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ICDDRB: Centre for Health & Population Research RRC APPLICATION FORM

RESEARCH PROTOCOL

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Protocol No: 2006-006 Date received:
RRCApproval: Yes/ No Date:
ERC Approval: Yes/No Date:

Project Title: Safety, dose, immunogenicity, and community transmission risk of a candidate Shigella flexneri 2a vaccine (SC602) among young children in rural Bangladesh.
Theme and key words: Vaccine evaluation Vaccine, Shigella, Immunogenicity

Principal Investigator: DR. Abdullah. H. Baqui **Division:** PHSD **Phone:**
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Co-Principal Investigators: Sheet attached
Co-Investigator(s): Sheet attached
Student Investigator/Intern: X

Population: Inclusion of special groups *(Check all that apply)*:

Gender
- ☑ Male
- ☑ Females

Age
- ☑ 0 – 5 years
- ☐ 5 – 9 years
- ☐ 10 – 19 years
- ☐ 20 +
- ☐ > 65

- ☐ Pregnant Women
- ☐ Fetuses
- ☐ Prisoners
- ☐ Destitutes
- ☐ Service providers
- ☐ Cognitively Impaired
- ☐ CSW
- ☐ Others (specify _____)

Project / study Site *(Check all the apply)*:
- ☐ Dhaka Hospital
- ☑ Matlab Hospital
- ☑ Matlab DSS area
- ☐ Matlab non-DSS area
- ☐ Mirzapur
- ☐ Dhaka Community
- ☐ Chakaria
- ☐ Abhoynagar
- ☐ Mirsarai
- ☐ Patyia
- ☐ Other areas in Bangladesh _____
- ☐ Outside Bangladesh
  name of country: _____
- ☐ Multi centre trial
  (Name other countries involved)
  _____

Type of Study *(Check all that apply)*:
- ☐ Case Control study
- ☐ Community based trial / intervention
- ☐ Program Project (Umbrella)
- ☐ Secondary Data Analysis
- ☑ Clinical Trial (Hospital/Clinic)
- ☑ Family follow-up study
- ☐ Cross sectional survey
- ☐ Longitudinal Study (cohort or follow-up)
- ☐ Record Review
- ☐ Prophylactic trial
- ☐ Surveillance / monitoring
- ☐ Others

Targeted Population *(Check all that apply)*:
- ☑ No ethnic selection (Bangladeshi)
- ☑ Bangalee
- ☐ Tribal groups
- ☐ Expatriates
- ☐ Immigrants
- ☐ Refugee

Consent Process *(Check all that apply)*:
- ☑ Written
- ☐ Oral
- ☐ None
- ☐ Bengali language
- ☑ English language

Proposed Sample size:
Sub-group _____ ☐
_____ ☐

Total sample size: 78 ☐
_____ ☐
_____ ☐

International Centre for Diarrhoeal Disease Research, Bangladesh
Walter Reed Army Institute of Research, USA

RESEARCH PROTOCOL

1. **Title of Project:** **Safety, dose, immunogenicity, and community transmission risk of a candidate *Shigella flexneri* 2a vaccine (SC602) among young children in rural Bangladesh**

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Name of Division/Programme of ICDDR,B under which the study will be carried out: Child Health Programme, Public Health Sciences Division

Medical Monitor: Dr. AN Alam, MBBS, PhD, Training & Education Dept., ICDDR,B

Dates of proposed period of support: 1 Apr 2000 to 31 Mar 2001

Costs: Direct: US$ 98,921.00 Total: US$ 123,651.00

Sponsors: Walter Reed Army Institute of Research, USA, and National Vaccine Program, USA

Investigational New Drug information: This study will be carried out under BB-IND 5465 application filed with the Center for Biologics Evaluation and Research of the US Food and Drug Administration, sponsored by the Office of the Surgeon General, US Department of the Army. United States Department of Defense Multiple Project Assurance Number 20010.

Approval of the project by the Division Director of the applicant

The above mentioned project has been discussed and reviewed at the Division level as well as by the external reviewers. The protocol has been revised according to the reviewers comments and is approved.

Professor Lars Ake Persson		7.2.2000
Name of Division Director	Signature	Date

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

11. Signature of PI: Malyn Date: 6-2-2000

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Project Summary

SC602 is an orally administered, live attenuated strain of *Shigella flexneri* 2a that has shown promise as a vaccine candidate for prevention of diarrhea and dysentery due to *S. flexneri* 2a. The vaccine strain is a mutant version of virulent *S. flexneri* 2a strain 454 from the Institute Pasteur collection that retains the ability to invade colonic mucosal cells, yet is unable to spread intra- and intercellularly or to utilize environmental iron normally.

Pre-clinical and clinical studies were conducted in naïve adult volunteers in the USA at Walter Reed Army Institute of Research (WRAIR). SC602 was found to be well tolerated and immunogenic in this population at a dose of 10^4 CFU given with bicarbonate. Significant protection was afforded 8 weeks after vaccination against challenge with virulent *S. flexneri* 2a. Outpatient studies demonstrated no secondary transmission to household contacts of vaccinees.

Clinical trials of SC602 were also conducted in adults and school age children in Bangladesh at the ICDDR,B's Matlab Diarrhoea Treatment Centre and the surveillance area. Both inpatient and community-based studies were conducted in focusing on personal and community safety, fecal shedding of vaccine, humoral immune markers and dose determination. Studies of dose levels of 10^4 to 10^6 CFU were conducted sequentially in 88 adults, then 81 school children age 5-15 years. Both adults and school children tolerated the vaccine well, demonstrating minimal dose-related vaccine shedding, minimal reactogenicity, no transmission risk, and little immune stimulation in this *Shigella*-endemic population. The present study is designed to build on this work, administering SC602 to the target population age group of 12 to 35 months old children.

The current study proposes to evaluate SC602 safety, fecal shedding, dose and immunogenicity in up to 78 children aged 12-35 months initially in inpatient followed by in outpatient trials at the Matlab field station. Two or three inpatient studies will be conducted sequentially. Each inpatient trial will admit 18 children 12-35 months old to the inpatient ward at the ICDDR,B Matlab hospital for 5 days. The first trial will randomize 3 groups to receive one of 2 single doses of SC602 (10^3 or 10^4 CFU) or placebo orally with bicarbonate. Baseline laboratory evaluation will include stool culture and microscopy, and immunoglobulin studies of serum and stool. Daily follow up will include assessment of symptoms, with culture of all stools for *Shigella*. Fecal colonization will be terminated, if needed, with antimicrobial therapy prior to discharge from the ward. Serum and stool samples will be obtained on days 14 and 28 for repeat immunoglobulin assays.

The study design for the 2nd inpatient trial (and 3rd if necessary) will be identical to the 1st, with the exception that dosing for the second trial will be determined after assessment of vaccine shedding and symptoms in trial 1. .The 3rd inpatient trial will be conducted only if necessary to examine higher vaccine doses based on results of trials

1 and 2. Following determination of optimal dose for this age group, the outpatient trial will commence using only that dose.

The outpatient trial will enroll 24 children 12-35 months of age, randomized to receive vaccine (16 subjects), or placebo (8 subjects). Daily follow up for symptoms in vaccinees and household contacts will commence for 5 days. Laboratory follow up will include routine rectal swab culture of vaccinees and household contacts, and fecal/serum immunoglobulins in vaccinees. Symptomatic diarrhea from study subjects and neighborhood children occurring during the study period will be cultured for SC602 in addition to standard evaluation.

Data will be analyzed to determine a) whether vaccination was associated with increased occurrence of adverse events; b) the extent of secondary spread of the vaccine; c) whether there was any vaccine induced outbreak in the household; and d) the immunoglubulin response to the vaccine.

Description of the Research Project

Hypotheses to be Tested:

1. Oral administration of SC602 to healthy Bangladeshi children from 12 to 35 months of age in dose levels less than or equal to 10^6 CFU will not lead to significant adverse events.

2. The dose that consistently colonizes vaccine recipients and is safe, will also induce evidence of immune stimulation.

3. There may be some secondary spread of vaccine to household contacts of vaccine recipients, but there will be no vaccine-induced occurrence of dysentery/diarrhea.

Specific Aims

1. To evaluate adverse events associated with oral administration of up to 4 dose levels of SC602 [10^3, 10^4, 10^5 or 10^6 CFU] relative to placebo in healthy children 12-35 months of age in a community endemic for *Shigella*.

2. To evaluate duration of fecal shedding of SC602 in these children for up to 28 days after vaccination.

3. To evaluate probability of, and symptoms associated with, secondary spread of SC602 to household contacts of vaccinated children as determined by stool culture of these contacts for the vaccine strain.

4. To evaluate humoral immune responses in serum and stool, manifested by changes in immunoglobulins.

Background of the Project

Epidemiology.

The 4 species of the genus *Shigella* are the most common bacterial cause of severe bloody diarrhea (bacillary dysentery) worldwide. In the developing world, *Shigella flexneri* predominates, with *S. flexneri* 2a specifically the predominant serotype. Studies from the Matlab, Bangladesh field research station of the ICDDR,B reveal that in 8-10% of all deaths in 1-4 year old children are due to bloody diarrhea. Stool cultures from subjects with diarrhea (all clinical variants) in this population reveal shigellae in 8-12% of specimens; about 60% of which are *S. flexneri*. In a community-based cohort study of 700 <5 years old children in this area, the attack rate of *Shigella*-

associated diarrhea was 25 episodes per 100 child-years, and 15 episodes per 100 child-years for *S. flexneri* (Baqui et al, 1992). Globally, 65% of *Shigella* cases occur in children <5 years old. In the developing world, overall, 60% of cases are associated with *S. flexneri*, with 32-58% being the *S. flexneri* 2a serotype. *S. sonnei* predominates in the developed world (77% of isolates) (Kotloff et al, unpublished). The incidence of clinical shigellosis peaks at 1-4 year old children and decreases to approximately 25% of peak levels in older children and adults living in endemic areas (Keusch et al, 1991; Slayers et al, 1994).

High attack rates of shigellosis also occur in individuals newly arrived to endemic areas, such as travelers, western expatriots and military populations on maneuvers (Bennish et al, 1990; Cohen et al, 1988). During Operation Desert Shield, 57% of US troops reported diarrhea over a 2 months interval, 26% of which were associated with shigellae (Hyams, 1991). During Operation Bright Star in Egypt, 30% of US troops per week were struck with diarrhea, 19% of which were shigellae-associated (Oldfield, 1991).

Biology.

Shigellae are characterized as gram-negative, rod-shaped, non-motile bacteria that are closely related to *Escherichia coli*. Shigellae are thought to elicit diarrhea by two distinct mechanisms (Hale, 1991). An early watery diarrhea commonly occurs, likely related to production of a toxin as the bacterium passes through the small intestine. Bloody diarrhea may follow, associated with pathologic lesions in the colon. Colitis is induced by endocytic uptake of shigellae by follicle-associated M cells, followed by intra- and intercellular spread to adjacent epithelial cells, with resultant inflammation and ulcer formation. Invasion of M cells is mediated by *ipa* proteins encoded on a critical 120-140 mD virulence plasmid; whereas intra- and intercellular spread is mediated by another plasmid gene, *icsA* (Hale, 1991). Cell to cell spread of intracellular shigellae is initiated by recruitment of host cell actin to form a cytoskeleton-based motor, which moves the organisms to areas of intermediate junctions between cells. A rigid protrusion containing shigellae is formed, mediated by cell adhesion molecules (CAM), that is taken up via active endocytosis by contiguous cells. Lysis of the endocytosed double membrane bound vessicle follows, brought about by plasmid-encoded proteins. Mutant shigellae that cannot recruit and organize an actin motor for intercellular spread are unable to elicit clinical symptoms in rabbit ileal loops or in intragastrically challenged rhesus monkeys (Sansonetti et al, 1991). Thus the active remodeling of the intestinal epithelium for the propagation and spread of shigellae in the intracellular environment is central to the dysenteric manifestations of shigellosis.

Clinical.

Dysentery, the definitive clinical manifestation of shigellosis, is defined as frequent passage of bloody stools with mucus and abdominal pain. Constitutional symptoms such as rectal tenesmus, fever, mild tenderness over the left colon upon palpation and

shigellosis peaks at 1-4 years and decreases to approximately 25% of peak levels in older children and adults living in endemic areas (Slayers et al, 1994). Also, individuals newly arrived to endemic areas with high pre-existing serotype-specific serum antibodies have significantly lower attack rates during *Shigella* outbreaks (Cohen et al, 1988).

