group, the safety data from the previous cohort(s) will be evaluated and reviewed by the IPST. These data include, but are not limited to, physical examinations, vital signs, and solicited reactogenicity symptoms. The IPST will convene after each cohort within an age group to review data through Day 3 after the second vaccine administration and make a recommendation to the Sponsor on whether to proceed to the initiation of the following cohort.

The DSMB will be composed of 3-4 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 meets before study initiation, prior to age de-escalation, and after the study closeout. 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 Officer in detail about the discussions of those meetings.

Study Pause

Study pause is defined as a decision to cease, temporarily or definitely, enrollment and all vaccinations. Study pause or final cessation of vaccinations will not eliminate any safety follow-up procedures specified by protocol. The Sponsor will pause vaccinations in the study if the IPST, DSMB or protocol team including the PATH Medical Officer and EMMES Medical Monitor, determines that study hold criteria have been met, or in the event of any other safety concerns.

Study Pause Rules

The following study pause rules will trigger automatic halt of further vaccination until the protocol team consisting of the PI, study physician, independent medical monitor, PATH Medical Officer and EMMES Medical Monitor, has performed a review.

Meeting one or more of the following criteria will automatically pause or halt further vaccinations in the study.

- One participant death from Day 0 to Day 42, unless judged definitely unrelated to vaccination
- One participant with a serious AE (SAE) judged as definitely or probably related to vaccine
- >2 participants in a cohort with the same ≥grade 3 (severe) solicited AE within three days following vaccination judged as definitely or probably related to vaccine
- >2 participants in a cohort with the same ≥grade 3 (severe) abnormal clinical monitoring laboratory value (7 days following vaccination) judged as definitely or probably related to vaccine
- >2 participants in a cohort with the same ≥grade 3 (severe) AE judged as definitely or probably related to vaccine

Study Pause Procedure

If pause criteria are met, the icddr,b DSMB will be notified and the protocol team consisting of the PI, study physician, independent medical monitor, PATH Medical Officer and EMMES Medical Monitor will convene expeditiously in person or by conference call to review all available and relevant information and provide recommendations to the Sponsor whether to permanently stop vaccinations, re-start vaccination and enrollment, or otherwise modify the study In addition, if the PI (or designee), the EMMES medical monitor, the PATH medical officer, any member of the IPST or any member of the DSMB identifies a safety signal of concern, the DSMB and IPST will be advised immediately for deliberation and recommendation as to whether vaccinations and enrollment be suspended.

At scheduled study review meetings, the IPST or DSMB may determine that pause criteria have been met.
If the Sponsor decides to restart the study after DSMB review and recommendation to restart, vaccinations may resume. Vaccinations that are delayed beyond the protocol-specified window may be administered at the discretion of the PI, in consultation with the Sponsor. The appropriate follow-up and subsequent vaccination schedule for participants with delayed study vaccinations will be determined by the Sponsor on a case-by-case basis.

At any time during the study, the Sponsor may hold vaccinations if it judges that there is a safety concern.

Ethical Assurance for Protection of Human rights

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

Informed Consent Process

Before any study-related activities and in agreement with applicable regulatory requirements, the PI must ensure that the participant or participant's parent is fully informed about the aims, procedures, potential risks, and potential benefits of the study. The participant or participant's parent will be given the written, icddr,b ERC/WIRB approved ICF, allowed ample time to read the consent form, encouraged to ask questions about the study, have the questions answered and then be given time to decide if s/he would like to participate in the study. It will be emphasized that participation is voluntary, and that the parent has the right to withdraw his/her child from the study at any time without prejudice.

The PI or designee must obtain the participant's or participant's parent's voluntary, signed and dated ICF (or, if the parent/guardian is unable to sign, independently witnessed and documented parental consent) before any study-related procedures are performed. Study staff must document the informed consent process. The original, signed ICF must be kept in the site study file.

Risk/Benefit

No benefits can be guaranteed to participants for their participation in this research study.

Previous experience with the second generation study product, currently formulated as ETVAX administered with and without dmLT adjuvant, is limited to the first in human trial recently conducted in Swedish adults. In that trial, the vaccine alone and in combination with dmLT was well-tolerated, with reactogenicity, when it occurred, predominantly mild in nature (with no difference between vaccine and placebo recipients) and no SAEs were observed. Furthermore, dmLT has been in two other clinical trials – DMID protocol 09-0066 evaluated alone in doses of up to 100 ug and VAC 006 evaluated with a live attenuated ETEC vaccine candidate– consistently demonstrating an acceptable safety profile with oral administration.

As with any vaccine, severe allergic reaction is a potential rare event. None were observed in the phase 1 trial of this vaccine.

