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ICDDRB: Centre for Health & Population Research
(RRC APPLICATION FORM)

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Protocol No: 2000-011 Date received: 3.4.2000
RRC Approval: Yes/ No Date:
ERC Approval: Yes/No Date:

RESEARCH PROTOCOL

Project Title: **CLINICAL EVALUATION OF GREEN BANANA (AMYLASE-RESISTANT STARCH) IN THE MANAGEMENT OF CHILDHOOD SHIGELLOSIS**

Theme and key words: Shigellosis, treatment, banana, colitis, amylase-resistant starch

Principal Investigator: **M. IQBAL HOSSAIN, MD, DCH.** **Division: CSD**
Phone: **8811751/Ext 2321**
Address: **ICDDR,B; GPO Box 128, Dhaka 1000, Bangladesh**
Email: **ihossain @icddrb.org**
Tel: **8802 8811751-60/Ext. 2333**

Co-Principal Investigator(s): G. H. RABBANI, MD, PhD, FACG
Scientist, Clinical Sciences Division
Tel: 8802 8811751-60/Ext. 2321
Email: rabbani@icddrb.org

Co-Investigator(s): **George Fuchs, MD**
Director, CSD, International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh
Fax: 880-2-8823116; E-mail: gfuchs@icddrb.org
Ali Miraj Khan, MD
Clinical Sciences Division
International Centre for Diarrhoeal Disease Research, Bangladesh
Wasif A Khan, MD
Clinical Sciences Division
International Centre for Diarrhoeal Disease Research, Bangladesh
Mrs. Nasheha Khatun, MSc, DAND
Dietitian and Nutritionist, Clinical Sciences Division

Student Investigator/Intern: Position open

Collaborating Institute(s):

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 6 months – 5 yrs

Project / study Site (*Check all the apply)*:

- [x] 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 up)
- [ ] Program Project (Umbrella)
- [ ] Secondary Data Analysis
- [x] Clinical Trial (Hospital/Clinic)
- [ ] Family follow-up study
- [ ] Cross sectional survey
- [ ] Longitudinal Study (cohort or follow-
- [ ] Record Review
- [ ] Prophylactic trial
- [ ] Surveillance / monitoring
- [ ] Others

Targeted Population *(Check all that apply)*:

- [ ] No ethnic selection (Bangladeshi)
- [x] Bangalee
- [ ] Tribal groups
- [ ] Expatriates
- [ ] Immigrants
- [ ] Refugee

Consent Process *(Check all that apply)*:

- [x] Written
- [x] Oral
- [ ] None
- [x] Bengali language
- [x] English language

Proposed Sample size:

Total sample size: N=220

Sub-group: N_1=110, N_2=110

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

☐ Human exposure to radioactive agents? ☐ Human exposure to infectious agents?
☐ Fetal tissue or abortus? ☐ Investigational new drug
☐ archives/source (specify_____) Pathological Investigational new device? ☐ Existing data available via public or diagnostic clinical specimen only
☐ Existing data available from Co-investigator ☐ Observation of public behavior
☑ New treatment regime

Yes/No

☐ ☑ Is the information recorded in such a manner that subjects can be identified from information provided directly or through identifiers linked to the subjects?

☐ ☑ Does the research deal with sensitive aspects of the subject's behavior; sexual behavior, alcohol use or illegal conduct such as drug use?

Could the information recorded about the individual if it became known outside of the research:

☐ ☑ a. place the subject at risk of criminal or civil liability?

☐ ☑ b. damage the subject's financial standing, reputation or employability; social rejection, lead to stigma, divorce etc.

Do you consider this research *(Check one)*:

☐ greater than minimal risk ☐ no more than minimal risk
☑ no risk ☐ only part of the diagnostic test

Minimal Risk is "a risk where the probability and magnitude of harm or discomfort anticipated in the proposed research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or tests. For example, the risk of drawing a small amount of blood from a healthy individual for research purposes is no greater than the risk of doing so as a part of routine physical examination".

Yes/No

☑ ☐ Is the proposal funded?

If yes, sponsor Name: USAID - W

Is the proposal being submitted for funding ?

☐ ☐ If yes, name of funding agency:

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

IF YES, submit a written statement of disclosure to the Director.

Dates of Proposed Period of Support	**Cost Required for the Budget Period ($)**
(Day, Month, Year - DD/MM/YY)	***a. Ist Year 2nd Year 3rd Year***
	$ 38,735 28925
	b. Direct Cost 67,660 Total Cost : ____
Beginning date May 2000	End date April 2002

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 by the external reviewers. The protocol has been revised according to the reviewer's comments and is approved.

George Fuchs, MD — 3 April 2000

Name of the Division Director — Signature — Date of Approval

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.

Signature of PI

Date: 1st April, 2000

Name of Contact Person
(if applicable)

DR. G. H. RABBANI

PROJECT SUMMARY

Background and Objectives: Better management of dysentery caused by *Shigella* spp. necessitates search for effective and safe alternative adjuvant therapy. A basis exists to consider dietary sources rich in amylase-resistant starch (ARS) fiber as a potential therapeutic regimen because of its beneficial effects on intestine, particularly colon, since shigellosis is predominantly a colonic disease. Dietary fibers including ARS have been found to be effective in many types of gastrointestinal disorders, however, their role in shigellosis has not been investigated. Green banana is a potential source of ARS and short-chain fatty acids (SCFA:acetate, propionate, butyrate) generated by fermentation by anaerobic colonic bacteria. SCFA have colonotrophic, anti inflammatory as well as antimicrobial activities. Two recent studies at ICDDR,B by one of our authors (Rabbani et al, 98, 99) documented the beneficial effects of SCFA and green banana in the treatment of experimental shigellosis in rabbits and in children with persistent diarrhoea respectively. Green banana is also inexpensive, culturally acceptable and accessible in many countries including Bangladesh.
We, therefore, propose a double-blind, controlled clinical trial with the primary objective to evaluate the therapeutic efficacy of green banana in the management of children with shigellosis.

Methods: Two hundred twenty children aged 6-59 months with *Shigella* dysentery of ≤48 hours duration will be hospitalized for 6 days in a study ward at ICDDR,B. Routine clinical care will be provided. All children will receive syrup pivmecillinum 60 mg/kg/day in four equal divided doses for five days. They will be randomized to either green banana-supplemented (n=110) or the standard diet group (n=110). For children in banana group, green banana will be cooked, peeled and pulp blended using a standard kitchen blender, and 200g of the pulp (wet weight) will be mixed with a milk-based diet (total volume will be 1L) and given by mouth. The amount of banana pulp to be provided is the same amount we have previously used with good results and without any side effects in children with persistent diarrhea. A detailed clinical history will be obtained, and a thorough physical examination including anthropometry will be performed. Vital signs will be recorded every 8 hours. During baseline observation and entire study period, the volume, frequency and type of stool, vomit, presence of straining at stools, rectal prolapse, body temperature, urine output, food and ORS intake, and requirement of unscheduled intravenous fluid will be obtained and compared between the groups. Breast feeding will be allowed and the amount will be recorded by test weighing. Main outcome variables e.g. clinical cure rate and bacteriologic cure rate according to pre-set criteria will be compared between the groups. Stool samples will be collected daily and stored at - 20°C until assay, and concentration of various SCFA will be determined by standard methods. No invasive procedures are planned as part of the study protocol. Informed written consents will be taken from the parents/guardians.

Rationale: If the study demonstrates efficacy of green banana as an adjunct in the management of shigellosis, it would simplify the treatment of shigellosis by using a locally available, culturally acceptable, cheap food material. This treatment can also be introduced as a home-treatment in the community.

HYPOTHESIS

We hypothesize that SCFA formed in the colon by the anaerobic fermentation of unabsorbed amylase-resistant starch (ARS) from ingested green banana will improve dysenteric illness in children caused by infection due to *Shigella* spp.

SPECIFIC AIMS AND OBJECTIVES

- Evaluate the therapeutic efficacy of cooked green banana in improving clinical and bacteriologic features of acute shigellosis by specifically assessing changes in stool characteristics and frequency and fecal excretion of *Shigellae* (clinical cure and bacteriologic cure according to pre-set criteria) in children population.

- To show that the therapeutic effects of green banana is mediated by the production of colonic SCFA.