Demonstration of an immune response to shigellae has relied upon measurement of serum antibodies. DuPont et al (1972) showed that the magnitude of the serum antibody response to O-antigen following challenge with *S. flexneri* correlated with the severity of the clinical illness. Since *Shigella* infections remain largely limited to colonic mucosa and rarely disseminate via the systemic route, it is the mucosal rather than systemic immunity that plays a major role in protecting the hosts. Secretory IgA constitutes the first line of defense against *Shigella* infections and has been found in local secretion after natural infections (Dinari et al, 1987). The role of *Shigella*-specific sIgA in the antibody-dependent cell-mediated antibacterial activity of intestinal lymphocytes has been clearly shown in *in vitro* studies (Tagliabue et al, 1983). In the murine pulmonary infection model of shigellosis, protection was found to be serotype specific and was dependent on local concentration of dimeric IgA (Phalipon et al, 1995).

SC602 characteristics.

Shigella vaccine candidates developed previously have frequently been excessively reactogenic or inadequately immunogenic (Hale, 1995). More recent efforts have focused on live attenuated mutants that retain the ability to invade the colonic epithelium and induce an immune response, yet fail to elicit clinical symptoms. The ability of the bacterium to spread from cell to cell following invasion has been shown to be important for eliciting clinical symptoms. In 1990, Philippe Sansonetti and his group at the Institute Pasteur developed the SC602 mutant strain (Barzu et al, 1996) which retains the virulence plasmid genes (encoding *ipa* proteins) that mediate cellular invasion, but is missing the gene encoding *icsA*, which mediates intra- and intercellular spread. This is accomplished via a 10 Kb deletion encompassing the entire 3.6 Kb *icsA* gene and extensive flanking regions on the virulence plasmid. Additionally, this strain has a second attenuating mutation of the aerobactin (*iuc*) gene, which is involved in iron utilization and survival in the extracellular space (Hale, 1991). This deletion, a 4 Kb deletion encompassing the *iuc* chromosomal locus, was pursued based on the observation that certain auxotrophs are unable to multiply intracellularly, indicating that at least some nutrients are limiting. It has been demonstrated that aerobactin negative mutants grow in cultured cells similar to the wild-type strain, however, subtle differences in the virulence of these mutants can be detected in animal models. With a relatively small inoculum, an *iuc* mutant produces a delayed-positive Sereny test (guinea pig keratoconjunctivitis model for assessing *Shigella*/EIEC invasiveness) relative to wild-type strains. When the inoculum is increased the *iuc* mutant is nearly as virulent as the parent strain. Thus it appears that aerobactin facilitates multiplication of shigellae within tissues, but attenuation associated with the loss of siderophore expression can be

overcome if the initial challenge includes high numbers of organisms (Hale, 1991). Virulence can be restored by an 8 Kb complementation of the icsA gene, demonstrating that this is the primary attenuating mutation. It is highly unlikely that the entire *icsA* region could be reconstituted by recombination with the virulence plasmid from another enteroinvasive pathogen in the environment, since the virulence plasmid is not self-transmissible by conjugation (Sansonetti et al, 1982).

SC602 preclinical testing.

In 1992, a research seed of SC602 was acquired from the Institute Pasteur under a Material Transfer Agreement with WRAIR. In October 1994, lot 0070 was fermented and lyophilized under current Good Manufacturing Practices (GMP) in the Department of Biologics Research, WRAIR. Approximately 150 ampules of lyophilized lot 0070 remain in storage at 75°+/-5° C at WRAIR. Reconstituted ampules of lot 0070 have consistently yielded 10^{10} CFU /ml since manufacture.

Preclinical testing of lot 0070 was performed in 1995 using guinea pigs and rhesus monkeys. In another study, SC602 was evaluated for efficacy using the Sereny test (guinea pig keratoconjunctivitis model of *Shigella*/EIEC invasiveness). The Sereny test resulted in only mild irritation with no real signs of keratoconjunctivitis, which suggested significant attenuation of this vaccine strain. These data indicate that in the guinea pig model, SC602 was not reactogenic and was protective.

Echeverria and Hale (unpublished data,1995) conducted a safety and efficacy trial of lot 0070. The vaccine group consisted of 16 monkeys that ingested sodium bicarbonate followed by 10^{11} CFU of SC602; 8 controls ingested bicarbonate alone. Within the first 48 h 7/16 (44%) of the animals that received SC602 had liquid stools with mucus. The stool volume was not increased, and none of the animals had liquid stools for more than 24 h. Two of 8 control animals (25%) had liquid stools with mucus. All vaccinated monkeys shed vaccine for at least 3 days. Neither lethargy nor anorexia was observed in either group of animals. Challenge of naïve rhesus monkeys with 10^{11} CFU wild type *S. flexneri* 2a elicited dysentery in all animals, and was lethal for 1/3. Within the limits of the rhesus monkey model, these data demonstrated substantial attenuation of SC602, and the safety and colonizing ability of the lot 0070 product.

SC602 human trials in the United States.

Four phase 1 human inpatient clinical trials and one outpatient trial of SC602 have been conducted in adults in the United States at WRAIR. The first of the inpatient studies was conducted in 1995, testing increasing doses from 10^2 to 10^8 in groups of 3 volunteers each. No product-related reactions were seen at doses through 10^6 CFU, however, fever or moderate constitutional symptoms was observed in 2/3 volunteers receiving 10^7 CFU, and severe shigellosis occurred in 2/3 subjects receiving 10^8 CFU. These data suggested that the dose of 10^6 CFU effectively colonized, was not reactogenic and merited further study.

Safety and immunogenicity of a single 10^6 CFU dose was further evaluated in 15 volunteers, monitored on the inpatient ward for 2 weeks. All volunteers shed SC602 shortly after vaccination. Moderate to severe symptoms were seen in 67% of subjects including 2 with diarrhea and fever, 5 with diarrhea but no fever, 3 with fever and constitutional symptoms but no diarrhea, and 1 with constitutional symptoms but no fever or diarrhea. The 10^8 CFU dose was felt to be too reactogenic and a 3rd trial was conducted using a dose of 10^4 CFU.

The 10^4 CFU dose was given to 12 volunteers in the inpatient ward. All of the volunteers shed SC602 at levels of 10^5-10^6 CFU/gm stool. Only 1 volunteer reported any adverse events (mild to moderate systemic symptoms and diarrhea). Seven of 12 subjects demonstrated significant IgA antibody secreting cell stimulation following vaccination. These data therefore warranted further testing of efficacy of the 10^4 CFU dose.

The 4th study (Coster et al, 1999) readmitted 7 of the 12 volunteers who had received the 10^4 CFU dose 8 weeks after vaccination, and another 7 controls. All subjects were challenged with 10^3 CFU virulent *S. flexneri* 2a strain 2457T given with bicarbonate. None of 7 vaccinees experienced severe disease (diarrhea with fever, dysentery or shigellosis), whereas 6 of 7 controls did become seriously ill ($p=<.005$, 2-tailed Fisher Exact Test). Three vaccinees experienced mild transient diarrhea without other associated symptoms or fever. Similar levels of protection against experimentally induced shigellosis have never been reported in the scientific literature, spurring further studies.

These inpatient studies truncated vaccine colonization with antibiotic therapy prior to discharge. Further investigation of transmission risk was thus pursued in an outpatient study of 35 volunteers at WRAIR. Thirty-three subjects were given 10^4 CFU SC602 and 2 placebo in this study. Vaccinees and household contacts were followed for symptoms and fecal shedding for up to 60 days before administration of antimicrobials to truncate intestinal carriage if necessary. No secondary transmission to household members was documented. Time to first vaccine-positive stool was a mean of 65 h after vaccination (range 18-172 h), and vaccinees shed SC602 for a mean of 12 days (range 1-32). Twenty-one percent of persons reported symptoms after vaccination, including 4 (12%) with generalized aches or headache, 3 (9%) with fever (T>39C), 3 (9%) with diarrhea (>2 loose stools in 24 h), and 2 (6%) with abdominal cramps. One of those with fever, headache and generalized aches (but no diarrhea) also had evidence of upper respiratory infection at the time. Overall, symptoms were short-lived (lasting only a few h), well tolerated, and not always due to the vaccine. These data suggest that about 10% of people will have some change in bowel pattern, and that about 10% will have low grade fever, not necessarily associated with GI symptoms or related to the vaccine.

SC602 human trials in Bangladesh.

The WRAIR studies did not answer important questions about secondary transmission in developing countries where clean water and sanitation resources are limited or unavailable, and population density is high. Neither did they address the issue of appropriate dosing in populations from an area endemic for *Shigella*. Adults and older children from these populations were felt likely to require a higher dose of vaccine than unexposed populations, though nutritional deficiencies might conversely raise the risk of the vaccine. For these reasons, a series of inpatient and community based outpatient studies were conducted at the ICDDR,B research facility in Matlab, Bangladesh. These were conducted in adults and older children in order to establish the safety of the vaccine prior to study of the vaccine target population of 1-2 year olds at greatest risk for *Shigella*.

The Matlab studies were conducted sequentially, first in adults, then school age children (5-15 years old). Each age group was studied in a series of 4 sequential randomized, double-blind, placebo-controlled clinical trials. The first study was an inpatient trial designed to closely evaluate safety and fecal shedding of 3 dose levels of the vaccine (10^4, 10^5 or 10^6 CFU of SC602 or placebo) given in a single dose. Volunteers were followed up for 5 days, then given antimicrobial therapy on day 6 if shedding of vaccine strain continued. Stool, blood and urine were collected on days 0, 14 and 56 for immunoglobulin assays. Following the inpatient trial, 3 outpatient trials were conducted to evaluate each dose level individually. Household contacts of vaccinees were followed additionally for symptoms and fecal shedding of vaccine for up to day 56. Stool, blood and urine of vaccinees were again collected for immunoglobulin assays.

Inpatient trial: Adults

The first of these trials admitted 20 healthy adult Bangladeshi men 20-39 years of age to the Matlab field station hospital. They were randomized to receive a single oral dose of 10^4, 10^5 or 10^6 CFU or placebo with bicarbonate solution in a double-blinded trial. Follow up included daily interview and collection of all stools for culture and characterization. Malaise (40%), anorexia (60%) and mild abdominal pain (35%) were the most commonly observed symptoms. There was no clear dose-relationship, and no fever, diarrhea, dysentery or severe symptoms were observed. Short-term fecal excretion of SC602 occurred in a dose related fashion (0/5 in the 4 log group, 1/5 in the 5 log group, and 2/5 in the 6 log group). All doses were felt safe to continue to outpatient evaluation.

Outpatient trials: Adults

Sixty-eight Bangladeshi adults were divided into 3 groups studied in sequence according to dose level (lowest to highest). The first group consisted of 12 subjects who received SC602 at a dose of 4 logs CFU with buffer, 4 received vaccine without

buffer, and 4 received buffer alone. This design was repeated for the 5 log and 6 log CFU doses, except that 4 additional subjects were given buffer alone. Symptoms and fecal excretion of SC602 were monitored among vaccinees and their household contacts for up to 56 days. Diarrhea was not reported at any of the vaccine dose levels. Minor symptoms were commonly reported among most study participants, but there was no difference between vaccinees and controls and no dose-relationship. In the 6 log CFU dose group, 1 vaccinee had low grade fever, and 2 reported body aches. No increase in symptoms was seen among household contacts. SC602 was not isolated from any vaccinee, household contact or diarrhea case occurring in the neighborhood. The adult trials suggest minimal vaccine shedding, minimal reactogenicity, no transmission risk, and little immune stimulation in this *Shigella*-exposed population.

Inpatient trial: schoolchildren

Nine subjects age 5-15 years old were randomized 3 each to receive 10^4, 10^5 or 10^6 CFU. No severe symptoms, fever or diarrhea followed vaccination, though 1-2 loose stools occurred in 4 of 9, and abdominal pain in 2 of 9. All symptoms were mild, except 1 report of moderate abdominal cramps in a 6 log CFU recipient.

Overall, symptoms were not serious and not dose related. One subject passed a few drops of blood of unclear origin on day 5 with normal stool and did not excrete SC602. Stool microscopy revealed <10 WBC's per HPF, hookworms and other worms. Rectal examination was unrevealing. The passage of few drops of blood for a day was considered due to some local lesion, e.g., anal fissure.

Outpatient trials: School children

Four, 5 or 6 logs CFU or placebo were given to a total of 70 children. Vaccinees and household contacts were followed for symptoms and shedding. Once again, no diarrhea, dysentery or severe symptoms were experienced. Loose stools occurred in 15% of vaccinees, but 9% of controls. Abdominal pain was reported in 21% of vaccinees, but 14% of controls. Symptoms were otherwise not more common in vaccinees than in controls, and no dose relationship was apparent. Secondary transmission to household contacts was not demonstrated.