Participants may experience fever and/or diarrhea post-vaccination, and possibly dehydration requiring oral rehydration. Nausea and abdominal cramps are also possible post-vaccination. Pregnancy is exclusionary for study participation and pregnancy status will be documented through testing prior to study interventions, along with discussion on methods to prevent pregnancy during study. 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. It will also inform investigators of the potential for this vaccine to move forward into larger infant studies to establish optimal dose and eventually into field efficacy trials. Finally, if the addition of dmLT improves overall vaccine immunogenicity, it offers the potential opportunity for further dose sparing of ETVAX, which is especially important when administering vaccines to paediatric populations, lowering the manufacturing and cost-of-goods, and ultimately making the vaccine available to low-resource country populations at a lower cost.

Protocol Review Process

Scientific review of this protocol will be conducted by icddr,b Research Review Committee and PATH. The protocol will be submitted under a new IND at the US FDA and to icddr,b. The IND Sponsor will be PVS. Protocol ethical review and oversight will be performed by the icddr,b ERC, DSMB, WIRB, and the Independent Ethical Committee' in the Gothenburg Region (IECGR). Continuing review will be undertaken in accordance with existing regulations. The Sponsor will be responsible for trial registration at ClinicalTrials.gov.

Copies of the approved continuing review and final study reports, along with the respective local IRB approval notifications, will be submitted to the Sponsor as soon as these documents become available.

Participant Confidentiality

The PI must ensure that participant confidentiality is maintained. Personal identifiers will not be included in any study reports. All study records will be kept confidential to the extent provided by national and local laws.

All study procedures will be conducted per GCP guidelines, and every effort will be made to protect participant privacy and confidentiality to the extent possible.

All study-related information will be stored securely at the field site at Mirpur or in the icddr,b main campus at Mohakhali. When not in use and under immediate control of study staff, all participant information will be stored in locked archive area at the field site with access limited to study staff. Data collection, process, and administrative forms, laboratory specimens, and other reports will be identified exclusively by a coded number to maintain participant confidentiality. All local databases will be secured with password-protected access systems. Participants' study information will not be released without written parental permission, except as necessary for monitoring or compliance with legal or regulatory requirements.

Medical records containing identifying information may be made available for review when the study is monitored by the Sponsor or an authorized regulatory agency. Direct access may include examining, analyzing, verifying, and reproducing any records and reports that are important to the evaluation of the study.

Reimbursement

Pending FDA, WIRB, and icddr,b ERC approval, adult participants or parents/guardians of enrolled children will be compensated for their travel costs in this study. The study ICF will state the plan for reimbursement. Participants or parents/guardians of study participants will not be charged for study vaccinations, research clinic visits, research-related examinations, or research-related laboratory tests.

Storage of Specimens

Stored study research samples (including samples retained for elective analysis) will be labelled by a code that only the study site can link to the participant. All stored research samples will be logged into a secure database and any use documented. Samples will be stored at the icddr,b bio repository and laboratories to complete the analyses required to meet study primary, secondary and exploratory analyses. For validation of results further, a sub sample of specimens will be shipped to Gothenburg University and to PVS. As a part of the informed consent process, participants will be informed of and asked to agree to long-term storage of specimens for use in future, related research. Specimens may be stored up to 5 years for these potential immunological and microbiological studies.

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 animal will be used in this study

Collaborative Arrangements

Describe if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization.

This project is a collaborative study of icddr,b with PATH Vaccine Solutions (PVS), USA, Sahlgrenska Academy of University of Gothenburg, Sweden, and Scandinavian Biopharma, Sweden, as well as other International experts in the vaccine field.

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.

Mirpur field office at Mirpur, Dhaka, diarrheal hospitals of icddr,b in Mohakhali and Mirpur and existing health facilities as well as laboratory facilities are available for the study.

Literature Cited

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

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APPENDIX I: Schedule of Events