BACKGROUND OF THE PROJECT INCLUDING PRELIMINARY OBSERVATIONS

Shigellosis remains a major cause of childhood morbidity and mortality in many developing countries including Bangladesh (1-3). Published reports reflect that at least 140 million cases of shigellosis, and almost 600,000 deaths due to shigellosis occurs worldwide annually among under 5 children, primarily in developing countries (4). It is estimated that approximately 3.8 million diarrhea related deaths that occurs worldwide in children annually (exclusive of China), 0.5 million are attributable to shigellosis (5). In the Matlab field study area of ICDDR,B approximately two thirds of all diarrhea related deaths are caused by dysentery (2). Based upon studies in other parts of Bangladesh (1), it is likely that more than half of these children with dysentery are infected with *Shigella* spp. Although malnourished population are more prone to this bacteria, it also induces malnutrition particularly in children. It causes anorexia and catabolism. The magnitude of protein loss in shigellosis is much higher than in acute watery diarrheas, and an adult may lose up to 500 ml of serum in stool in a day during severe shigellosis (6-8). In children of developing countries, who are already in a marginal nutritional status, this loss of serum protein is detrimental. The risk of death increase in sepsis, and in patients with severe protein energy malnutrition. Treatment of children with shigellosis with an effective antimicrobial agent is known to shorten both the duration of illness as well as the duration of fecal excretion of the pathogen (9). It is also likely that early treatment may prevent deaths (10), and complications, such as toxic colitis, hemolytic-uremic syndrome (11), and sepsis (12), occur late in the course of the illness.

Effective treatment of shigellosis has been complicated by the recent emergence of multiresistant strains (13-15). These strains are resistant to ampicillin, co-trimoxazole and nalidixic acid, which until recently had been the drugs of choice for treating shigellosis (9). In-vitro resistance to pivmecillinum (selexid) has been only occasionally encountered (105 of 2005 *Shigellae* isolates at ICDDR,B: J Albert, personal communication), and the drug is used for management of multiresistant *Shigellae* infections, irrespective of age of patients without any notable side effect, at hospitals of ICDDR,B and other places of Bangladesh. It is likely that, as with the other ß-lactam agents, resistance will also develop to this agent with its wider use. Thus, there is a pressing need to find additional effective antimicrobial as well as adjuvant therapies for shigellosis.

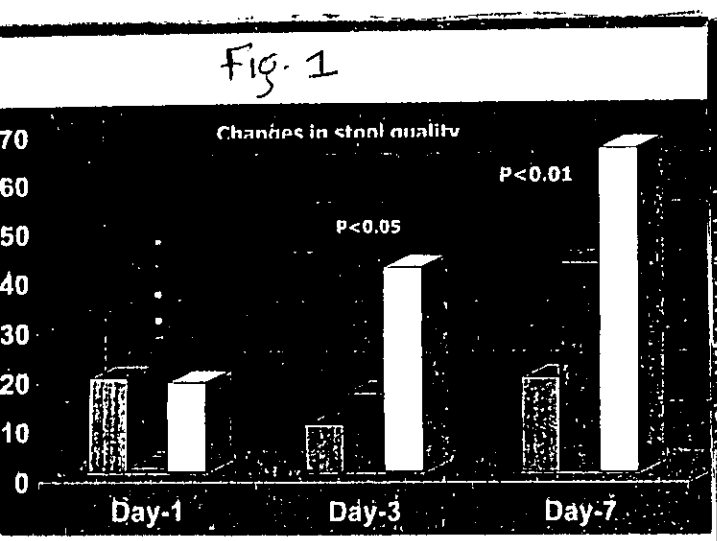
During the last decade, the beneficial role of ARS in the management of a variety of gastrointestinal disorders has been the subject of increased interest. Many types of fibers including wheat bran, oat bran, cellulose, guar gum, ispaghula and pectin have been shown to be useful in the symptomatic control through a variety of mechanisms in inflammatory bowel diseases, diversion colitis, diabetes mellitus, and gall stone disease (16,17). It has been postulated that the therapeutic effects of fibers in certain diseases are mediated by the effect of SCFA produced in the colon. SCFA (mainly acetate, propionate, and butyrate), are generated in the colon by bacterial fermentation, especially by anaerobic organisms, of dietary fibers, polysaccharides, and sugars that escape absorption in the small bowel. SCFA are the major anions in the human colon, present at concentrations of 50-70 mmol/kg for acetate, 20-25 mmol/kg for propionate, and 18-26 mmol/kg for butyrate (18). Acetate, propionate, and butyrate account for approximately 95% of colonic SCFA contents and butyrate provides 60% to 70% of the colonocytes energy needs (19, 20). They play a significant role in the overall regulation of colonic function including fluid and electrolytes transport, protein-synthesis, and they also have antibacterial actions (21, 22).

Inhibition of SCFA-stimulated Na^+-H^+ exchange and SCFA absorption contribute to the diarrheal fluid loses observed in acute colitis, and may reduce colonocyte energy supply (23). Recent evidence suggest that SCFA contribute to maintaining the health and integrity of the colonic epithelium (20). Daily perfusion of SCFA for two weeks through the defunctionalized colon in patients with diversion colitis resulted in clinical and endoscopic remission (24). SCFA increase colonic mucosal blood flow and mucous secretion, and particularly through butyrate controlling colonocyte growth and differentiation (25). Scheppach et al. also reported the direct colonotrophic effect of SCFA (26). The mechanism for locally-mediated colonotrophism is probably multifactorial, and may include increase in visceral blood flow (27-29), aerobic oxidation of SCFA for energy (30), increased production of enterotrophic hormones (31), and stimulation of the enteric nervous system (32). SCFA reduce the severity of diversion colitis and distal ulcerative colitis in humans (25, 33, 34). Butyrate and other SCFA have a marked effect on crypt cell proliferation in patients with active ulcerative colitis (35).

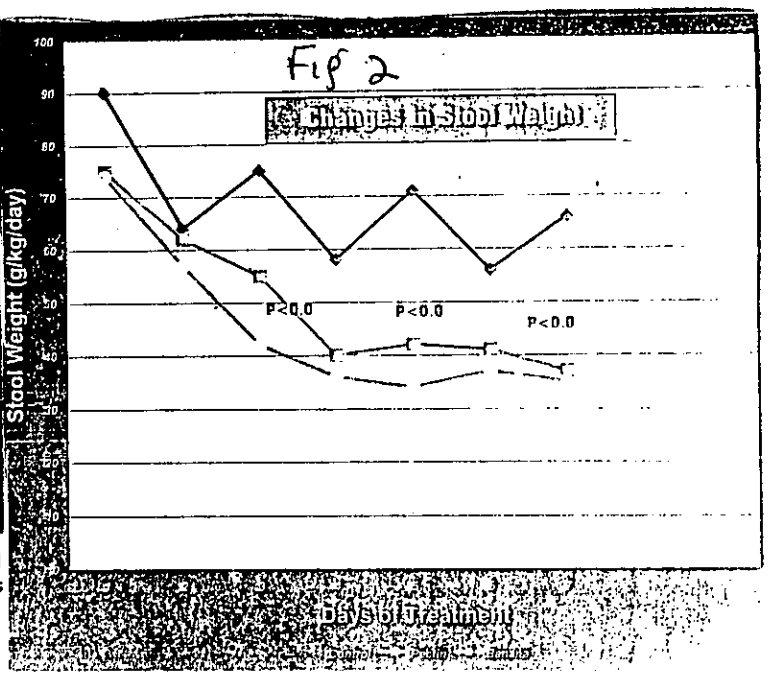
Preliminary observations from ICDDR,B

In a placebo controlled, metabolic balance study done in ICDDR,B, 62 children with persistent diarrhea including shigellosis were randomly given cooked green banana (n=22) or pectin (n=19) or a placebo (n=21) daily for 7 days. Both banana and pectin were found clinically useful in the dietary management of persistent diarrhea in children, and no unpleasant effects were observed. [*Rabbani et al. Beneficial effects of pectin and raw banana in the dietary management of persistent diarrhea in children. Digestive Disease Week' 1998 (Abstract)]. See P8.*

In another study at ICDDR,B, the same group (36: Rabbani et al) demonstrated that SCFA have therapeutic properties in the treatment of *Shigella* colitis in animals. In this animal model acute colitis was induced in adult rabbits by intracolonic administration of *Shigella flexneri* 2a. After 24 h, rabbits were given 6-h colonic infusions of SCFA (acetate, propionate, n-butyrate; 60:30:40 m*M*) or SCFA-free solution (control). Rabbits were killed in



Bar chart indicating percent of children who has soft or normal stool on day-1, day-3, or day-7 after starting treatment. The differences between the control and the treatment groups (banana, pectin) are significantly different (p<0.05) on day-3 and day-7.