Signs and symptoms in inpatient school children:

Symptoms	4 log (n=3)	5 log (n=3)	6 log (n=3)	Total (n=9)
Diarrhea	0	0	0	0
Loose stool	0	3	1	4
Bloody stool	0	1	0	1
Nausea	1	0	1	2
Vomiting	1	0	0	1
Abdominal pain	1	0	1	2
Fever*	0	0	0	0
Headache	1	0	1	2
Body aches	0	0	0	0
Malaise	1	0	2	3
Joint pain	0	0	0	0
Gas	2	0	0	2
Anorexia	1	1	2	4
Others	2	0	0	2
Summary of symptoms				
Mild	3	3	1	7
Moderate	0	0	1	1
Severe	0	0	0	0
Any symptom	3	3	2	8

Signs and symptoms in outpatient schoolchildren:

	Dose(CFU)			
Symptoms (%)	4 log (n=16)	5 log (n-=16)	6 log (n=16)	Placebo (n=22)
Diarrhea	0	0	0	0
Dysentery	0	0	0	0
Loose stools	1	4	2	2
Abdominal pain	4	3	3	3
Headaches	5	2	3	3
Fever	1	0	0	2
Any mild	8	9	6	9
Any moderate	0	1	1	2
Any severe	0	0	0	0

In summary, the school children trials demonstrated SC602 to be well tolerated, with mild symptoms in about half, and moderate symptoms rarely. Loose stools and mild abdominal cramps may occur somewhat more commonly than in controls, though not remarkably so. Secondary transmission of vaccine was not evident.

The combined trials in adults and school children support further efforts to evaluate the vaccine in small numbers of children in the target population age range of 1-5 years. This study is designed towards that end.

Research Plan

STUDY DESIGN

A randomized, double-blind, placebo-controlled clinical trial is proposed, conducted in 4 phases. The first 3 phases will be inpatient trials directed primarily at determining the safety and optimal dose of SC602 in the target age group of 12-35 months old children. Healthy children will admitted in ICDDRB Matlab research facility after screening and taking consent. Children will be followed closely for symptoms, fecal shedding of vaccine, and immune markers. After 5 days of observation, they will be treated with an antimicrobial if shedding *Shigella* to truncate intestinal colonization prior to discharge. The volunteers will be requested to come to Matlab on days 14 and 28 for submission of blood and stool specimens. On the basis of these studies, the optimal dose in this age group will be chosen. The outpatient trial will follow, in 12-35 months old children, and providing only the single dose chosen in the inpatient trials, or placebo. The additional intent of the outpatient trial is to assess risk for secondary transmission from vaccine recipients to household contacts. These trials will administer vaccine or placebo to 78 children, and monitor about 100 household contacts for secondary transmission. These studies will form the basis for consideration of further safety and/or efficacy trials of SC602 in this population.

Number of subjects enrolled in the trials

Trial	Vaccine	Placebo	Household contacts	Dose
Inpatient #1	12	6		10(3) or 10(4) CFU
Inpatient #2	12	6		10(4) or 10(5) CFU
Inpatient #3	12	6		10(5) or 10(6) CFU
Outpatient	16	8	100	To be determined
Totals	52	26	100	

1. Inpatient trial #1:

The first inpatient trial will admit 18 healthy children age 12-35 months of age with a caretaker, to the ICDDR,B clinical treatment and research facility in Matlab, Bangladesh. Baseline stool and serum specimens will be collected for stool culture and

characterization, and fecal and serum immunoglobulin studies. Children will be randomized to 3 dose groups: 6 will receive a single oral dose of 10^3 CFU SC602 with bicarbonate buffer; 6 will receive 10^4 CFU with buffer; and 6 will receive a placebo (sterile water) and buffer. Close daily monitoring of symptoms and physical examination will be conducted. All stools will be collected for culture and characterization. On day 6, all children will be given antimicrobial therapy prior to discharge. Health workers will collect stool and serum specimens for immunoglobulin assay during home visits on days 14 and 28 following vaccination. Secretory IgA specific for *S. flexneri* 2a LPS will be assayed in stool samples. Anti-*S. flexneri* 2a LPS IgA and IgG will be assayed in serum samples.

Inpatient trials data/specimen collection

Study Day	0	1	2	3	4	5	6	14	28
Vaccinate	X								
Symptoms	X	X	X	X	X	X	X		
Stool cx	X	X	X	X	X	X	X		
Stool/Blood for Ig	X							X	X

2. Inpatient Trial #2

Approximately 2 weeks after the 1st trial is begun, the 2nd inpatient trial will again admit 18 children with their caretaker to the ward. The chosen dose(s) will be administered to 12 children (6 each if 2 doses), and placebo to 6. Children will be followed similarly to trial 1. Stool specimens for culture, and stool and serum specimens for Ig studies will be collected according to the same schedule as in the 1st trial.

Interpretation of optimal dose to be administered in the 2nd inpatient trial will be based on analysis of rates of fecal shedding of SC602, and on rates and severity of symptoms demonstrated during follow up in this 1st trial. The possible doses administered in the 2nd trial will be 10^3, 10^4 or 10^5 CFU.

Inpatient Trial No. 2 Dose Selection Guidelines:

% Shedding Trial 1	**Symptoms Trial 1**	**Dose 1 (CFU)**	**Dose 2 (CFU)**
<34%	Minimal	10^5	-
<34%	Mild	10^4	10^5
<34%	Mod (few subjects)	10^4	-
<34%	Mod (many subjects)	Stop study	-
35-67%	Minimal - mild	10^4	10^5
35-67%	Mod (few)	10^4	-
35-67%	Mod (many) or Severe (any)	Stop study	-
>68%	Minimal-mild	10^4	10^5
>68%	Mod (few)	10^3	10^4
>68%	Mod (many) or Severe (any)	Stop study	-

3. Inpatient Trial #3:

If considered to be necessary based on the absence of significant fecal shedding/symptoms in 10^5 CFU recipients, then a 3rd inpatient trial of 10^6 CFU will commence. The design will be identical to the earlier trials, though 10^5 and/or 10^6 CFU will be given to 12 subjects, and placebo to 6. Baseline studies and follow up procedures will be identical. The optimal dose to be administered in the outpatient trial will be determined from the combined data from the inpatient trials.

Inpatient trial No. 3 dose selection guidelines:

% Shedding Trial 2	Symptoms Trial 2	Dose 1	Dose 2
<34%	Minimal	10(6)	-
<34%	Mild	10(5)	10(6)
<34%	Moderate (few)	10(5)	-
34-67%	Minimal-mild	10(5)	10(6)
34-67%	Mod (few)	10(5)	
34-67%	Mod (many), or Severe (any)	Proceed to OP trial with 10(4)	
>68%	Minimal or mild	Proceed to OP trial with 10(5)	

4. Outpatient Trial:

The outpatient trial will enroll 24 children from neighborhoods proximal to the clinical facility, vaccinating them approximately 3 weeks after beginning the 3rd inpatient trial. Additionally, household contacts of these children will be recruited and followed for intestinal symptoms and fecal shedding of SC602. Baseline stool and serum specimens will be collected from vaccine recipients, and processed as before.

Children will be randomized into 2 groups; 16 will receive the single best dose of vaccine identified in the inpatient trials (with buffer), and 8 will receive placebo with buffer. Every day for the first 5 days following vaccination, a study physician will visit the children at home, record symptoms and signs of illness, and perform directed physical examination. Symptoms in household contacts will be recorded as well. A health worker will collect stool samples from vaccinees on days 2, 4, 6, 8 and 10 for culture and characterization. If persistently culture-positive *for Shigella flexneri* on day 10, stools will continue to be collected each 3 days until 2 consecutive stools are culture-negative or until day 28, at which time antimicrobial therapy will be administered to truncate intestinal carriage. Household contacts will have stool collected on days 6 and 10 for culture, and will be asked about intestinal symptoms since the last visit. Stool and serum specimens for immunoglobulin assays will be collected on days 14 and 28 from vaccine/placebo recipients.

Diarrhea occurring in non-enrolled children < 5 years old from neighborhoods of vaccinees, who seek treatment at the clinical center, will be routinely cultured for the

vaccine strain in addition to the usual pathogens sought. Study subjects developing intestinal illness during the trial will have a stool specimen collected for culture prior to institution of therapy. Diarrhea treatment will follow standard WHO guidelines.

Outpatient trial symptom/specimen collection schedule:

Day	0	1	2	3	4	5	6	8	10	14	28
Vaccinees											
Symptoms	X	X	X	X	X	X					
Rectal swab cx	X		X		X		X		X		
Stool/Blood for Ig	X									X	X
HH Contacts											
Symptoms	X	X	X	X	X	X	X		X		
Rectal swab cx							X		X		

STUDY SUBJECTS

Population.

The trial will be carried out at the Matlab field research area of ICDDR,B. The field area was originally developed for field evaluation of cholera vaccines. To support field studies, a Health & Demographic Surveillance System (HDSS) was established in 1966. Over the years, the system has been refined and is currently operational in 142 villages containing about 210,000 population. An average village under surveillance has about 250 households divided into clusters of households (neighborhoods or *bari*); typically *baris* are comprised of 5-8 households. The HDSS gathers vital event information, such as births, deaths, and migrations on a regular basis through home visits. The demographic database is computerized and updated regularly.

Since the inception of work in the Matlab area, a diarrhea hospital has been in operation at Matlab to treat diarrhea patients from the locality. In addition to Matlab hospital, three community-operated diarrhea treatment centers also provide treatment to diarrhea patients. Oral rehydration therapy (ORT) and referral services for diarrhea are provided in the community by a cadre of community health workers (CHW -- one per 1,800 population in half of the area and one per 3,000 population in the remaining area) and *Bari Mothers* (these are community volunteers who serve as depot-holders of ORS packets, distribute ORS and provide advice to diarrhea patients -- there is one for about 50 persons).

Matlab is fairly representative of most parts of rural Bangladesh. In 1995, the crude birth rate and the crude death rate were 26.5 and 7.9 per 1000 populations and infant mortality rate was 65.3 per 1,000 live births. Although there has been a decline in diarrheal death rates over time, the rates are still high. In 1995, diarrhea accounted for about 12% of infant deaths and about a third of all deaths in children 1-4 years of age. When diarrheal deaths were dis-aggregated into deaths due to acute watery diarrhea, dysentery or persistent diarrhea, it became evident that dysentery and persistent diarrhea were more important causes of deaths than acute watery diarrhea in children over one year of age. Little data is available on older age groups from the Matlab area,

however in those < 60 months, *Shigella* incidence peaks from 18 to 42 months of age, declining to less than half of the peak rates between 43 and 60 months. The vaccine being tested is thus intended for use in the target population of children < 5 years of age in diarrhea endemic countries, and in adult and child travelers to those areas.

Age range and gender.
Gender will not be a selection criterion. Age range will be 12-35 months of age for both inpatient and outpatient trials. The age group most likely to benefit from this vaccine. *Shigella* incidence in this population is very low prior to 12 months of age, rises after that to peak at 18-42 months, then drops off to 25% or so of peak levels by 48 months. Though rates are lower before 12 months, severity of disease is greater. Age <12 months has been identified as a risk factor for mortality in cases of *Shigella*-associated diarrhea. This very young group will be excluded in this study because the vaccine is a living attenuated strain, and safety has not yet been demonstrated. After 36 months of age, most children in this community will have been exposed to *Shigella*, have some degree of immunity. Baseline Ig levels at this age are likely to be higher, and post-vaccination titers less likely to rise significantly compared to younger children. Given the small sample size, including children older than 36 months may then underestimate both risk and immunogenicity in the younger target population. The inpatient trials primary goal is to evaluate safety, immunogenicity and dose. The outpatient trial will focus on transmission risk.

Selection method.
Villages from the Matlab DSS area that are proximal to the clinical facility will be selected for the study. *Baris* of average size (5-7 households) will be identified from the selected villages. The Heads of villages in which the baris belong will be approached by one of the study investigators for permission to recruit subjects from among his village for participation in the trial. If permission is provided, the representative of the chosen *bari* will be approached for his approval as well. Once this is accomplished, parents of children in the target age range will be approached for consent to screen their child for inclusion in the study. Written consent will be obtained prior to proceeding with screening.