		Screening	1st Dose	F/U Visit	2nd Dose	F/U Visits			F/U Call
Visit Number		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Study Day	**Note**	**-7 to -4**	**Day 0**	**Day 7 ± 1** (6-8 days after first dose)	**Day 14 ± 2** (12-16 days after first dose)	**Day 19 + 1** (5-6 days after second dose)	**Day 28 ± 2** (14 ± 2 days after the second dose)	**Day 42 ± 4** (28 ± 4 days after the second dose)	**Day 182 ± 14** (6 months ± 14 days after the first dose)
Informed Consent		X							
Inclusion/Exclusion Criteria	1	X	X*		X*				
Medical History		X	X*						
Physical Exam	2	X						X	
Vital Signs	3	X	X*	X	X	X		X	
Screening serology	4	X							
Hematology	5	X		X					
Clinical Chemistry	6	X		X					
Urine Pregnancy Test (Part A only)		X			X*				
Randomization			X*						
Vaccine Dosing			X		X				
Interim Medical Interview, Concomitant Medications review, and AE Review			X*	X	X*	X		X	X
Reactogenicity Assessment by TSS	8		X----------X		X----------X				
Home visits by TSS	9		X----------X		X----------X		X		X
Stool for culturing		X							
Stool for SIgA (ELISA) 5 gm – Part A 2-3 gm Parts B, C & D		X	X*	X		X	X		
Plasma Sample for Serology (ELISA)		X		X		X			
Whole Blood for ALS		X		X		X			
SAE/AE follow-up				X	X	X		X	X
Total blood volume: Part A		10 ml		10 ml		10 ml			
Total blood volume: Parts B, C, D		3.5 ml		3.5 ml		3.5 ml			

*Prior to vaccination

Note	
1	Fulfillment of all screening eligibility criteria 1 is required to accept any participant into the study, and should be assessed during the screening period (Day -7 to -4). Fulfilment of all of the following continuing eligibility criteria is required for all participants to receive their second vaccination (Day 14). If a participant does not meet the following continuing eligibility criteria prior to receiving their second immunization, vaccination can be deferred by up to two days. However, if a participant falls outside this deferred window, he/she will not receive the second vaccination but encouraged to continue all safety follow-up activities per protocol.
2	Full physical examination will include assessment of head, eyes, ears, nose, oropharynx, neck, chest (auscultation), lymph nodes (neck, supraclavicular, axillary, inguinal), abdomen (auscultation and palpation), musculoskeletal, skin, and neurological A brief physical exam will include assessment of head, eyes, ears, nose, oropharynx, neck and chest (auscultation) A targeted physical exam may be conducted at the physician's discretion and if clinically indicated.
3	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.
4	Screening serology will include HBsAg, and HCV
5	Complete blood count (CBC), including WBC with differential (neutrophils and lymphocytes only), hemoglobin, and platelet count
6	Screening Chemistry Panel (Day -7 to -4): ALT, albumin, bilirubin and creatinine Safety Monitoring Chemistry Panel (Day 7): ALT and creatinine
7	Females of child bearing potential only
8	Reactogenicity assessments by TSS will only occur for 7 days following each administration. The TSS will be responsible for that source document and bring it to and from the participant's home. Solicited reactogenicity symptoms will include the following: fever, nausea (Part A only), abdominal pain/stomach ache (Parts A and B only), vomiting, and diarrhea.
9	Home visits by TSS will occur for 7 days after each vaccination; i.e., Day 0-7 and Day 14-21.

APPENDIX II:

PART A (ADULTS) Clinical Toxicity Grading

Systemic Illness	Mild (Grade 1)	(Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Illness or clinical AE (as defined according to applicable regulations)	No or minimal interference with usual activities; no medical intervention/therapy required	Greater than minimal interference with usual activities; no or minimal medical intervention/therapy required	Marked limitation in ability to perform usual activities; medical intervention/therapy required.	Inability to perform basic functions OR Medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death
Acute systemic allergic reaction	Localized urticaria (wheals) with no medical intervention indicated	Localized urticaria with medical intervention indicated OR Mild angioedema with no medical intervention indicated	Generalized urticaria OR Angioedema with medical intervention indicated OR Symptomatic mild bronchospasm	Acute anaphylaxis OR Life-threatening bronchospasm OR laryngeal edema
Fever (non-axillary)	37.7 – 38.6°C	38.7 – 39.3°C	39.4 – 40.5°C	> 40.5°C
Vomiting	Transient or intermittent vomiting with no or minimal interference with oral intake	Frequent episodes of vomiting with no or mild dehydration	Persistent vomiting resulting in greater than mild dehydration OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Diarrhea	At least 3 looser-than-normal stools without dehydration	Diarrhea with some dehydration	Diarrhea with severe dehydration	Diarrhea with hypovolemic shock
Nausea	1-2 episodes within a 24-hour period	3-4 episodes within a 24-hour period	≥5 episodes within a 24-hour period	
Abdominal Pain	Discomfort noted, but no disruption of normal daily activities; slightly bothersome; relieved with or without symptomatic treatment	Discomfort sufficient to reduce or affect normal daily activity to some degree; bothersome; interferes with activities, only partially relieved with symptomatic treatment.	Discomfort sufficient to reduce or affect normal daily activity considerably; prevents regular activities; not relieved with symptomatic treatment.	