(Fig 1 and fig 2,Rabbani et al. Beneficial effects of pectin and raw banana in the dietary management of persistent diarrhea in children)

batches of 2 or 3 animals at 24, 48, 72, and 96 h after treatment for histologic and bacteriologic assessment. SCFA significantly reduced fecal blood and mucus and improved clinical symptoms. Histologically, SCFA significantly ($p<.01$) reduced mucosal congestion, cellular infiltration, and necrotic changes. SCFA significantly ($p<.01$) reduced the number of *Shigellae* in the colon. These effects were not observed in the control animals. Effects of SCFA on histologic improvement of colitis, its antibacterial and bactericidal effects are shown in annexure 1, 2 and 3 respectively. It was suggested that SCFA may be useful agents in improving clinicopathologic features of shigellosis and should be clinically evaluated (36).

Composition of green, unripe banana

Green banana (*Musa paraidisiaca sapientum*) has been analysed by modern methods and found to have the following chemical composition (Faisant 95): (g/kg): protein 38, dietary fiber 92 (corresponding to cell materials) and total α-glucans 770, including oligosaccharides 60 (free glucose 47 plus oligosaccharides of DP 2 to 12, 13) and 'insoluble' starch 710. Thus, 30g banana flour contained 23.1 g α-glucans of which 1.8 g were oligosaccharides (including 1.4 g glucose) and 21.3 g 'insoluble' starch (Faisant 95).

Rationale for the use of green banana

Green banana is a potential source of ARS and SCFA (Faisant 95). ARS reduces frequency of diarrheic stools (37). Studies observed that absorption of calcium, magnesium, copper and zinc utilization remains unchanged (38, 39).

Green banana has been reported to have a protective effect against acute aspirin-induced gastric mucosal injury in rats (41). Banana powder has also been demonstrated to protect against aspirin, indomethacin, phenylbutazone, prednisolone, and histamine induced ulcer in guinea pigs (42). Beneficial effects of dried banana powder on patients with non-ulcer dyspepsia and peptic ulcer have been reported (43, 44). It has been hypothesized that bananas enhance mucosal resistance and promote healing of ulcers due to the presence of water-soluble polysaccharides (e.g. pectin) in green bananas and surface-active phospholipids in ripe sweet bananas (44). Approximately 75% of resistant starch (45) of banana origin, i.e. starch that escapes digestion in the human small intestine, undergoes fermentation in the colon (46). Anaerobic breakdown of these starch yields SCFA, CO_2, H_2 (methane production minimal) and energy. In addition to ARS, bananas also contain phospholipids and other ingredients which have the potential to directly affect the physiology and biochemical functions of humans and animals. Green banana has been used as an antidiarrheal agent in many traditional communities of Asia and Africa. It is speculated that the mechanism of action of banana is related to its high content of ARS and fiber (47).

In summary, we believe that there is convincing evidence proving a rationale to study green banana as a potential inexpensive, safe, culturally acceptable, and accessible therapeutic agent in children with invasive diarrhea, particularly shigellosis. We, therefore, propose the current study to evaluate the role of green banana in the management of *Shigella* dysentery in children.

Significance

The study will address two important issues. First, whether cooked green banana improves the clinical outcome in children with acute shigellosis. Second, we shall get some information on the levels of fecal SCFA (baseline) as well as effect of green banana supplementation on fecal SCFA in the course of acute shigellosis in children. If the study demonstrates significant effect of green banana, this could be a major development in the field of child survival. Green banana could then be used in the treatment of shigellosis, both at hospital and community settings.

RESEARCH DESIGN AND METHODS

Study design and sample size: Double-blind study

This study will be a double-blind, controlled clinical trial involving 220 children, both boys and girls, aged 6 - 59 months with *Shigella* dysentery who will be hospitalized for 6 days in a research ward of Dhaka Hospital of ICDDR,B. All children will receive syrup pivmecillinum 60 mg/kg per day in four equal divided doses, for five days. They will be randomly assigned to either milk-suji supplemented with green banana (test diet) (n=110) or the milk-suji alone (control diet) without any supplementation (n=110). In a recent study, Salam et al. (48) found 65% clinical cure rate with pivmecillinum in shigellosis in children population. The sample size estimation is based on an assumption that addition of green banana will result in 85% improvement (30% specific) in the treatment groups at a 5% significant level with 80% power and assuming a drop out rate of ~56%. Actually we have to recruit 140 evaluable children (70 in each group). Additional 80 (56%) children (40 in each group) will be recruited to compensate for drop-outs, non-compliance and incomplete study. All the children will be selected from the outpatient department of Dhaka Hospital of ICDDR,B.

The sample size is calculated using the following statistical formula.

$$\frac{P_1(100-P_1) + P_2(100-P_2)}{(P_1 - P_2)^2} \times \alpha\,\beta$$

where: P_1 = 65% (observed clinical cure rate in std treatment)
P_2 = 85% (expected clinical cure rate in study (experimental) treatment)
α = 0.05 (level of significance)
β = 80% (statistical power)

Selection criteria

1. Age: 6 - 59 months.
2. Both boys and girls will be included.
3. Stool characteristics: Frankly bloody or bloody mucoid on inspection.
4. Duration of dysentery: $\leq$ 48 hours.
5. Optional symptoms: Fever, abd pain, anorexia, dehydration, vomiting.

Exclusion criteria

1. Failure to obtain parental consent.
2. Prior treatment with an antimicrobial and/ or antidiarrhoeal agent (loperamide, metronidazole).
3. Demonstration of erythrophagocytic trophozoite of *Entamoeba histolytica* or cyst of Giardia.
4. Severe malnutrition (weight for age $\leq$ 60% of NCHS median), and/or presence of nutritional edema.

5. Presence of any associated condition such as: pneumonia, sepsis, meningitis, severe dyselectrolytemia, hemolytic uremic syndrome, renal failure and toxic megacolon.
6. Failure to isolate any *Shigella* spp. from admission fecal/rectal swab culture.

Informed consent

Written, voluntary informed consent will be required from the parents or legal guardian of the patients before the child is admitted into the study.

Randomization

Patients will be assigned a study number upon enrollment into the study on admission, i,e, before starting the banana therapy. Such study numbers will be assigned consecutively, and a study diet will have been randomly pre assigned to each study number using a computer generated table of random numbers.

Clinical management

On admission, a clinical history and physical examination will be completed to assure that each patient fulfils the entry criteria of the study. Information obtained on admission will also be used to compare the treatment groups as baseline. The history and physical examination will include the followings:

a. Patients' identification, age, gender, date and time of admission, study random number.
b. Description and duration of symptoms prior to admission including stool character, amount and frequency of vomiting, abdominal pain, straining, fever, change of appetite etc.
c. Treatment provided prior to admission including ORS, intravenous fluid and drugs.
d. Physical examination to assess hydration status, nutritional status, and abnormal physical findings.

The children will be placed on a 'cholera cot' to facilitate quantitative collection of stool and urine. Urine will be collected separately from stool by using Pediatric Urine Collection (PUC) bags. All stool will be collected and frequency will be recorded by the attending mother and staff nurses. Patient status, body weight, weight of the stool and urine and physical characteristics of the stool will be assessed and recorded by well experienced staff nurses. A physician will evaluate the patients on each day, to specially assess the severity of a set of defined clinical symptoms. All intake-output and vital signs will be recorded in eight hourly schedule. Patients will be hospitalized for six days following initiation of study diets. Initial and subsequent dehydration if any, will be corrected using ORS or intravenous cholera saline as appropriate. Each child will receive syrup pivmecillinum 60 mg/kg per day in four equal divided doses for five days. Pivmecillinum resistant cases (resistant to pivmecillinum in this hospital in last year was ~5%) will receive alternate sensitive drug (e.g. ciprofloxacin).

Laboratory Procedures

1. **On admission**

   Stool microscopy for erythrocytes, pus cells and parasites. Stool/rectal swab cultures for isolation and identification of *Shigella* spp. *Salmonella* spp. *and V. cholerae.*

   Complete blood count (routine test).

2. **After 72 hours (study day 3)**

   Stool /rectal swab culture for *Shigella* spp. and microscopic examination of stool.

3. **After 120 hours (study day 5)**

   Stool/rectal swab culture for *Shigella* spp. and microscopic examination of stool.

4. **From admission to day-6**, all stool passed each day will be saved, homogenized, and its volume recorded; an aliquot of 50 mL from this sample will be stored at -20°C with added magnesium chloride as a bactericidal agent to prevent fermentation during storage, this sample will be subsequently analyzed for SCFA.

5. **Other tests** like serum electrolytes, creatinine, blood culture, urine analysis etc. will be performed if and when clinically indicated.

   [No additional blood will be drawn or any invasive procedure will be done specially for this study].