Inclusion criteria.
Age: 12-35 months.
Baseline bowel habits: normal. $\geq$ 3 stools per week; $\leq$ 3 stools per day.
Parent/guardian has good understanding of the study, subjectively determined after study briefing and individual discussion.
Child indicates assent to participate when questioned.
Parent/guardian willing to follow guidelines of the study.
Outpatient trial: At least 50% of household contacts must be willing to participate in monitoring for secondary transmission.

Exclusion criteria.
Age <12 months or >35 months.
Current or recent diarrhea (within 2 weeks of vaccination).
Chronic gastrointestinal disease
Current use of stomach acid neutralizers
Need to take medication containing zinc/iron during the period of study
Use of antibiotics within 7 days of dosing
Known or suspected clinically significant illness
Allergic to available antibiotics
Febrile within 48 h of vaccination
Outpatient trial: Fewer than 50% of household contacts willing to participate in monitoring for secondary transmission.

Subject identification
Following collection of baseline biographic data, a unique study identifying number will be assigned to each volunteer, which will be utilized in identifying all data collected on that subject and in the study data base.

SAMPLE SIZE
Four small trials will be conducted in sequence as part of this protocol. Up to three inpatient trials will be followed by an outpatient trial. The inpatient trials will enroll 18 subjects each, of whom 12 will receive vaccine and 6 placebo. The outpatient trial will enroll 24 subjects, providing vaccine to 16 and placebo to 8. Additionally, as part of assessment for secondary transmission risk, household members will be recruited for participation in a minimal risk aspect of the outpatient trial, collecting only 2 stool samples and symptom responses. No medication or vaccine will be provided to this population, unless symptomatic intestinal disease is apparent and/or the vaccine strain is cultured from their stool. In that case, standard antibiotic treatment will be provided. Assuming 5 household members per vaccinee in the outpatient study, about 100 household members will be enrolled.

As a phase 1 trial in young children, sample size considerations are relatively less important than safety concerns. Our sample size has been determined based on three considerations:

We feel an ethical obligation to perform the trial in as safe a manner as possible. That is why we escalate the dose sequentially in the inpatient studies, providing only the very lowest doses initially, rather than expose all subjects at once. That is also why we expose the fewest number of volunteers possible.

Logistical considerations. How many volunteers are available to participate, and how many can be accommodated and monitored adequately in the facilities at our disposal?

Statistical considerations. With at least 12 volunteers receiving the chosen dose, and

20 placebo, if none of the placebo recipients and a third of the vaccinees experience an adverse event, there will be >80% power to detect this difference with >95% significance level by Fisher Exact Test. Similarly, if none of the placebo recipients sero-convert, and at least one third of the vaccinees at the chosen dose sero-convert, we will be able to detect that difference with >80% power and >95% significance by Fisher Exact Test.

OUTCOME VARIABLES

Clinical

Adverse events in study subjects.
Secondary transmission of SC602 to household contacts of vaccinees.
Adverse events in household contacts of vaccinees.

Laboratory

Occurrence and duration of fecal shedding of the vaccine strain.
Immune response as measured by changes in anti-*S. flexneri* 2a LPS secretory IgA in stool, and IgA and IgG in serum.

DATA COLLECTION METHODS

Baseline

Baseline socio-demographic data and laboratory specimens will be collected during the week prior to vaccination. The laboratory assays will include serum (about 4 ml), stool and urine for Ig assays; and stool for O&P and culture. Forms for these data are included in appendix.

Follow up

Inpatient trials. Following vaccination, study subjects will be monitored every day until discharge by study physicians. Information to be obtained during follow-up will include vital signs, responses to an interview regarding symptoms experienced by the subject during the last 24 h, and a physical examination of the study subjects. Laboratory follow up will include culture and characterization of all stools each day until discharge. Discharge will be allowed beginning on day 6 if at least 2 sequential stools are culture negative for SC602. All subjects will receive antibiotics on day 6 prior to discharge. For those who are culture positive for the vaccine, they will be retained on the ward until 2 sequential culture negative stools are received, but in no case longer than 10 days after vaccination. If still shedding SC602 72 h after initiating antimicrobial therapy, a different antibiotic will be given, and the first drug discontinued. On days 14 and 28, subjects will be asked to provide stool and serum collection for Ig assays.

Oupatient trial. Following vaccination study subjects will be monitored daily for 5 days by the teams of one physician and one CHW. Information obtained will again include vital signs, physical exam and responses to a symptom interview. Additionally, symptoms occurring in household contacts will also be sought out and recorded. Beginning on day 6, subjects will be visited every other day by a team of one health assistant (HA) and one CHW who will collect data on adverse events in the subjects

and their contacts using a pre-tested questionnaire. After day 10 until conclusion of the study on day 28, these teams will continue to visit vaccinees every 3 days only if the stool is persistently culture-positive for SC602. Teams will visit all vaccinees on days 14 and 28 for stool and serum collection for Ig assays.

Specimens collected
Blood: 3 samples of 4 ml each will be collected in total; one sample at baseline and the others on days 14 and 28 respectively for Ig assays. Specimens will be collected in clotting tubes by experienced personnel, labeled as to name, date, study day and study ID. Specimens will be stored at 4 degrees C until transport to ICDDR,B Dhaka for processing.

Stools: All stools from inpatients will be collected. Rectal swabs from outpatient vaccinees will be collected every other day to day 10 and each 3rd day thereafter to day 28 if *S. flexneri* culture positive. All vaccinees will also have stool specimen collected at baseline and on days 14 and 28 for secretory IgA assay. Household contacts will have a rectal swab collected on days 6 and 10. Each volunteer and household contact will receive a reusable stool container for collection of specimens. Specimens will be taken to the laboratory and processed within 2 h of collection for culture and characterization. A 1 gm sample of the first stool collected each day from inpatients will be stored for later PCR processing for *S. flexneri* gene sequences. All *S. flexneri* isolates will be saved and tested for the SC602 gene deletion by PCR.

MEDICATIONS USED
Vaccine and buffer agents:
Lyophilized ampules of SC602 were manufactured and stored at Forest Glen (Bldg 501) of WRAIR, USA. The ampules will be transported on dry ice to ICDDR,B and maintained at -70+/-5 degree C until used in the vaccine study. At each prolonged stop during transportation of the vaccine, the container will be checked to verify that adequate dry ice is present and that the vaccine is frozen. A signature page will be utilized indicating name, date, time and condition of the vaccine whenever a change of possession occurs.

The vaccine received from the sponsor will have the following label:

> *Shigella flexneri* 2a SC602, Production Record No. BPR-047-00, volume 5.0 ml
> Lot No. 0070, Date Manufactured 10/21/94, Storage B70+/-5C
> Manufactured by WRAIR, Washington DC 20047
> Caution: New Drug: Limited by Federal Law to Investigational Use

There is no expiration date for this vaccine. WRAIR continues to monitor CFU. To date, there has been no change.

On the day of vaccination, frozen SC602 lypholate will be transported on dry ice from storage at ICDDR,B Dhaka to the vaccination site in Matlab. The contents of the

ampule will be reconstituted in cold sterile deionized water, and the ampule will be placed on wet ice for about thirty minutes. The concentrated reconstituted vaccine will be diluted with enough sterile saline to make the desired concentration. Replica spread plate quantitative cultures will be made of the inocula before and after vaccination to confirm viability and inoculum size. The reconstituted vaccine inoculum will be maintained on wet ice until vaccination. The buffer solution concentration represents 2 g NaHCO3 dissolved in 150 ml sterile deionized water. Vaccine and buffer will be prepared with appropriate labeling. The vaccination details will be held by the Study Monitor in a randomization sheet which will include the following: volunteer name, study ID#, date, vaccine name, route, dose given (CFU), IND#, FY#, Lot#, Prod#, manufacturer and vial#.

The vaccine will be reconstituted and doses prepared as appropriate in individual cups. One ml of vaccine solution at the appropriate concentration will be mixed in 30 ml sterile water for ingestion. Sterile water (30 ml) without vaccine will serve as the placebo, also placed in similar cups. There is no perceptible difference in taste of the 2 solutions (personal communication, Dr. Hale), thus blinding will be maintained. Bicarbonate solution will be prepared and volume of ingestion determined according to a schedule based on body weight (appendix). This will be placed in a second cup. Volunteers will be randomized and vaccine doses assigned by an investigator not involved in clinical care of volunteers. The record of randomization will be maintained in a locked cabinet, unavailable to investigators until recording and analysis of data has been completed.

At the indicated time, all subjects will be gathered together and doses distributed. The bicarbonate will be ingested first, followed one minute later by the vaccine/placebo. Immediate adverse events will be recorded after 1 hour. Subjects will then be returned either to the ward (inpatient trials) or will be allowed to return home (outpatient trial).

All unused vaccine strain will be accounted for and stored at -70 +/- 5C at ICDDR,B and then either destroyed by incineration or returned to WRAIR for storage. All reconstituted vaccine and culture plates will be bagged and tape-sealed in a hazardous waste container until disposed of by autoclave.

Antibiotics & ORT

Inpatient trials: All subjects will be treated with nalidixic acid (55 mg/kg/day orally in 4 divided doses for 5 days) beginning on day 6, and discharged when 2 consecutive stools are negative by culture for *Shigella*. If stools are persistently positive for *Shigella* after 72 h of nalidixic acid therapy, an alternative antibiotic will be chosen. The vaccine strain is sensitive to all standard antimicrobial agents including quinolones, ampicillin and trimethoprim-sulfamethoxazole, all of which are available for use in Matlab. Additionally, for subjects that have symptoms of intestinal infection, medical management will follow standard WHO guidelines, including oral rehydration therapy (ORT) and antibiotics as appropriate. The antibiotic of choice for symptomatic subjects will again be naladixic acid.

Outpatient trial: Vaccinees and household contacts symptomatic of intestinal infection during the study period (28 days of follow up) will be treated according to WHO guidelines as referred to above. Additionally, vaccinees persistently culture positive for *S. flexneri* on day 28 of follow up, will be treated with naladixic acid to terminate intestinal colonization. Household contacts culturing *S. flexneri* from stool samples at any time will also be treated with naladixic acid. Adult household contacts will be treated with a dose of 1 gm orally every 6 h for 5 days; children will be dosed as above.

Oral rehydration therapy will be started as soon as subjects develop significant diarrhea or have signs or symptoms suggestive of volume depletion. Subjects who cannot adequately be rehydrated with oral fluids will be treated with intravenous solution at the ICDDR,B Matlab Hospital.

LABORATORY METHODS

SC602 Shedding:

Inpatient trials: All stool samples will be collected for culture and characterization. Stools will be brought immediately after collection to the laboratory, and evaluated for weight, gross appearance of blood or mucus and consistency. Loose stools or stools with gross blood will have microscopy for evaluation of pus cells and RBC=s. Stools will be cultured by two methods. A streak plate of the original specimen will be performed on both hektoen enteric agar (HEA) and *Salmonella-Shigella* agar (SSA). Additionally once daily, 4 stool swab specimens will be placed in 10 ml saline (approximately 1:10 dilution), from which 100 ul will be resuspended in another 10 ml saline (1:1000 dilution). 100 ul of each suspension will be spread plated onto HEA (a -4 log dilution plate) for counting. Dilutions may be adjusted based on early counts. All plates will be incubated at 37C for 24 B 48 h. Non-lactose fermenting colonies resembling *Shigella* will be tested biochemically then serologically (using poly Group B antisera) to identify *S. flexneri.* Isolates of *S. flexneri* will be inoculated onto nutrient agar slants and maintained at room temperature for subsequent identification by PCR of the SC602 deletion. Absence of typical *Shigella* colonies will be considered prima facie evidence of an absence of SC602 shedding.

Outpatient trial: Rectal swab specimens will be collected from vaccinees and household contacts. Swabs will be placed into Cary Blair transport media and maintained on wet ice until culture. Specimens will be streak-plated within 6 hours of collection onto HEA and SSA, then incubated 24-48 hours. NLF colonies will be identified and tested as above for *S. flexneri*, then those identified isolates processed further by PCR to identify the vaccine strain.

Immunogenicity:

On days 0, 14 and 28 about 4 ml blood will be drawn for anti-*S. flexneri* 2a LPS IgG and IgA determinations by ELISA. Stool specimens will also be collected on those days and tested for secretory IgA by ELISA.

Antigen preparations: LPS will be used as the target antigen in this study. LPS from *S. flexneri* 2a will be extracted by the phenol-water method from batch-grown bacteria as described by Luderitz *et al.* The LPS will be purified by treatment with proteinase K and DNAse.

Determination of total immunoglobulins: t-IgG, t-IgA concentrations in sera and t-IgG concentrations in fecal extracts will be measured by nephelometry with reagents and a nephelometer (Beckman Instruments Inc. Galway, Ireland).