APPENDIX III

PARTS B, C & D (TODDLERS, YOUNG CHILDREN & INFANTS) Clinical Toxicity Grading

Systemic Illness	Mild (Grade 1)	(Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Illness or clinical AE (as defined according to applicable regulations)	No or minimal interference with usual activities; no medical intervention/therapy required	Greater than minimal interference with usual activities; no or minimal medical intervention/therapy required	Marked limitation in ability to perform usual activities; medical intervention/therapy required.	Inability to perform basic functions OR Medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death
Acute systemic allergic reaction	Localized urticaria (wheals) with no medical intervention indicated	Localized urticaria with medical intervention indicated OR Mild angioedema with no medical intervention indicated	Generalized urticaria OR Angioedema with medical intervention indicated OR Symptomatic mild bronchospasm	Acute anaphylaxis OR Life-threatening bronchospasm OR laryngeal edema
Fever (axillary/orally)	≥37.5 - <38.5°C	≥38.5 - <39.5°C	≥39.5 – <40.5°C	≥ 40.5°C
Vomiting	Transient or intermittent vomiting with no or minimal interference with oral intake	≥5 episodes within a 24-hour period	Persistent vomiting resulting in greater than mild dehydration OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Diarrhea (≥3 loose stools/day)	At least 3 looser-than-normal stools without dehydration	Diarrhea with some dehydration (per IMNCI definition)	Diarrhea with severe dehydration (per IMNCI definition)	Diarrhea with hypovolemic shock
Abdominal Pain	Discomfort noted, but no disruption of normal daily activities; slightly bothersome; relieved with or without symptomatic treatment	Discomfort sufficient to reduce or affect normal daily activity to some degree; bothersome; interferes with activities, only partially relieved with symptomatic treatment.	Discomfort sufficient to reduce or affect normal daily activity considerably; prevents regular activities; not relieved with symptomatic treatment.	

APPENDIX IV: Laboratory Toxicity Grading Table

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 Study Physician, Principal Investigator and either the medical monitor or the study sponsor. This consensus will be documented with a note in the participant's chart by the PI or designee.

Adults 18-45 Years				
Solicited Lab AEs	**Grade 1 – Mild**	**Grade 2 – Moderate**	**Grade 3 - Severe**	**Grade 4 – Potentially Life Threatening**
Hemoglobin (gm/dl) (female)	9.5 - 11.4	8.0 - 9.4	6.5 – 7.9	< 6.5
Hemoglobin (gm/dl) (male)	11.0 - 12.4	9.0 – 10.9	7.0 – 8.9	< 7.0
Leukocytes (cells/mm^3)				
Decreased	2,000 – 3,999	1,500 - 1,999	1,000 - 1,499	< 1,000
Increased	11,001-15,000	15,001-20,000	20,001-25,000	>25,000
Platelets/mm^3	100,000-125,000	50,000-99,999	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
Albumin g/dL	2.8 - 3.1 g/dL	2.5 - 2.7 g/dL	< 2.5 g/dL	NA

12-59 months				
Solicited Lab AEs	**Grade 1 – Mild**	**Grade 2 – Moderate**	**Grade 3 - Severe**	**Grade 4 – Potentially Life Threatening**
Hemoglobin (gm/dl)	10.0-10.9	7.0-9.9	<7.0	Cardiac failure secondary to anaemia
Leukocytes (cells/mm^3)				
Decreased	2,000 - 2,500	1,500 - 1,999	1,000 - 1,499	< 1,000
Increased	>1.0-1.3 ULN 17,501 – 22,750	>1.3-1.7 ULN 22,751 – 29,750	>1.7-2.2 ULN 29,751 – 38,500	>2.2 ULN >38,500
Platelets/mm^3	100,000-125,000	50,000-99,999	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
Albumin	2.8 - 3.1 g/dL	2.5 - 2.7 g/dL	< 2.5 g/dL	NA

6-11 months				
Solicited Lab AEs	**Grade 1 – Mild**	**Grade 2 – Moderate**	**Grade 3 - Severe**	**Grade 4 – Potentially Life Threatening**

Hemoglobin (gm/dl)	9.0-9.9	7.0-8.9	<7.0	Cardiac failure secondary to anaemia
Leukocytes (cells/mm^3)				
Decreased	2,000 - 2,500	1,500 - 1,999	1,000 - 1,499	< 1,000
Increased	>1.0-1.3 ULN 17,501 – 22,750	>1.3-1.7 ULN 22,751 – 29,750	>1.7-2.2 ULN 29,751 – 38,500	>2.2 ULN >38,500
Platelets/mm^3	100,000-125,000	50,000-99,999	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
Albumin	2.8 - 3.1 g/dL	2.5 - 2.7 g/dL	< 2.5 g/dL	NA