Study diets and double-blinding

Children will be fed milk suji (a standard milk based diet of this hospital), and for older children additional meals of steamed rice, chicken, potato, refined white bread and eggs. Breast-fed child will be allowed to take breast milk but each time there will be test weighing to calculate the amount of breast milk ingested by the child. During the study known amounts of food will be given with their known calorie contents. Since the plant fibers of many vegetables normally consumed by the patients may confound the effects of the dietary intervention, the children will not be given vegetable containing food, particularly curries containing leafy vegetables during the study period, the diets will be standardized according to fiber content (apart from interventions of green banana) as well as the proportion of carbohydrate, fat, and protein.

Green banana *(Musa sapientum)* will be procured locally and stored in the kitchen refrizerator. The fruits will be boiled, peeled, and the pulp blended using a standard kitchen blender, 200 g of banana pulp (wet weight) will be mixed with milk-suji (total volume 1000 mL) and will be offered freely to children (target 20 to 30g banana /kg body weight/day). For older children, over 2 years of age who usually take lesser amount of milk-suji, additional amount of banana pulp (to meet 20 to 30 g/kg/d) will be given in the form of 'chop' (fried banana pulp) made by banana pulp and mild

spices. For the purpose of double-blinding, chop will be made of banana or potato by adding an inert food colour (cocoa dust), so that the chops can not be distinguished by their appearances. The amount of banana pulp to be provided is the same amount we have previously used with good results and without any side effects in studies in children with persistent diarrhea (Rabbani et al. 1998). The approximate macronutrient composition of banana is carbohydrate 17.8 g, protein 1.2 g, and fat 0.1 g with an energy value of 75 kcal per 100 g (analyzed from ICDDR,B Nutrition Laboratory). The control group of children will receive the same diet but without supplemental banana. The older children (more than 2 years old) in this group will receive same amount of chop (as green banana group) made only with potato without any banana. The dietitian will prepare the diets and will keep the blind codes of the diets without any knowledge of the investigators and nursing personnel.

COMPOSITION OF DIFFERENT DIETS*

Ingredients/per 1000 ml	Milk Suji† (Control diet)	Banana diet
Full cream milk powder (g)	40	40
Rice powder (g)	40	5
Sugar (g)	25	25
Oil (Soya) (g)	25	25
Magnesium chloride (g)	0.5	0.5
Potassium chloride (g)	1	1
Calcium (g)	2	2
Green banana pulp (g)	0	200
Energy-kcal /100 ml	67	67
Protein g /100 ml	1.4	1.4
Protein energy ratio	8%	8%
Fat energy ratio	47%	47%

* Plus water as required to make it 1000 mL
†Standard milk based diet for patients 6 - 60 months old, in Dhaka hospital of ICDDR,B

Fecal short chain fatty acids

All stools will be collected daily for 6 days and their volume recorded. An aliquot of 50 mL of homogenized stool will be stored for SCFA assay. Specimen will be stored at - 20°C until analysis. Magnesium chloride ($MgCl_2$) will be added to the stool sample as a bactericidal agent to prevent fermentation during storage (23).The following SCFA will be determined by gas chromatography:

acetate, propionate, butyrate, valerate, and isovalerate. Briefly, homogenate of 2 - 10 gram of fecal sample will be prepared by the vacuum-distillation method described by Zijlstra et al. and modified by Treem et al. (49,50).The distillate is subsequently analyzed by gas liquid chromatography using a gas chromatograph (Hewlett-Packard (HP) Model 5890, Palo Alto, CA, USA) attached to an integrator (HP 3396 series II). Different concentrations of SCFA are prepared by using acetate, propionate, butyrate, valerate, and caproate, which are utilized as internal standards. All determinations of fecal SCFA will be run in triplicate and the mean expressed as the final value.
Fecal SCFA concentrations will be determined using HPLC either at the ICDDR,B laboratory or at the Christian Medical College, Vellore.
The possibility of determining SCFA concentrations in fecal specimens using HPLC at the ICDDR,B Nutritional Biochemistry Laboratory is currently being explored. It has been suggested that if we can buy a SCFA-specific column, it can be done here. In view of this the cost of a HPLC column has been included in the budget.

Evaluation of outcome (outcome variables)

A. Primary response variables:

Clinical cure

Bacteriologic cure

B. Secondary response variables:

- Stool frequency: Daily, and total stool frequency
- Stool characteristics: Time to last watery, and first formed stools, and last bloody-mucoid and mucus in stools.
- Fever: Time to last fever (rectal temp >38°C)
- Straining/tenesmus: Presence of straining/tenesmus on every study day, and time to last straining/tenesmus
- Number of WBCs and RBCs in stool microscopic examination after 72 and 120 hours
- Number of cases with negative culture for *Shigella* spp. after 72 and 120 hours
- Adverse events: Abdominal distention etc. (if any).

Definitions

Bacteriologic cure: Bacteriologic cure will be defined if *Shigella* are not isolated after study day 3, with subsequent stool samples remaining culture negative for *Shigella*.

Clinical cure: This will be judged on the basis of patient's status on study day 5. Patients having the following features will be considered to have clinical cure, and those having "Slight-improvement", or "Failure" will be considered to have clinical failure. Grading the responses into the above mentioned categories will be done as follows:

Clinical cure:

1. No frank bloody mucoid stool and
2. One or zero watery stool and
3. < 6 stool and
4. Afebrile (no rectal temperature >38^{0}C, or no more than one measurement of 38^{0}C)

Slight improvement:

1. One or zero bloody mucoid stool
2. 3 or less number watery stools
3. 9 or less number total stools
4. Afebrile (no rectal temperature measurement >38^{0}C, or no more than one measurement of 38^{0}C).

Failure:

1. More than 1 bloody mucoid stool, or
2. More than 3 watery stools, or
3. More than 9 total stools, or
4. Febrile (rectal temperature > 38^{0}C, or more than one measurement of 38^{0}C)

Study Medical Officer, Principal and/or other Investigators will assess and evaluate the above features/grading who will remain blinded about the type of the given diet.

Management of treatment failure and complications

Patients who will fail to respond by five days treatment, or develop complication will be transferred to General Ward or Special Care Unit of this hospital or other hospitals as necessary for clinical management.

Organization of the trial

Patients will be selected from the outpatient department of Dhaka Hospital of ICDDR,B, and will be admitted to the study ward if they fulfill the admission criteria. The Research Physician with the assistance of Principal and/or other investigators will take care of the patients for clinical management. The research data will be recorded in coded forms and will be preserved securely after completion of the study. Patients will be admitted in the study from 8:30 am to 5 pm.

DATA ANALYSIS

SPSS windows version 7 program will be used. Clinical cure rate, bacteriologic cure rate and other secondary outcome variables e.g. frequency and type of stool, presence/absence of fever, straining, rectal prolapse, etc. will be compared by chi-square test or Fisher's exact test if indicated. Means of stool volume, fluid and calorie intake, intravenous fluid/ ORS requirement between two groups will be compared by Student's t-test or Mann-Whitney U test. A p value < 0.05 will be considered

significant. Depending on the type of distribution of data, we will also apply Poissons's curve analysis, Rank Sum test (non-parametric) and Kaplan-Meiyer survival analysis.
Dropout patients (after randomization and before completion of the study) will also be included in analysis, and the data of those patients as long as they will maintain the protocolized management will be analyzed.

FACILITIES AVAILABLE

Site: The Research Ward of the Dhaka Hospital of ICDDR,B will be used for hospitalization of the study patients. The facility is being used for similar clinical studies for decades.
Study population: The Dhaka Hospital provides treatment to over 120,000 diarrheal patients each year, about 70% of whom are <5 years of age. Cholera and *Shigella* dysentery are endemic in Bangladesh, and this hospital is well known as the hospital for treatment for diarrhea and dysentery in and around Dhaka city (capital of Bangladesh) where many of the patients do come with shigellosis each year; study subjects will be selected from these patients' population.
Laboratory facilities: The Clinical Laboratory Services and Nutrition Biochemistry Laboratory of the Laboratory Sciences Division of ICDDR, B will be used for all study related laboratory investigations (test), including hematology, bacteriology, clinical chemistry, and other pathological tests that may be necessary. These laboratories are competent enough and regularly used for all study protocols.
Standard kitchen facility: The hospital has excellent kitchen facilities and qualified nutritionists, they use to serve different types of diet as required both for the general- and study patients.