EIA analyses: All enzyme immunoassay (EIA) results related to antigen-specific responses will be expressed as relative titers, which will be defined as optical density (OD) multiplied by the dilution factor of the sample. The EIA method is described in brief:

EIA plates (Costar Corporation, Cambridge, Mass.) will be coated overnight with antigens in 0.1 M carbonate-bicarbonate buffer (pH 9.6) at 22ÊC. The plates will be saturated with PBS with 0.05% Tween 20 (PBST) and 1% bovine serum albumin (PBST-BSA). Fivefold dilutions of serum or fecal samples in PBST-BSA will be incubated at 22ÊC overnight. Enzyme-linked secondary antibodies will be incubated for 4 h at 22ÊC. Between the various incubation steps, the plates will be rinsed three times with PBST. The enzyme reaction will be developed for 100 min after addition of the substrate p-nitrophenyl phosphate (Sigma), and the OD will be measured at 405 nm. Multiple samples from the volunteers will be always tested in the same plate. The mean OD observed when the sample is replaced with PBST-BSA will define the background level.

Antigen-specific IgG and IgA subclasses of antibodies in serum: In brief, EIA plates will be coated with (i) LPS (10 Fg/ml) (in Nunc MaxiSorp plates [A/S Nunc, Roskilde, Denmark]). Serum samples will be applied. As secondary antibodies, biotinylated murine anti-human IgG1 (8c/6-39; Sigma), IgG2(HP-6014); Sigma), IgG3(HP-6050; Sigma), IgG4 (HP-6025; Sigma), IgA1 (A1-18; Sigma), IgA2 (A9604D2; Southern Biotechnology, Birmingham, Ala.), will be used. After incubation and washing, the plates will be incubated with avidin-alkaline phosphates (Sigma) in a 1:4,000 dilution.

Antigen-specific fecal s-IgA antibodies: Diluted fecal samples will be added to the wells of plates coated with the antigen (LPS) (similar to that for serum), and after incubation, a rabbit anti-human secretory component conjugated to horseradish peroxidase (1:3,000 dilution) (Dakopatts, Copenhagen, Denmark) will be used. The enzyme reaction will be developed by adding the substrate (o-phenyline diamine [Dako] in 0.1 M citrate-phosphate buffer containing (0.005% H_2O^2 pH5.0), and the OD will be measured at 492 nm.

Fecal t-IgA and s-IgA concentrations: Quantitation of IgA and S-IgA will be also done by EIA. Fecal samples will be added to the plates coated with anti-human IgA (á chain specific), or anti-secretory component (Dako). After incubation, an alkaline

phosphatese-conjugated rabbit anti-human IgA (1:1,000 dilution) will be added. The reaction product will be developed as described above. OD readings for the standard curve and samples will be analyzed by using a four-parameter model in the Delta Soft II program (Bio Metallics, Inc. Princeton, N.J.). The results will be expressed as grams per liter of original stool volume.

QUALITY ASSURANCE

Data

To ensure data quality, the study supervisors and investigators will make random spot checks of data forms, including the clinical record, baseline and follow up lab collection form checklist, laboratory results form, household contact lab data collection form and household contact questionnaire. In addition, a 5% sample of study subjects will be re-interviewed and re-assessed within one day of the original interview/assessment.

All questionnaires and data forms will be reviewed by one of the investigators for accuracy, consistency and completeness. Whenever necessary, the CHW/HA will make additional field visits to clarify inconsistencies or collect missing information. After editing, the data will be double entered in databases using on-line custom designed data entry programs. Necessary range and consistency checks will be built in. Data will be periodically checked by running and reviewing frequency distributions and cross-tabulations.

Laboratory assays

Standard operating procedures (SOPs) and quality control measures will be operative for all laboratory assays, equipment and reagents.

DATA MANAGEMENT

Disposition of data

Data will be double-entered into a custom-designed computer data base specific for this trial at the ICDDR,B. Original data collection instruments will be maintained there as well. Copies of original instruments and summarized data tables will be disseminated to the investigators as necessary to complete analysis, presentation and manuscript preparation.

2. Analysis

Baseline characteristics of the subjects receiving vaccine and placebo will be examined for group comparability. Any significant baseline differences will be controlled for during data analysis. The frequency distribution will be examined to assess the distribution of data.

The data from the vaccine and placebo groups will be compared to determine: a) whether vaccination was associated with increased occurrence of adverse events; b) the extent of secondary spread of the vaccine strain; c) whether there was any vaccine induced outbreak in a bari or neighborhood; and d) the immune response to the vaccine.

The titer of a serum ELISA is the reciprocal of the last dilution with a positive optical density (OD). To show that an individual has responded to the *Shigella* vaccine, a 3-fold increase in titer over the day 0 serum is required for any subsequent sample. In the primary analysis of stool or serum ELISA data, a significant LPS antibody response will be defined as a 3-fold or higher increase in titer after vaccination. In a secondary analysis, the geometric mean titer plus 3 standard deviations of the day 0 samples will be used as a cutoff for significance of antibody response after vaccination.

Use
Combined assessment of shedding, safety and immunogenicity data will serve as the basis for consideration of further investigation of SC602 in expanded safety and efficacy studies of SC602. If personal and community safety, and immunogenicity are demonstrated, then a subsequent protocol will be considered to further evaluate field protective efficacy and safety in larger numbers of children.

MODIFICATIONS TO/DEVIATIONS FROM THE PROTOCOL
Minor modifications necessitated during the course of the trial, will be made on site as needed, and documented for subsequent review by the chairman of the research review committees at the respective institutions within a reasonable time period. Important methodologic changes that are emergent in nature will be made on site, but the research review committees will be notified within 72 h by oral communication, with written communication following as soon as is reasonably possible thereafter (no later than 2 weeks). Important but non-emergent changes to the protocol will be detailed in writing to the research review committee chairman at least 1 week in advance of the change, with oral communication necessary concomitantly. Incorporation of non-emergent changes will be dependent upon preliminary approval of the changes by the research review committee chairman. If it is felt by the chairman that full committee review is indicated, the trial will be placed on hold until such approval can be granted by the committee, or until 30 days have passed. After 30 days, approval to proceed will be assumed.

Ethical Assurance for Protection of Human Rights

SAFETY MONITORING COMMITTEE
The administration of subsequent doses following the initial inpatient trial will be contingent upon the safety record of the doses, in combination with fecal shedding data. Prior to proceeding with the outpatient trial, an assessment of safety will be made by an independent Safety Monitoring Committee comprised of senior professionals from within and outside ICDDR,B who are in no way involved in the trial.

Causal relationship of vaccine to symptoms will be determined individually, however a causal relationship will generally be implied in vaccinees if temporally associated during the period of observation, regardless of whether or not SC602 is culturable from the stool. In household contacts, the presence of SC602 cultured from the stool will be necessary to establish causality with the above symptoms.

SIGNIFICANT HEALTH EVENTS

A significant health event occurring during the surveillance period of the study will be defined in the two categories denoted below. These events will be immediately (within 24 h) reported to the Chairman of ICDDR,B Ethical Review Committee and the USAMRMC Deputy Chief of Staff for Regulatory Compliance and Quality (301) 619-2165 (non-duty hours call 301 619-2165 and send fax to 301 619-7803). A written report will follow the initial telephone call within 3 working days. Address the written report to the US Army Medical Research and Materiel Command, ATTN: MCMR-RCQ-HR, 504 Scott St., Ft. Detrick, Maryland 21702-5012.

Serious adverse experience:

A serious adverse experience is any event that is life-threatening or permanently disabling.. An adverse event is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Unexpected adverse experience:

Any adverse event that is not identified in nature, severity or frequency in the current investigator=s brochure or general investigational plan.

RISKS AND BENEFITS TO VOLUNTEERS

Signs and symptoms associated with the ingestion of the vaccine may include abdominal cramping, diarrhea with or without blood/mucus, fever, malaise, muscle and/or joint aches, headache, tenesmus, nausea and vomiting. In previous studies at the same dose, these signs and symptoms were generally mild or did not occur. Reactive arthritis due to *Shigella flexneri* has not been demonstrated in Bangladesh and is felt to represent an extremely low risk, nevertheless, should sterile inflammatory arthritis develop in any vaccinee, HLA B27 testing will be performed. Arthritis has never been documented in any SC602 vaccine recipient in either US or Bangladeshi residents. Mild gastrointestinal symptoms may also be experienced with ingestion of the bicarbonate buffer alone. Clinical dysentery (bloody diarrhea with fever) will be considered as a severe adverse event. All other signs/symptoms mentioned will be graded as mild, moderate or severe.

Nalidixic acid is generally well tolerated, but nausea, vomiting and abdominal pain may occur. Allergic reactions such as pruritus, urticaria, various rashes, photosensitivity, eosinophilia and fever occasionally occur and cholestasis, thrombocytopenia, leukopenia and haemolytic anemia rarely occur (Goodman and Gilman, 1996). Co-trimoxazole (trimethoprim-sulfamethoxazole) and ampiciliin/amoxicillin are also well-tolerated; the most frequent adverse reactions associated with their use include diarrhea, nausea and abdominal pain. Headache and dizziness occur occasionally, as do hypersensitivity reactions.

There is also the risk of pain, hematoma, or infection at the site of venopuncture.

Recipients may benefit from increased surveillance for diarrheal disease. Diarrhea is highly prevalent in Matlab, and study participants may very well become infected with other wild-type diarrheal pathogens during the course of this study. Diarrhea is eminently treatable in most cases when identified early in the course of disease, however much morbidity and mortality still occur in part due to failure to be promptly evaluated and treated. Monitoring as planned in this study is likely to recognize diarrhea earlier than may otherwise be the case, and thus allow initiation of prompt and appropriate therapy.

MEASURES TAKEN TO MINIMIZE RISK

As mentioned previously, a Safety Monitoring Committee will review the inpatient safety data prior to proceeding with the outpatient trial. In addition, a study monitor will review data after each trial and must concur with study physicians that the planned dose is safe prior to continuing.

All vaccinees will be monitored closely by study physicians for at least 5 days after vaccination. Adverse events reflective of gastrointestinal infection will be promptly addressed according to standard management criteria delineated by WHO guidelines. Non-gastrointestinal illness will be managed according to the standard of medical care provided in Bangladesh. The ICDDR,B treatment center already provides routine medical care for residents of Matlab, and will continue to do so for volunteers in this trial. Adverse events will be reported promptly as per previous discussion of significant health events.

Should an outbreak of symptomatic diarrhea occur in the *baris* of vaccinees, with SC602 isolated from those subjects, the study will be discontinued and all symptomatic subjects will be offered treatment with antimicrobial agents.

Adverse reactions to antimicrobial agents will be managed with discontinuation of the drug, and institution of a drug from another class to which the vaccine is sensitive if necessary.

MEDICAL CARE AND EQUIPMENT REQUIRED

Medical care of gastrointestinal infection with shigellae is expertly managed by staff of the ICDDR,B treatment facility. No special equipment is required for this study.

Literature Cited

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Title of Project: Safety, dose, immunogenicity and community transmission risk of a candidate *Shigella flexneri* 2a vaccine (SC602) among young children in rural Bangladesh

Budget Period: April 2000 to March 2001

SALARY

Position	Pay Level	# of Staff	% of effort	Monthly Rate	Man/ Month	Sub-total	Total (US $)
Dr. Abdullah H. Baqui	P-5/7	1	20%	11,356	12	27,254	
Dr. Md. Yunus	NO-D/21	1	5%	2,125	12	1,275	
Dr. Shams El Arifeen	NO-C/7	1	20%	1,307	12	3,137	
Dr. K. Zaman	NO-C/11	1	20%	1,382	12	3,317	
Mr. J. Chakrabarty	NO-C/10	1	5%	1,370	12	822	
Dr. Mahbubur Rahman (Microbiology)	NO-C/7	1	20%	1,220	12	2,928	
Dr. Rubhana Raquib (Immun)	NO-C/1	1	10%	1,025	12	1,230	
Dr. Hafizur Rahman	NO-C/1	1	10%	1,125	12	1,350	
Programmer	GS-6/2	1	50%	505	12	3,030	
Field Research Officer	GS-5/1	1	100%	379	6	2,274	
Research Technician (Microbiology)	GS-4/1	1	100%	287	6	1,722	
Research Technician (Immun)	GS-4/1	1	50%	287	6	861	
Data Manager	NO-A/6	1	10%	744	12	893	
DMA Gr I	GS-4/1	1	50%	287	6	861	
Project Office Manager	NO-A/2	1	10%	704	12	845	
Admin Assistant	GS-4/1	1	20%	287	6	344	
Research Physician	Spl Lvl	2	100%	280	5	2,800	
Senior Health Assistant	GS-4/1	1	50%	287	5	718	
Health Assistant	GS-3/1	2	100%	247	5	2,470	
Health Assistant	GS-3/1	4	100%	247	1	988	
Office attendant	GS-1/1	1	100%	185	12	2,220	
Field Workers	Spl Lvl	2	100%	82	5	820	
Field Workers	Spl Lvl	4	100%	82	1	328	
SUB-TOTAL:							62,487

TRAVEL COSTS

Conveyance cost	1,160	
SUB-TOTAL:		1,160

SUPPLIES & OTHER COSTS

Vaccine	-	
Office & field supplies	1,000	
Communications	500	
Printing & Publications	500	
SUB-TOTAL:		2,000

INTER-DEPARTMENTAL SERVICES

Compensation for wage lost and conveyance of outpatient volunteers			2,500
Hospitalization costs for inpatient study including compensation to volunteers			5,400
Supplies and other costs for microbiological studies including PCR testing (Estimated number of R/S or stool culture for shigella is 4,500)			10,000
Stool microscopy	120	1.22	146
Blood CBC	120	2.30	276
Blood, stool and Urine Ig assays including supplies			12,450
Land Transport for Matlab			435
Water Transport for Matlab			435
Guest house costs - Matlab			632
Service charges (DW/CSA)			1,000
SUB-TOTAL:			33,274
TOTAL DIRECT COSTS:			98,921
OVERHEAD @25%:			24,730
TOTAL STUDY COSTS:			$123,651

M. Rahman Chowdhury
Senior Budget & Cost Officer
ICDDR, B, Mohakhali
Dhaka-1212, Bangladesh.

Biography of the investigators

Biographical sketch

Name: Abdullah H Baqui, MBBS, MPH, DrPH Birth date: 03/31/53

Title: Senior Epidemiologist and Head, Child Health Program
Public Health Sciences Division, ICDDRB

Education:

Institution and Location	Degree	Year conferred	Field of study
Dhaka Medical College, Bangladesh Bangladesh	MBBS	1976	Medicine
Johns Hopkins University, USA	MPH	1985	International Health
Johns Hopkins University, USA	DrPH	1990	International Health

Research and or Professional Experience:

1977-1978 Medical Intern, Dhaka Medical College, Dhaka, Bangladesh
1978-1981 Medical Officer, Matlab Health Research Station, ICDDR,B, Bangladesh
1981-1987 Physician-in-Charge, Clinical Services, Matlab Health Research Station, ICDDR,B
1987-1990 Senior Medical Officer/Assistant Scientist, Department of Epidemiology, ICDDR,B
1990-1994 Head, Research and Evaluation, Urban Health Extension Project, ICDDR,B
1994-1994 Associate Project Director, Urban Health Extension Project, ICDDR,B
1990-present Research Associate, Assistant Scientist, Dept of Int. Health, Johns Hopkins Univ.
1994-present Project Director, RISC Project, ICDDR,B
1994-1997 Project Director, MCH-FP Extension Project (Urban), ICDDR,B
1998-present Senior Epidemiologist & Head, Child Health Program, PHSD, ICDDR,B

SELECTED PUBLICATIONS:

Baqui AH, Black RE, Sack RB, Yunus M, Siddique AK and Chowdhury HR. "Epidemiologic and clinical characteristics of Acute and Persistent Diarrhoea in Rural Bangladeshi Children." Acta Pediat Scand Suppl 381:15-21, 1992

Baqui AH, Sack RB, Black RE, Yunus M, Haider K, Alim ARM, Siddique AK. "Enteropathogens associated with Acute and Persistent Diarrhoea in Rural Bangladeshi Children". The Journal of Infectious Disease 1992; 166:792-6

Baqui AH, Black RE, Sack RB, Chowdhury HR, Yunus M, Siddique AK. Malnutrition, Cell-Mediated immune deficiency and diarrhoea: A community-based longitudinal study in rural Bangladeshi children. Am J Epidemiol 1993; 137(3):355-65.

Baqui AH, Black RE, Yunus M, Haque ARMA, Chowdhury HR, and Sack RB. "Methodologic Issues in Diarrhoeal Diseases Epidemiology : Definition of Diarrhoeal Episodes." International Journal of Epidemiology 1991;20(4).

Baqui AH, Sack RB, Black RE et al. Malnutrition and cell-mediate immune deficiency are independent risk factors for persistent diarrhoea in Bangladeshi children. Am J Clin Nutr 1993; 58:453-8.

Baqui AH, Yunus M, and Zaman K. "Community-Operated Treatment Centers Prevented Many Cholera Deaths". J Diar Dis Research 1984; 2(2): 92-98.

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Baqui AH, Yunus M, Zaman K. "Surveillance of Patients attending a rural diarrhea hospital in Bangladesh". Tropical and Geographical Medicine 1991; 43(1-2):17-22.

Baqui AH, Zaman K, Yunus M, Mitra AK, Hossain KMB and Banu H."Epidemiological and Clinical Characteristics of Shigellosis in Rural Bangladesh". J Diar Dis Research 1988; 6(1):21-28.

Baqui AH, Arifeen SA, Amin S, Black RE. Levels and Correlates of Maternal Nutritional Status and Consequences for Child Survival in Urban Bangladesh. Eur J Clin Nutr 1994, 48,349-357

Baqui AH, Francisco A de, Arifeen SE, Siddique AK and Sack RB. Bulging fontanelle after supplementation with 25,000 IU vitamin A in infancy using EPI contacts. Acta Paed Scand 1995, 84:863-6

Biographical Sketch:

Name: Daniel W. Isenbarger, MD, MPH Birth date: 11 May 1961

Title: Major, Medical Corps, US Army
Internal Medicine Officer
Department of Enteric Infections, Division of Communicable Diseases and Immunology
Walter Reed Army Institute of Research, WRAMC, Washington, DC

Education:

Institution:	Degree:	Date conferred:	Field of Study:
USUHS	MPH	1997	Public Health Biometrics
Georgetown University	MD	1991	Medicine
Whittier College	BA	1983	Political Science

Research and/or professional experience:

1987-88	Malaria immunology laboratory investigations, Yale University and WRAIR
1991-92	Internal Medicine internship, WRAMC, Washington DC
1992-94	Internal Medicine residency, WRAMC, Washington DC
1992	Combat Casualty Care Course, Advanced Trauma Life Support Course, Brooke Army Medical Center, San Antonio, Texas
1993	Tropical Medicine Course, WRAIR, Washington DC
1994-96	Staff Internist, Internal Medicine Clinic, WBAMC, El Paso, Texas
1996-97	Internal Medicine Officer, Department of Enteric Infections, WRAIR
1997-99	Internal Medicine Officer, Department of Bacteriology, AFRIMS, Thailand
1999-present	Internal Medicine Officer, Department of Enteric Infections, WRAIR

Publications:

Sanders J, Isenbarger DW, Walz S, Pang L, Scott D, Tamminga C, Echeverria P, Oyofo B, Hewitson W, Sanchez J, Tribble D. An observational clinic-based study of diarrheal illness in deployed U.S. Military personnel in Thailand: presentation and outcome of *Campylobacter* infection. Abstract, ASTM&H Conference 1999.

Sethabutr O, Venkatesan M, Yam S, Pang LW, Smoak BL, Sang WK, Echeverria P, Taylor DN, Isenbarger DW. Detection of PCR products of the *ipaH* gene from *Shigella* and enteroinvasive *Escherichia coli* by enzyme linked immunosorbent assay. Abstract, ASTM&H Conference Proceedings 1998.

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Isenbarger DW, Chapin BL. Osteoporosis: current pharmacologic options for prevention and treatment. Postgrad Med 1997;101(1):129-142.

Hospenthal DR, Hicks CB, Green DL, Wiesen AR, Byrd JC, Isenbarger DW, Salzberg DJ. Trends in the incidence and diversity of fungi recovered from urine. Mycopathologia 1995;132:15-19.

Isenbarger DW, Aronson N. Lytic vertebral lesions: an unusual manifestation of AIDS-associated Kaposi=s Sarcoma. Clin Infect Dis 1994;19:751-5.

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Hoffman SL, Isenbarger D, Long GW, Sedegah M, Szarfman A, Waters L, Hollingdale MR, van der Meide PH, Finbloom DS, Ballou WR. Sporozoite vaccine induces genetically restricted T cell elimination of malaria from hepatocytes. Science 1989;244(4908):1078-81. Oral presentation to American Tropical Medicine Society, annual meeting, 1988.

Hoffman SL, Berzofsky JA, Isenbarger D, Zeltser E, Majarian WR, Gross M, Ballou WR. Immune response gene regulation of immunity to Plasmodium berghei sporozoites and Circumsporozoite Protein vaccines. J Immunol. 1989;142(10):3581-4.

Biographical Sketch

Name: Dr. Ahmed Nurul Alam, MBBS & Ph.D Date of Birth: 08.01.19941

Title at ICDDR,B: Consultant Physician & Head,
Training and Education Department
International Centre for Diarrhoeal Disease Research,
Bangladesh (ICDDR,B)

Education:

Institution and Location	Degree	Year conferred	Field of Study
Dhaka Medical College	MBBS	1963	Medicine, Surgery & Gynae/Obstetrics
Kings College Hospital Medical School, UK	Ph.D.	1978	Medicine (Gastroenterology)

Research and Professional Experience:

1964 to 1965 Junior and Senior House Physician, Department of Medicine, Dhaka Medical College Hospital, Bangladesh

1965 Additional Clinical Assistant, Department of Medicine, Dhaka Medical College Hospital

1966 Outpatients Medical Officer, Department of Medicine

1966 to 1967 Clinical Assistant, Department of Medicine, Dhaka Medical College Hospital and Institute of Post-graduate Medicine & Research, Dhaka.

1967 to 1972 Registrar, Department of Medicine, Dhaka Medical College Hospital.

1972 to 1973 Resident Physician, Dhaka Medical College Hospital.

1973 to 1979 Research Fellow at Liver and Gastroenterology Research Unit and Clinical Assistant, Hepatology and General Medicine, Kings College Hospital and Medical School, London, UK.

1980 to 1981 Associate Professor of Medicine, Gastroenterology, Institute of Post-graduate Medicine and Research, Dhaka.

1981 to 1984 Associate Scientist, International Centre for Diarrhoeal Disease Research, Bangladesh.

1984 to 1992 Head, Clinical Research Centre, ICDDR,B.

1992 to till date Consultant Physician and Head, Training and Education Department, ICDDR,B,

PUBLICATIONS

1. Alam AN, Wilkinson SP, Poston L, Moodie H, and Williams R. Intraccellular electrolyte abnormalities in fulminant hepatic falure. Gastroenterology. (1977); 914-917.

2. Alam AN, Wheeler P, Wilkinson SP, Poston L, Golindano C, & Williams R. Changes in the electrolyte content of l eucocytes at different clinical stages of cirrhosis. Gut. 1978; 19: 650-654.

3. Alam AN, Poston L, Wilkinson SP, Golindano CG, and Williams R. A study in vitro of the sodium pump in fulfilment hepatic failure. Clinical Scences and Molecular Medicine. 1978; 55: 355-363.

4. Alam AN, Khanum S, Khatun M, Molla A, Rahaman MM. Acceptability and digestibility of wheet syrup.

Nutrition Rports International. 1985; 31; 2: 463-468.

5. Alam AN, Sarker SA, Molla AM, Rahaman MM and Greenough WB III (1987). Hydrolysed wheat based oral rehydration solution for acute diarrhoea. Arch. Dis. of Childhood. 1987; 62: 440-444.

6. Alam AN, Saha JR, Bobkin JF, Lindenbaum J. Interethnic variation in the metablic inactivation of digoxin by the Gut Flora. Gastroenterology. 1988; 95: 117-23.

7. Alam AN, Alam NH, Ahmed T, & Sack DA. Randomized Double-blind trial of single dose doxycycline for treating cholera in adults. British Medical Journal. 1990; 300: 1619-21.

8. Alam AN, Goff PA, Abdal NM, Rashid MA, Rahaman MM. Serum ferritin and cholera: A prospective study. Trop. and Geog. Med. 1991; 43: 12-16.

9. Alam K, Bradford K, Alam AN. Isolation of aeromonas hydrophila from two fatal cases of septicemia hospitalized with diarrhoea. Bangladesh J.Microbiol. 1991; 8: 51-53.