ETHICAL ASSURANCE FOR PROTECTION OF HUMAN RIGHTS

This study will be done on children, aged 6 -59 months. Half of them will be provided with a diet supplemented with boiled green banana which is a constituent of normal food. The potential for adverse effects is very low, but would include mild abdominal distention. No invasive clinical procedures are planned as part of the study protocol. The clinical record of each patient will be kept confidential, and the final report will be published without reference to the identify of the individual.
As study patients are usually more closely monitored than the other inpatient children, a high degree of clinical care will be ensured. Normal hospital guidelines will be strictly adhered to with regard to the management of the Shigella patients. Finally, informed parental consent will be obtained prior to recruitment of each subject.

C V OF DR. MD. IQBAL HOSSAIN (PRINCIPAL INVESTIGATOR)

Educational Qualifications:

Examination		Institute Board/University	Year	Division/Class
a)	**D.C.H.**	Bangladesh Institute of Child Health, (Dhaka University) Dhaka, Bangladesh	1990	3rd position
b)	**M.B.B.S.**	Rajshahi Medical College, (Rajshahi University) Rajshahi, Bangladesh	May, 1985	Regular
c)	**H.S.C.**	Sirajganj College, Rajshahi, Bangladesh.	1979	First
d)	**S.S.C.**	Gnanadyini High School, Rajshahi, Bangladesh	1977	First

Experience:

1. Served as Medical Officer at Sirajganj Children Hospital (MCHC Centre) from 1.8.1986 to 31.3.1991.
2. Served as Junior Consultant and Chief Physician at Sirajganj Children Hospital (MCHC Centre) from 1.4.1991 to 30.4.1993.
3. Served as Medical Officer in ICDDR,B from 6.5.1993 to 30.4.1999.
4. Has been serving as Assistant Scientist in ICDDR,B since 1.5.1999.

Publications:

1. **Hossain MI,** Kabir AKMI, Khan WA and Fuchs GJ. Acinetobacter bacteraemia in patients with diarrhoeal disease. Epidemiol Infect 1998;120:139-42.
2. **Hossain MI,** Kabir I, Fuchs GJ, McCutcheon MJ. Alvarez JO, and Khaled MA. Intra and Extra-Cellular Water Dynamics on Rehydration in Cholera and non-Cholera Patients. Digestive Diseases and Sciences 1998;43:663-7.
3. **Hossain MI,** Yasmin R, Faruque ASG. Morbidity and morbidity pattern of children in a district paediatric hospital of Bangladesh. Bangladesh J Child Health 1997;21(1&2):1-5.
4. **Hossain MI,** Yasmin R, Kabir I. Nutritional and immunisation status, weaning practices and socioeconomic conditions of under five children in three rural villages of Bangladesh. Indian Journal of Public Health 1999;43(1):37-41.
5. Teka T, Faruque ASG, **Hossain MI** and Fuchs GJ. Aeromonas-associated diarrhoea in Bangladeshi children: clinical and epidemiological characteristics. Annals of Tropical Paediatrics 1999;19:15-20.

CV OF DR. G. H. RABBANI, CO-PRINCIPAL INVESTIGATOR

Academic Qualifications

1966 Secondary School Certificate Examination (SSC), (1st Division), Rajshahi Education Board, Rajshahi, Bangladesh.

1968 Higher Secondary Certificate Examination (HSC), (1st Division with distinction), Rajshahi Education Board, Rajshahi, Bangladesh.

1974 Bachelor of Arts (B.A.), Dhaka University, Bangladesh.

1975 Bachelor of Medicine & Surgery (MB, BS), Dhaka Medical College, University of Dhaka, Bangladesh.

1980 Master of Science (M.Sc., with distinction) in Community Health in Developing Countries, London School of Hygiene and Tropical Medicine, University of London, England.

1981 Diploma in Public Health (D.P.H), Royal Tropical Institute of Public Health and Hygiene, London, England.

1989 Doctor of Medicine (MD) and Fellowship of the American College of Gastroenterology (FACG).

1994 Ph.D from the University of Copenhagen, Denmark.

Professional Training and Experience

1975-76 Internship (House Job), Dhaka Medical College and Hospital, University of Dhaka, Bangladesh.

1976-77 Lecturer in the Department of Medical Physiology and Biochemistry, Dhaka Medical College, University of Dhaka, Bangladesh.

1977-78 Working experience on animal models of intestinal secretion with special reference to inhibition of secretion using pharmacological agents. Department of Medical Microbiology, University of Goteborg, Sweden.
(Reference: Professor Jan Holmgren MD).

1981-82 Clinical experience and virological studies at the Kenyatta Hospital, Nairobi, Kenya and Department of Microbiology, Free University of Brussels, Belgium.
(Reference: Professor George Zissis).

1982
1983 Certificate Course in Computer Programming, University of Engineering and Technology, Dhaka, Bangladesh.

1984 Certificate Course in Research Methodology of Clinical Trials in Diarrhoeal Diseases, Organized by the World Health Organization, ICDDR,B Dhaka, Bangladesh.

1988 Two years Fellowship in Pediatric Gastroenterology and Pediatric Gastroenterology And Nutrition, International Institute of Infant Nutrition and Gastrointestinal Diseases, State University of New York School of Medicine at Buffalo, New York, USA (Ref. Prof. Emanuel Lebenthal).

1989 Post Doctoral Training: Research Associate in Gastroenterology (1-Year Faculty Position), Yale University School of Medicine, Department of Internal Medicine, New Haven, Connecticut (Ref. Prof. Henry Binder, MD).

Selected Bibliography:

1. Rabbani GH, Lu RB, Horvat K, Lebenthal E. Short chain glucose polymer and anthracene-9-carboxycilic acid inhibit water and electrolyte secretion induced by dibutyryl cyclic AMP in the small intestine. **Gastroenterology**, 101:1046-1053, 1991.

2. Rabbani GH and Binder H. Evidence of active butyrate absorption by rat distal colon. **Acta Vet Scand** 1989;86:195.

3. Rabbani GH, Albert MJ, Rahman H et al. Development of an improved animal model of shigellosis in the adult rabbit by colonic infection with *Shigella flexneri* 2a. **Infection and Immunity** 1995;63:4350-4357.

5. Rabbani GH, Albert MJ, Rahman H, Islam M, Alam K. Short-chain fatty acids improve clinical, pathologic, and microbiologic features of experimental shigellosis. **J Infect Dis** 1999; 179: 390-397.

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3. Bennish ML and Wojtyniak BJ.Mortality from shigellosis. Community and hospital data. Rev Infect Dis 1991;13(suppl4):S245-51.
4. Keusch GT, Shigellosis. In? Fauci AS, Braunwald E, Martin JB. ... [et al.] (editors). Harrison's Principles of internal medicine. 14th ed. New York: McGrraw-Hill, 1998:957.
5. Anonymous. The burden of disease resulting from diarrhoea. In Division of Health Promotion and Disease prevention, Division of International Health, Institute of Medicine, New vaccine development: establishing priorities. Vol.2. Disease of importance in developing countries, Washington, D.C. National Academy Press, 1986:159-69.
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epithelium to refeeding in the rat and relationship to plasma gastrin, enteroglucagon, and PYY concentrations. Gut 1987;28:171-80.

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DISSEMINATION AND USE OF FINDING

The findings of the study will be disseminated as follows:

1. Presentation(s) at Scientific Forums, ICDDR,B for dissemination among scientists of the Centre.
2. Presentation at the Annual Scientific Conference (ASCON) of ICDDR,B for dissemination among scientists and health officials of the Govt. of Bangladesh and of the Non-Govt. Organizations.
3. Presentation(s) at Regional and International Scientific Conferences.
4. Publication in peer-reviewed international medical journal.

VOLUNTARY CONSENT FORM

The following information will be read out to parents or the legal guardians of patients in Bangla.

1. Your child is suffering from invasive diarrhea / dysentery which is probably due to infection with a germ named *Shigellae*. Management of this disease requires the use of effective drugs. Unfortunately the *Shigellae* germs have become resistant to most of the drugs once useful in its treatment. Therefore, we are attempting to find effective adjuvant therapy.

2. In the present study we are evaluation the therapeutic effects of green banana in the management of *shigellosis* in children.

3. Your child will be admitted into the hospital study ward for 6 days. During this period the child will be given the standard treatment for the illness, which may include normal feed, ORS, iv fluid, and drugs (e.g. syrup pivmecillinum 60 mg/kg body weight/day in four equal divided doses for 5 days).

4. Your child will be randomly assigned to any of the two different diet groups and there is equal chance that the child will get green banana supplemented or the normal hospital diet, milk-based (milk suji) and specially for the older children additional rice-chicken-based diet. As you may know that different preparations of cooked green banana (curry, bharta, chop etc.) is normally eaten in this country. If your child will assign to banana group he will be given a similar preparation of green cooked banana which will be mixed with milk based diet and/or given in chop form (for older children) and fed freely during the study period. The amount of cooked banana that will be given approximately half to one full banana per child per day (~ 20 to 30g/kg body weight per day). We do not expect any unpleasant effects due to feeding banana in children above the age of 5 months.

5. During the study, all stools, urine, and vomit will be collected and quantitated daily. Daily body weights will be measured.

6. If you have your child admitted into the study, you will still possess the right to withdraw from the study at any point in time. In that case, you will not be penalized anyway and your child will not be prevented from getting standard care of the hospital.

7. All medical records of your child's treatment will be kept confidential and will be published without reference to the name and identity of the child.

8. If you are confident that you have understood all these points and all your questions have been answered to your satisfaction, and you wish to have your child admitted into the study, please indicate your consent by signing your name or thumb printing in the space below.

_____ _____

Signature of the Investigator | Signature of Parent/Guardian

Date: _____ | Date: _____

Patient's Name _____ | Adm # _____ Date _____

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র
মহাখালী, বাংলাদেশ

সম্মতি পত্র

নিম্নলিখিত তথ্যাবলী রোগীর পিতা বা প্রকৃত অভিভাবককে পড়ে শোনাতে হবেঃ-

১। আপনার শিশু সম্ভবত সিগেলা নামক একধরণের জীবাণু দ্বারা আক্রান্ত হয়ে আমাশয়ে ভুগছে। এই রোগের চিকিৎসার জন্য কার্যকরী ওষুধের প্রয়োজন। দুর্ভাগ্যজনক যে, যে ওষুধের দ্বারা আগে চিকিৎসা করা হতো, সিগেলা জীবাণু সেই ওষুধগুলির বিরুদ্ধে প্রতিরোধী হয়ে উঠেছে। সুতরাং আমরা একটি কার্যকরী সহায়ক চিকিৎসা ব্যবস্থা খুঁজে বের করার চেষ্ঠা করছি।

২। বর্তমান গবেষণায় আমরা শিশুদের সিগেলা দ্বারা সংক্রমিত আমাশয়ের চিকিৎসায় কাঁচা কলার আরোগ্যকর ক্ষমতার মূল্যায়ন করছি।

৩। আপনার শিশুকে হাসপাতালের গবেষণা ওয়ার্ডে ৬ দিন ভর্তি রাখা হবে। এই সময়ে আপনার শিশুকে অসুস্থতার জন্য আদর্শ চিকিৎসা দেয়া হবে; যার মধ্যে থাকবে সাধারণ খাবার, খাওয়ার স্যালাইন, শিরাপথের স্যালাইন (যদি লাগে) এবং ওষুধ (পিভমিসিলিনাম, ৬০ মিগ্রা/কেঃজি/দিন, দিনে ৪ বার-৫ দিন)।

৪। আপনার শিশুকে দুইটি ভিন্ন ধরণের খাবারের যে কোন একটি খাবার দৈবচয়ন পদ্ধতিতে দেয়া হবে। আপনার শিশুর সমান সম্ভাবনা আছে কাঁচা কলার তৈরী খাবার অথবা হাসপাতালের খাবার যা দুধ জাতীয় খাবার পাওয়ার এবং অপেক্ষাকৃত বড় শিশুদের জন্য চাউল-মুরগীর মাংস সহযোগে তৈরী খাবার দেয়া হবে। আপনি জানেন যে, সাধারণতঃ আমাদের দেশে কাঁচা কলা রান্না করে বিভিন্ন ভাবে খাওয়া হয়, যেমন: তরকারী, ভর্তা, চপ ইত্যাদি। যদি আপনার শিশুকে কাঁচা কলা দেয়া হয়, তবে একইভাবে রান্না করা কাঁচা কলা দুধ জাতীয় খাবারের সাথে মিশিয়ে দেয়া হবে অথবা অপেক্ষাকৃত বড় শিশুদের চপ আকারে খেতে দেয়া হবে। একজন শিশুকে প্রতিদিন আনুমাণিক অর্ধেক থেকে একটি কাঁচা কলার খাবার দেয়া হবে (~২০ - ৩০ গ্রাম/কেজি/দিন)। পাঁচ মাসের অধিক শিশুদের কলা খাওয়ানোর ফলে কোন অপ্রীতিকর ফলাফল আমরা আশা করছি না।

৫। এই গবেষণা চলাকালে, প্রতিদিন আপনার শিশুর পায়খানা, প্রস্রাব এবং বমি মাপা হবে ও সংগ্রহ করা হবে। প্রতিদিন আপনার শিশুর ওজনও নেয়া হবে।

৬। যদি আপনি, আপনার শিশুকে এই গবেষণায় অন্তর্ভুক্ত করেন, তারপরও আপনি আপনার শিশুকে যে কোন সময় প্রত্যাহার করতে পারবেন। সেক্ষেত্রে আপনার কোন ক্ষতি হবে না এবং আপনার শিশুর হাসপাতালের আদর্শ চিকিৎসা গ্রহণে কোন বাধা থাকবে না।

৭। আপনার শিশুর চিকিৎসা সম্বন্ধীয় সকল তথ্য গোপন থাকবে এবং আপনার শিশুর নাম ও পরিচয় ছাড়া তা প্রকাশিত হবে।

৮। যদি আপনি মনে করেন যে, আপনি এই গবেষণার প্রতিটি শর্ত বুঝেছেন, আপনার সকল প্রশ্নের সন্তোষজনক উত্তর আপনি পেয়েছেন এবং আপনি আপনার শিশুকে এই গবেষণায় অন্তর্ভুক্ত করতে চান, তবে আপনার সম্মতি স্বরূপ আপনি নিচে স্বাক্ষর করুন অথবা টিপ সহি দিন।

গবেষকের স্বাক্ষর	সাক্ষীর স্বাক্ষর	পিতা-মাতা/অভিভাবকের স্বাক্ষর/টিপ সহি
তারিখঃ	তারিখঃ	তারিখঃ

রোগীর নাম	ভর্তি নং	তারিখঃ

Budget
Project Title: Clinical evaluation of green banana (amylase-resistant starch) in the management of childhood shigellosis

P.I: M.Iqbal Hossain,MBBS,DCH
Co-PI: G. H. Rabbani, MD, PhD
Duration: 2 years
Donor- USAID/W

Personnel	**% effort**	**1st year**	**2nd year**	**US$ Total**
Dr. M. Iqbal Hossain, PI	20	2,100	2,190	4,290
Dr. G. H.Rabbani, Co-PI	15	1,050	1,100	2,150
Dr. Ali Miraj Khan,Co-Investigator	10	665	685	1,350
Mrs. Naseha Khatun, Co-Investigator	10	520	550	1,070
Dr.George J Fuchs, Consultant	5	-	-	-
Research Physician, MD	100	2,350	2,400	4,750
Research Assistant-1, MSc	100	2,000	2,050	4,050
Health Worker-3	100	1,800	1,900	3,700
Sub-total:		**10,485**	**10,875**	**21,360**
International Travel				
(To disseminate study findings at Seminar/Conference)		3000	3000	6,000
Sub-total:		**3000**	**3000**	**6000**
Supplies and materials:				
Office stationery		350	50	400
Drugs		400	200	600
Materials for study i.e., (raw banana, fibre test kits, stool/urine collection bags etc.)		800	400	1,200 -
Others		300	100	400
Sub-total:		**1,850**	**750**	**2,600**
Other Contractuals				
Fax,phone,DHL,postage etc		100	50	150
Xerox & mimeography		100	50	150
Sub-total:		**200**	**100**	**300**
Interdepartmental Services				
Laboratory cost (Stool M/E, C/S, CBC, Electrolytes, etc.)		2850	2,000	4,850 -
SCFA test in Stool, HPLC column etc.		4,000	1,000	5,000
Local transport		800	500	1,300
Patient hospitalization				
Evaluable case (140 pt x 6 d x $ 25/d)		11,000	10,000	21,000
Absconded / drop out (22 pt x 3 d x $ 25/d)		950	700	1,650
Sub-total:		**19,600**	**14,200**	**33,800**
Capital Expenditure				
Refrigerator		600	0	600
Computer, Printer, UPS etc.		3,000	0	3,000
Sub-total:		**3,600**	**-**	**3,600**
Total Direct Cost		**38,735**	**28,925**	**67,660**

Budget Justification:

Budget Justification:

Personnel expenses: In this trial the PI and Co-PI will contribute 20% and 15% of their efforts respectively. There will be one Co-investigator, dietitian, one medical officer, one research officer and 3 hospital assistants. A total amount of $21,360 for 2 years have been allocated for all personnel expenses according to the salary structure of ICDDR,B. We think this amount is justified.