10. Alam AN, Abdal NM, Wahed MA, Rao B, Kawser CA, Hoque M. Rahaman MM. Prostacyclin concentrations in haemolytic uraemic syndrome after acute shigellosis in children. Archives of Disease in Childhood 1991;66:1231-1234.

11. Alam AN. A useful guide to giving ORT at home. Dialogue on Diarrhoea. 1993; 52:7.

12. Alam AN, Islam MR, Hossain MS, Mahalanabis D, Hye HMKA. Comparison of pivmecillinam and nalidixic acid in the treatment of acute shigellosis in children. Scand J Gastroenterol. 1994; 29: 313-317.

E. BOOK CHAPTERS

1. Conn's Current Therapy, 1997
   Edited by Robert E. Rakel,
   W.B Sanders Company, Philadelphia,
   Pennsylvania, 19106,

   Chapter on:
   Cholera method of
   A. N. Alam

2. Medical Diagnosis and Treatment, 3rd & 4th Edition (1987 & 1997)
   Author: Prof. N. Islam
   i. Cholera
   ii. Shigellosis

3. Essence of Paediatrics: 2nd Edition, (1993) Prof. M.R. Khan and Dr. M.E. Rahman Chapter on: Diarrhoeal Disease pp 96-110.

F. ADDITIONAL EXPERIENCES:

1. Worked as a consultant for Aga Khan Foundation in Kenya, 1984 and Republic of China, 1987 & 1988.
2. Worked as a cousultant for USAID (ADDR) in Peru, 1992 & 1994.
3. Worked as a consultant for WHO in Republic of Yemen & Iran, 1992.
4. Woked as consultant for Worlc Vision International in Cambodia, 1993 & 1995.
5. Supervised thesis for Masters in Philosophy (M.Phil) from Dhaka University
6. Worked as examiner for the University of Dhaka for M.Phil and M.D.(Gastroenterology) Examinations

Worked as examiner for the Bangladesh College of Physicians and Surgeons for M.C.P.S. examination

Detailed Budget

Appendices

Appendix 1: Consent form, Inpatient Trial Vaccinees

Appendix 2: Consent form, Outpatient Trial Vaccinees

Appendix 3: Consent form, Household Contacts

INPATIENT CONSENT FORM

Project title: **Safety, dose, immunogenicity and community transmission risk of candidate *Shigella flexneri* 2a vaccine SC602 among young children in rural Bangladesh.**

Principal investigator: **Dr. Abdullah H. Baqui, Head, Child Health Program, Public Health Sciences Division, International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B).**

As a parent / legal guardian of an eligible child, you are being invited to allow your child to participate in a research study evaluating the safety and immunogenicity of an experimental vaccine for *Shigella*. This study is sponsored by ICDDR,B and the U.S. Army Medical Research and Materiel Command (USAMRMC) under the direction of Dr. Abdullah Baqui. It is essential that you understand that:

a) Taking part of your child in the study is entirely voluntary;
b) Personal benefit may not result to you from taking part in the study, but knowledge may be gained that will benefit others;
c) You may withdraw your child from the study at any time without penalty or loss of benefits to which you are otherwise entitled;

The nature of the investigational vaccine and of the study, the risks, inconveniences, discomforts and other pertinent information are discussed below. You may discuss any questions you may have about this study with the staff of the study group.

1. **Information on diarrhea-producing Shigella:** Diarrhea is an important cause of illness, particularly among children living in developing countries. Bloody diarrhea, known as dysentery, is a particularly severe form of this disease, and is most often caused by bacteria called *Shigella*. Vaccines are being developed and tested for their effectiveness in preventing diarrhea caused by these bacteria. This study involves a new vaccine designed to protect against *Shigella flexneri 2a*, which is one of the most common *Shigella* strains found worldwide.

2. **Purpose of Study:** The purpose of this study is to test this new vaccine in Bangladeshi healthy children to determine whether the vaccine is safe and can stimulate the production of protective antibodies in the blood. If this vaccine is safe and stimulates a good antibody response, we will perform additional studies in other volunteers to see if they are protected against this important cause of diarrhea.

3. **Experimental vaccine:** There are no licensed *Shigella flexneri* vaccines. The safety and ability of this vaccine to stimulate the formation of antibodies to *Shigella flexneri* is being tested in this study. The vaccine is an attenuated strain of *Shigella flexneri 2a* in which two parts of the bacteria that are important for its ability to cause disease have been removed. As such, the vaccine strain is much less able to cause disease, though at high doses this is still possible. Our goal is to determine the dose that is safe, yet still induces an optimal immune response. Later studies will be undertaken evaluating the efficacy of the vaccine based in part on the results of this trial. The vaccine that will be tested in this study is investigational and is not licensed by the U.S. Food and Drug Administration (FDA) for general use. The FDA has reviewed the plans for this study and has allowed the use of this vaccine in this research study.

4. **Design of the Experimental Vaccine Study:** To qualify for this clinical trial you will be asked to give a medical history and a small amount of blood of your child. This blood will be tested to evaluate your child's general health.

Once the screening tests are completed, participation will include five days' hospital admission and follow up home visits by members of the study team over the next month following administration of the vaccine. On the day of administration of the vaccine, all children will be admitted at the ICDDR,B hospital at Matlab. Eligible children will then receive one of two formulations of the vaccine or the placebo. We do not know which formulation they are receiving. After receiving the vaccine you will be asked to remain in the hospital with your child for five days to monitor for side effects, then return to home. Home visits by study personnel will commence the next day (day 6) to check for any side effects. Beginning with the first vaccination, blood will be obtained up to 3 times, 4 ml at a time (on admission, on Day 14 and day 28). Stool will also be collected daily during the stay in hospital and on Day 14 and 28 at home. If needed, your child will get medicine to kill the *Shigella* before being discharged from the hospital.

5. **Potential benefits.** There is no health benefit that can be guaranteed to your child as an individual as a result of his/her participation in this clinical trial. The vaccine may induce an immune response to *Shigella*. It is our hope that this study will enhance our understanding of these vaccines and eventually lead to safe and effective vaccines that can be used to prevent these important diseases.

6. **Potential risks.** Your child may develop diarrhoea from the vaccine. Facilities and medications for treating this are available and in case of any adverse event we will provide all medications free of cost. Another inconvenience to your child may be the pain and occasional bruising caused by the needle sticks in your

child's arm from the blood draws. Among the first 169 volunteers in Matlab who received this vaccine there were no serious side effects noted.

7. **Safeguards for your protection**. There will be a study physician available to answer your questions and concerns. A doctor will be available any time of day or night should your child experiences any symptoms, which you think might be related to participating in this clinical trial. You should report any medical problems that occur in your child promptly to doctor at the clinical facility of ICDDR,B at Matlab.

8. **Confidentiality**: Your identity will remain confidential in any publications resulting from this study. Officials of the ICDDR,B, USAMRMC and the Food and Drug Administration (FDA) may inspect all records from this study due to their support and interest in this vaccine. By signing this consent document, you agree to such inspection and disclosure. Records will be used by these institutions only in connection with carrying out their obligations relating to the clinical trial and every effort will be made to keep the records as confidential as possible. Complete confidentiality cannot be promised.

9. **Right to Withdraw**: You may refuse to participate in or may withdraw your child from this clinical trial at any time without penalty or loss of benefits to which you or your family would normally be entitled. The physician in-charge of this study may elect to end your involvement at any time for medical reasons. Counseling will be provided about your medical status if you are asked to leave the study for a medical reason.

10. **Conditions under which you should not participate or your participation may be terminated or rescheduled without your consent:**

    a) Fever detected on the day scheduled for vaccination.
    b) Any clinically significant illness, determined by your physician, that would in the physician's opinion place you at medical risk if you continued to participate in the study.

11. **Significant new findings**: Any significant information regarding new findings that develop during the study will be made available to you. You will be notified about any important medical information having an impact on your health or your willingness to continue participation in this study.

12. **Cost and Compensation**: All necessary medical care resulting from your child's participation in this research study will be provided to you without cost. Other than medical care, you will not receive any compensation for your participation in this research study.

13. **Consent**: I have read this document and feel that I have had enough time to consider the decision to allow my child to participate in this clinical trial. I have asked and received satisfactory answers to all of my questions. I have been given a copy of this form and am aware that a copy will remain in the files at ICDDR,B. I hereby give my consent on behalf of my child (name _____) to participate in this clinical trial. I understand that there is a possibility that the blood, urine and stool samples collected under this study may also be used in other research studies.

14. **Points of contact are**: Dr. Abdullah Baqui, ICDDR,B, GPO Box 1000, Bangladesh. Tel: 881 0115, Fax: 8826050, Telex: 675612 ICDD BJ, Email: ahbaqui@icddrb.org

I voluntarily and freely consent to donate any and all blood and stool collected from my child as part of this study and hereby relinquish all right, title, and interest in said items.

Parent/Guardian's Initials:_____ Date:_____

Name (in block letters) _____

Name of the child: _____

Relationship : Father / Mother / Legal guardian / _____

Signature of Witness_____ Date_____

Name of witness (in block letters) _____

OUTPATIENT VOLUNTEER AGREEMENT AFFIDAVIT

Project Title: Safety, dose, immunogenicity and community transmission risk of candidate *Shigella flexneri* 2a vaccine SC602 among young children in rural Bangladesh.

Principal Investigator: Dr. Abdullah H. Baqui, Head, Child Health Programme, Public Health Sciences Division, International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). GPO Box 128, Dhaka 1000, Bangladesh. Tel: 881 0115, Fax: 882 6050. Email: ahbaqui@icddrb.org

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As a parent/legal guardian of an eligible child, you have been asked to allow your child to participate in a research study evaluating the safety of an experimental vaccine for *Shigella*. This study is sponsored by the U.S. Army Medical Research and Materiel Command (USAMRMC) under the direction of Dr. Abdullah Baqui. It is essential that you understand that:

a) Taking part in the study is entirely voluntary;
b) Personal benefit may not result to you from taking part in the study, but knowledge may be gained that will benefit others;
c) You may withdraw your child from the study at any time without penalty or loss of benefits to which you are otherwise entitled;

Information on diarrhea-producing Shigella. Diarrhea is an important cause of illness among children living in developing countries, as well as travelers to developing countries. Bloody diarrhea, known as dysentery, is a particularly severe form of this disease, and is most often caused by bacteria called *Shigella*. Vaccines are being developed and tested for their effectiveness in preventing diarrhea caused by these bacteria. This study involves a new vaccine designed to protect against *Shigella flexneri 2a*, which is one of the most common *Shigella* strains found worldwide.

Purpose of Study. The purpose of this study is to test this new vaccine in Bangladesh volunteers to determine whether the vaccine is safe and can stimulate the production of protective antibodies in the blood. If this vaccine is safe and stimulates a good antibody response, we will perform additional studies in other volunteers to see if they are protected against this important cause of diarrhea.

Experimental vaccine.

There are no licensed *Shigella flexneri* vaccines. The safety and ability of this vaccine to stimulate the formation of antibodies to *Shigella flexneri* is being tested in this study. The vaccine is an attenuated strain of *Shigella flexneri 2a* in which two parts of the bacteria that are important for its ability to cause disease have been removed. As such, the vaccine strain is much less able to cause disease, though at high doses this is still possible. Our goal is to determine the dose that is safe, yet still induces an optimal immune response. Later studies will be undertaken evaluating the effectiveness of the vaccine based in part on the results of this trial. The vaccine that will be tested in this study is investigational and is not licensed by the U.S. Food and Drug Administration (FDA) for general use. The FDA has reviewed the plans for this study and has allowed the use of this vaccine in this research study.

Design of the Experimental Vaccine Study

To qualify for this clinical trial you will be asked to give a medical history and a small amount of blood of your child. The blood will be tested to evaluate your child's general health.

Once the screening tests are completed, participation will include your home visits by members of the study team over the one month following administration of the vaccine. On the day of administration of the vaccine, all participants will meet at the ICDDR,B clinical facility for final screening. Eligible children will then receive either the vaccine or the placebo. Volunteers will not be told which formulation they are receiving. After receiving the vaccine you will be asked to remain in the clinic with your child for up to one hour to monitor for side effects, then return to your home. Home visits by study personnel will commence the next day to check for any side effects. They will visit everyday for five days and then on the every other day for next ten days. You will be asked to give information of any symptoms that your child experiences to review with the study personnel during your home visit. Beginning with the first vaccination, blood will be obtained up to 3 times (4 ml every time on Day 0, Day 14 and day 28). Stool

and rectal swabs will also be collected every other day for up to day 10, and then on every third day up to day 28.