Patients' Hospitalization: This is a major line item which would require $33,800 for hospital bed and treatment including laboratory cost of investigations of 162 patients over the 2 year period at the present rate of expenses at the Centre ($25/day).

Supplies and materials: Items like office stationery, drugs, banana, clinical supplies will require $2,600 for 2 years.

Contractual services, including fax, phones, DHL services will need $300 for 2 years.

Capital expenses: An amount of $3,600 has been budgeted for procuring necessary capital items including a refrizerator and a PC with printer and UPS.

Travel: An amount of $6,000 has been budgeted for international travel for the investigators to present the results of the study at important international meetings/conferences/seminars as a means for disseminating the study findings.

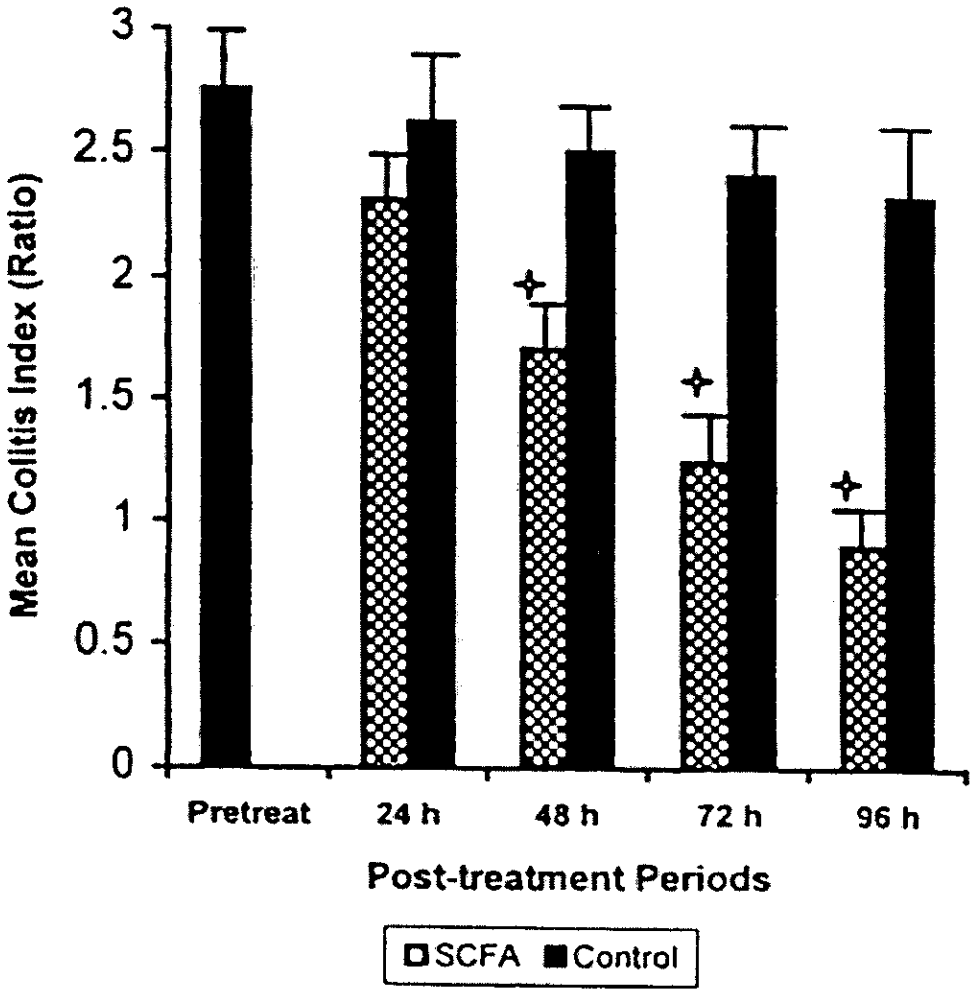

Figure 1. Effects of SCFA treatment on histologic improvement of colitis due to *S. flexneri* 2a infection in rabbits as assessed by colitis index (CI). CI is established by assigning numeric scores according to severity of inflammation: mild = 1.0, moderate = 2.0, and severe = 3.0 points. CI is defined as ratio between sum of histologic scores and total no. of autopsy specimens. Solid bars show mean CI of control rabbits; patterned bars show those of SCFA-treated rabbits. Asterisks indicate statistically significant *P* values (<.05 or <.01). Gradual decline of CI values in SCFA-treated rabbits indicates significant improvement of colitis; marginal decrease in CI values in control rabbits indicates no improvement.

Source (Rabbani et al. Ref- 36)

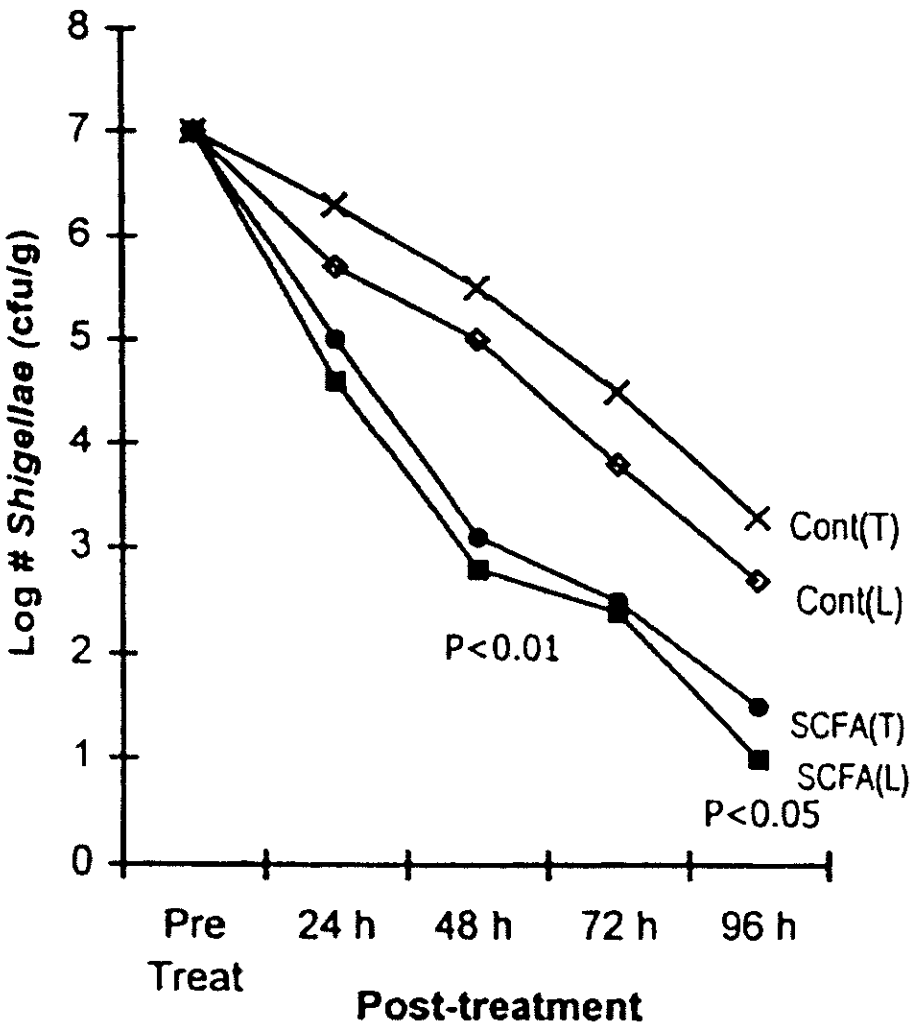

Figure 2. Antibacterial effects of SCFA treatment in experimental shigellosis in rabbits. Each point represents mean count (cfu/g) of viable *S. flexneri* 2a recovered from colonic lumen (L) or tissue (T) of SCFA-treated or control (untreated) rabbits. SCFA treatment significantly reduced bacterial counts (cfu/g) in colon compared with untreated animals at 48 h and 96 h after starting treatment.