Potential benefits. There is no health benefit that can be guaranteed to you as an individual as a result of your participation in this clinical trial. The vaccine may induce an immune response to *Shigella*. It is our hope that this study will enhance our understanding of these vaccines and eventually lead to safe and effective vaccines that can be used to prevent these important diseases.

Potential risks. Your child may develop a diarrhoeal disease from the vaccine. Facilities and medications for treating this are available where the vaccine will be given. In natural *Shigella* infection, some individuals develop an inflammatory condition known as Reiter's Syndrome, which includes inflammation of the joints, conjunctivae and urethra. This condition has not been seen in the 45 volunteers in the United States who have received the vaccine, but it remains a possibility. This condition generally resolves on its own with time, though occasionally long term disability has resulted.

Another inconvenience to you may be the pain and occasional bruising caused by the needle sticks in your arm from the blood draws. Among the first 45 volunteers who received this vaccine there were no serious side effects noted other than dysentery, which was treatable in all cases.

Safeguards for your protection. There will be a study physician available to answer your questions and concerns. A doctor will be available any time day or night should you experience any symptoms which you think might be related to participating in this clinical trial. You should report any medical problems that occur promptly to Dr. Baqui or another doctor at the clinical facility.

Confidentiality: Your or your child's identity will remain confidential in any publications resulting from this study. Officials of the ICDDR,B, USAMRMC and the Food and Drug Administration (FDA) may inspect all records from this study due to their support and interest in this vaccine. By signing this consent document, you agree to such inspection and disclosure. Records will be used by these institutions only in connection with carrying out their obligations relating to the clinical trial and every effort will be made to keep the records as confidential as possible, within the limits of the law. Complete confidentiality cannot be promised.

Right to Withdraw: You may refuse to participate in or may withdraw your child from this clinical trial at any time without penalty or loss of benefits to which you or your family would normally be entitled. The physician in-charge of this study may elect to end your involvement at any time for medical reasons. Counseling will be provided about your medical status if you are asked to leave the study for a medical reason.

Conditions under which your child should not participate or your participation may be terminated or rescheduled without your consent:

a) Fever detected on the day scheduled for vaccination.
b) Any clinically significant illness, determined by the physician, that would in the physician's opinion place your child at medical risk if the child continues to participate in the study.

Significant new findings: Any significant information regarding new findings that develop during the study will be made available to you or your own physician(s) at your request. You will be notified about any important medical information having an impact on your health or your willingness to continue participation in this study.

Cost and Compensation: All necessary medical care resulting from your participation in this research study will be provided to you without cost. Other than medical care, you will not receive any compensation for your participation in this research study.

Points of contact are: Dr. Abdullah Baqui, ICDDR,B, GPO Box 1000, Bangladesh. Tel: 870115, Fax: 886050, Telex: 675612 ICDD BJ, Email: ahbaqui@ icddrb.org

Consent for study Project title: **Safety, dose, immunogenicity and community transmission risk of candidate *Shigella flexneri* 2a vaccine SC602 among young children in rural Bangladesh.**

I have been given an opportunity to ask questions concerning this investigational study. Any such questions were answered to my full and complete satisfaction. Should any further questions arise concerning my rights on study-related injury, I may contact Dr. Baqui or one of his co-investigators.

I voluntarily allow my child (name) _____ to participate in this study. I understand that I may at any time during the course of this study revoke my consent and withdraw my child from the study without further penalty or loss of benefits. However, I may be requested to undergo certain examinations if, in the opinion of the attending physician such examinations are necessary for my child's health and well-being. My refusal to participate will involve no penalty or loss of benefits to which I am otherwise entitled.

I voluntarily and freely donate any and all blood, urine and stool collected as part of this study and hereby relinquish all right, title, and interest in said items.

Name of Volunteer _____ Age: _____

Name of Parent / guardian _____ Relationship: _____

Signature of Parent / guardian _____ Date_____

Signature of Witness_____ Date_____

HOUSEHOLD CONTACTS' VOLUNTEER AGREEMENT

Project title: "Safety, dose, immunogenicity and community transmission risk of candidate *Shigella flexneri* 2a vaccine SC602 among young children in rural Bangladesh."

Principal Investigator: **Dr. Abdullah H. Baqui, Head, Child Health Programme, Public Health Sciences Division, International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). GPO Box 128, Dhaka 1000, Bangladesh.**

The *Shigella flexneri* SC602 vaccine is made of living bacteria whose ability to cause disease has been weakened. The vaccine is given by mouth and is often present in the stool of a volunteer vaccinee for 2 or more weeks after vaccination. Because it is in the excreted stool, it may contaminate the hands of the volunteer's caretaker after using the toilet. Household and neighborhood contacts of volunteers who have received the *Shigella flexneri* SC602 vaccine may be at risk for also getting this vaccine. This would occur as a result of transmission of the vaccine from the hands of the vaccine recipient to food or water followed by the family member or household contact eating or drinking this contaminated food or water. Transmission which might occur in this way can easily be prevented by washing of the hands after using the toilet. Our vaccine could cause the following stomach or intestinal symptoms: loose stools, diarrhea, bloody diarrhea, nausea, and/or cramps or gurgling in the stomach. These stomach or intestinal symptoms can be associated with any or all of the following additional symptoms: fever, headache, joint aches, muscle aches, and/ or loss of appetite.

Purpose of the Study: To determine if you have acquired the study vaccine bacteria from a volunteer enrolled in the protocol entitled " Safety, dose, immunogenicity and community transmission risk of candidate *Shigella flexneri* 2a vaccine SC602 among young children in rural Bangladesh.."

As an adult family member or household contact of a child volunteer enrolled in the protocol " Safety, dose, immunogenicity and community transmission risk of candidate *Shigella flexneri* 2a vaccine SC602 among young children in rural Bangladesh.", you have been asked to volunteer to participate in a protocol that will be evaluating the possibility that household or neighborhood contacts of vaccinees may become infected with our vaccine bacteria . This study (which includes the household contact stool collection and investigational protocols) is sponsored by the U.S. Army Medical Research and Materiel Command (USAMRMC) and the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) under the direction Dr. Abdullah Baqui. It is essential that you understand that:

a) Taking part in this screening study is entirely voluntary;
b) Personal benefit to you will not result from participating in the study, but knowledge gained may benefit others;
c) Your child/dependent may be withdrawn from the study at any time without penalty or loss of benefits to which you are otherwise entitled.

You are encouraged to ask questions regarding the purpose, procedures, risks, and benefits of participating in this screening study. After you read or heard the information below, if you still have questions feel free to ask for further explanation from the investigators or staff until you are satisfied and understand the nature of the study and the role you have in it.

Study Procedures: A physician will see you or your child daily for first five days and will ask some questions. The answers will be recorded on a report. The answers will let us know if you have any symptoms that suggest that you have acquired our *Shigella* vaccine. On days 6 and 10, we request that you provide a stool sample for culture. This is all the participation that is necessary unless you become ill.

For all participants, if you develop diarrhoea (3 loose stools in 24 hours) or become ill (loose stools, diarrhea, bloody diarrhea, nausea, or stomach cramps/ gurgling associated with fever, headache, joint aches, muscle aches, and/ or loss of appetite) you may come to the ICDDR,B clinic to be evaluated. If you receive medical care from your regular physician, you can inform him/her that you **may** have been exposed to *Shigella flexneri*, and if your doctor confirms this tell the doctor that the following antibiotics can be used: Ampicillin, Bactrim, Nalidixic Acid, Ciprofloxacin or Azithromycin. If you become ill with other symptoms that are not related to your stomach or intestines (such as sore throat, chest pain, cough) we will **not** evaluate you and you need to see your regular physician or go to where you normally seek medical care. If you come to the ICDDR,B clinic for evaluation of your diarrhoeal illness, we will need to test (culture) your stool(s) for the vaccine bacteria. Stools must be cultured within 1-2 hours after passing the stool . Stools should be collected in the "stool cups" then placed into the ice chest and brought to the clinic. We ask that you also use the "spoon" provided in the "stool kit" and scoop a small amount of stool and place it into the bottle with the screw top. We request that you bring a sample of stool from a single diarrhea stool for each day you have diarrhea as explained above to the clinic. We request this to determine if the investigational vaccine bacteria is present in your stool. If the vaccine bacteria is not present, the study is completed and no further participation from you is necessary unless you again develop diarrhea or become ill through day 28. If you again become ill or develop diarrhea, then we request the above be repeated. If your stool culture contains the investigational vaccine bacteria, you will be offered antibiotic treatment to eliminate it. If your stool has the vaccine bacteria, then we need to have you bring to the clinic your stool, transported as described above, on a scheduled date after starting the antibiotic so we can culture your stool and make sure the antibiotic has killed the bacteria. The antibiotic to be used will be Nalidixic Acid 1 gm by mouth 4 times a day for 5 days, or Ciprofloxacin, 500 mg by mouth twice a day for 5 days. If you are unable to take Nalidixic Acid or Ciprofloxacin, you will be treated with Azithromycin (500 mg day 1, followed by 250 mg once daily for 4 days). If your stool is positive for another bacteria or parasite, we can offer you initial treatment and then you need to follow-up with your regular system of medical care. If we cannot culture from your stool *Shigella flexneri*, a common bacteria or identify a common parasite in your stool, we will not provide further medical care and you will need to follow-up with your regular system of medical care.

Exclusion You may not participate in this study if any of the following conditions apply to you:

a) You have an illness or condition that the study physicians think pose a risk for your participation
b) You are unwilling to submit stool samples when requested

Potential Risks and Discomforts: The risks to you are those associated with collection of a stool culture. You **must wash your hands thoroughly with soap and water after using the toilet** while your stool culture is positive for the vaccine strain to eliminate the risk of transmitting the vaccine strain to other individuals. Nalidixic Acid and Ciprofloxacin treatment are usually well-tolerated; the most frequent side effects associated with their use include nausea, vomiting, diarrhea, abdominal pain/discomfort, rash, headache, and effects on the central nervous system, such as restlessness. Azithromycin treatment is also well-tolerated; the most frequent side effects being diarrhea, nausea, and stomach pain

Safeguards for Your Protection: Good clinical practices and professional patient care will be used during this study.

Potential Benefits: There is no benefit that can be guaranteed to you for your participation in this research study. However, if the *Shigella flexneri* SC602 vaccine strain is present in your stool, the antibiotic therapy will eliminate it.

Compensation: Other than medical care that will be provided for study-related illness or injury, you will not receive any other compensation. No blood draws are anticipated and if done for medical indications, will not be reimbursed.

Significant New Findings: Any significant new findings that develop during the study which could affect your willingness to continue participation will be made available to you.

Right to Withdraw: You may refuse to participate in or you may withdraw from this study at any time without penalty or loss of benefits to which you would normally be entitled.

Circumstances Under Which Your Participation May Be Terminated Without Your Consent:

a) Any condition(s) that would make further participation detrimental to your own health. Counseling will be provided about your health if you are asked to leave the study for a medical reason.
b) Failure to comply with the procedures as outlined in this volunteer agreement affidavit.

Confidentiality: All data and medical information obtained about you as an individual will be considered privileged and held in confidence. You will not be identified by name in any published report or presentation of the results. Representatives of the ICDDR,B, U.S. Army Medical Research and Materiel Command and the FDA may inspect the records of this research as part of their responsibility to oversee research and ensure the protection of volunteers. **By signing the consent form you agree to such inspection and disclosure.** Complete confidentiality cannot be promised to volunteers.

Obtaining Additional Information and Points of Contact: At any time you are entitled to ask questions relating to any aspect of this study and your right to medical care if you sustain a study-related injury. If you need to reach a physician, you can contact Dr. Abdullah Baqui or one of the other study physicians at ICDDR,B, Matlab Hospital or at Dhaka – Phone: 881 0115.

Consent:

I have read this consent form / everything have been described to me and feel that I have had enough time to consider the decision to participate in this research screening study. I have asked and received satisfactory answers to all of my questions. I have been given a copy of this form and I am aware that a copy will remain in the files at the ICDDR,B. My voluntary participation in the study; the purpose, length, and procedures by which the study is to be conducted; and potential risks, benefits, and compensation, have been fully explained to me to my satisfaction. I hereby consent to participate in this research screening study.

I voluntarily consent to participate / allow my child to participate in this study. I also voluntarily and freely donate any and all stool and blood products and hereby relinquish all right, title, and interest to said items.

NAME OF VOLUNTEER _____

NAME OF GUARDIAN _____ (if volunteer is a child)

SIGNATURE OF VOLUNTEER/GUARDIAN _____ DATE _____

Permanent Address: _____

_____

NAME OF WITNESS _____

SIGNATURE OF WITNESS _____ DATE _____