Source (Rabbani et al. Ref- 36)

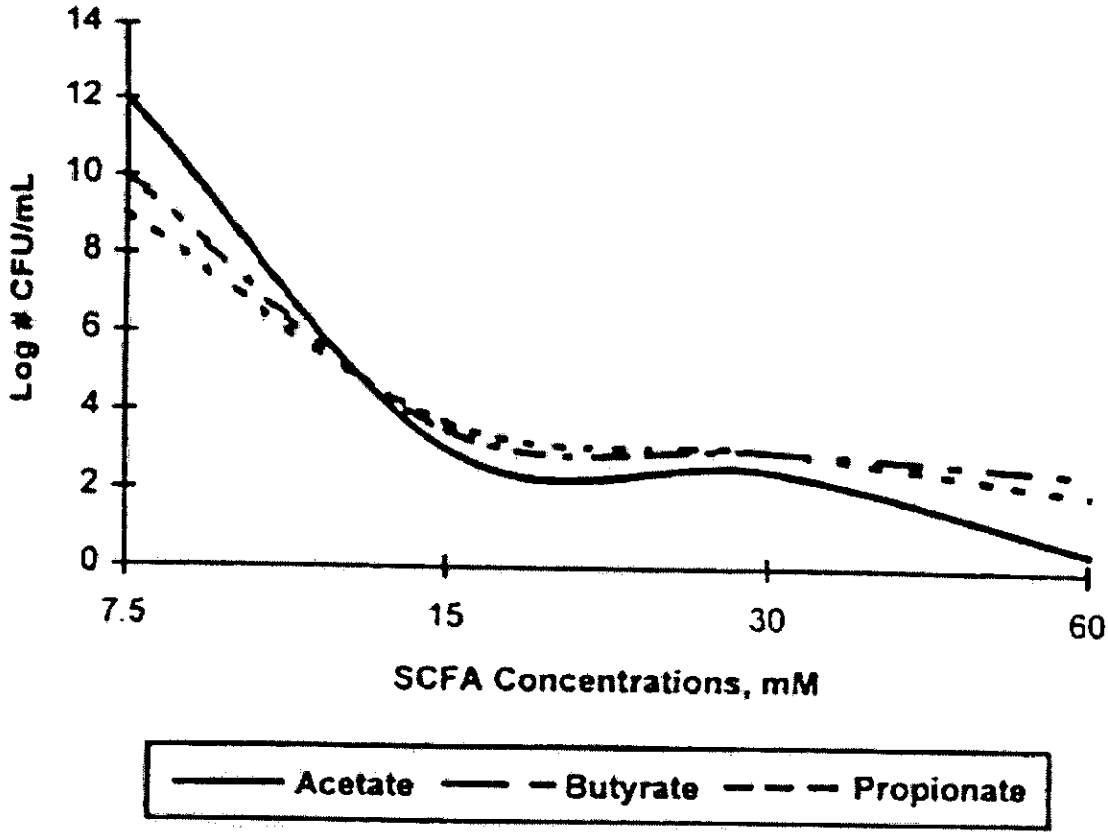

Figure 3. In vitro bactericidal effects of different SCFA on *S. flexneri* 2a. Bacteria were grown on Mueller-Hinton broth containing different SCFA at different molar concentrations and then plated on Mac-Conkey agar. After overnight incubation at 37°C, colonies were counted and expressed as no. of colony-forming units (cfu/mL). There is dose-dependent antibacterial effects of all 3 SCFA; 60 m*M* acetate produced highest inhibition, followed by 40 m*M* propionate and 30 m*M* n-butyrate.

Source (Rabbani et al. Ref- 36)

Responses to Reviewer's Comments

Reviewer A: Dr. Dilip Mahalanabis

1. We agree with the suggestion of this reviewer that the title does not seem to be appropriate and accordingly we have changed the title into: **Clinical evaluation of green banana (amylase-resistant starch) in the treatment of childhood shigellosis.** (Please see the Title Page).
2. The study will be a double-blind study, the test diet will contain milk-suji with cooked banana (test), and in the control diet, banana will be replaced by rice powder, providing equivalent calories. These diets can not be distinguished by their appearances or taste.
3. As rightly suspected by the reviewer, green banana could cause digestive problem in children less than 6 months old; to avoid this problem, we will give this diet to children between 6 months and 60 months of age. Please see the inclusion criteria in the Method Section of the revised protocol.
4. As suggested, for patients dropped after enrollment will have their information and outcome recorded and will be replaced by a new entry from the random list. Please see Method Section of the revised protocol.
5. As suggested, concealing of the patients' random number will not be necessary because now we will carry out the trial as a complete double-blind study.
6. Children's stool will not be collected in diapers but in the cholera-bed and thus plastic diapers will not be needed.
7. Further clarification about the definition of bacteriologic cure with regard to stool culture methods has been provided in the Methods Section of the revised protocol.
8. Confusions about the definitions of clinical cure on the basis of stool characteristics have been clarified in the revised protocol.

Reviewer B: Prof. B. S. Ramakrishna

1. As mentioned earlier, we have changed the title of this protocol according to the suggestions of both the reviewers.
2. As suggested by the reviewer, we will now measure total SCFA out in feces by collecting all stools daily and taking known aliquot for measuring SCFA concentrations. The methods have been mentioned in detail in the Methods Section of the revised protocol.

We hope that our responses to the reviewers' comments and corresponding revisions thereto would be acceptable to the Research Review Committee.
Thanks.

G. H. Rabbani, MD, PhD
Co-Principle Investigator

Title: Dietary fibers and short-chain fatty acids in the management of childhood shigellosis.

Summary of the Referee's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate page.

	Rank Score		
	High	Medium	Low
Quality of project	✓		
Adequacy of project design		✓	
Suitability of methodology	✓		
Feasibility within the time period		✓	
Appropriateness of budget	✓		
Potential value of field of knowledge	✓		

CONCLUSIONS

I support the application:

a. Without qualification ☐

b. With qualification ☑

- on technical grounds ☐
- on level of financial support ☐

I do not support the application ☐

Name of Referee: DILIP MAHALANABIS

Signature: Mahalanabis. Date: 13.3.2000

Position: DIRECTOR

Institution: Society for Applied Studies, Calcutta

Detailed Comments:

Please briefly provide your opinion of this protocol, giving special attention to the originality and feasibility of the project, its potential for providing new knowledge and the justification of the financial support sought; including suggestions for modifications (scientific or financial) where you feel they are justified.

Title: Dietary fibers and short-chain fatty acids....childhood shigellosis.

PI: Dr. M. Iqbal Hossain

The study is important both from the biomedical and public health point of view. The investigators propose to use an edible source of 'fiber' which would generate SCFA in the colon when ingested as a food in patients with shigellosis. The investigators are competent to conduct such a study. I fully support this proposal subject to their attending to the following comments:

I have the following comments which the PI may address:

1. It is a controlled trial of cooked green banana in childhood shigellosis; but the title does not indicate it.
2. It should be stated that the study cannot be blinded.
3. Could cooked green banana cause any problem in infants i.e. <1 yr ? The investigators may have data on this.
4. Page 6: For patients dropped after enrollment, full record on cause of illness and outcome should be kept. This is necessary for all clinical trials.
5. Page 7, Randomization: Because the study is not double-blind, it is important that the information on patient assignment to treatment is not known to the investigators until a patient has been definitely enrolled. This can be done by sealing the assignment code in serially numbered envelopes; the serial numbers then correspond to the patient ID numbers.
6. Page 7, Clinical Management: If the stool is collected in plastic diapers, what is the need for the cholera-cot collection method?
7. Page 7 & 10, Stool Culture and bacteriologic cure: Culture will be done on day 3 and day 5, but the definition for bacteriologic cure is culture negative after day 3 and consecutive days.
8. Page 10, clinical cure definition: The definition for "Resolution of Illness" mentions " ..... Dysenteric stool" (no mention of bloody/bloody-mucrid). But, the definition for marked/slight improvement and failure are all based on watery stool. This should be recitified.

In conclusion, I fully support this research proposal. The comments above should not be difficult for the PI to attend to.

13/3/2000

Title: Dietary fibers and short-chain fatty acids in the management of childhood shigellosis

Summary of Referee's Opinions:

Quality of project	High
Adequacy of project design	High
Suitability of methodology	High (see comments about SCFA)
Feasibility within time period	High
Appropriateness of budget	High
Potential of field of knowledge	Medium to high

CONCLUSIONS

I support the application with minimal qualification (on technical grounds).

Name of Referee:

Signature: (*this is a copy sent by e-mail)* Date: November 22, 1999

Position:

Institution:

Detailed Comments

Title: Dietary fibers and short-chain fatty acids in the management of childhood shigellosis

PI: Dr. M. Iqbal Hossain

The proposed study will evaluate the use of supplemental cooked green banana on the clinical and bacteriological outcome of shigellosis in children. The investigators correctly state that green banana is (1) commonly used as a traditional remedy for diarrhea; and (2) that green banana would provide a good source of pectin and starch for fermentation in the colon to short chain fatty acids. There is thus a rationale for trying out the efficacy of green banana in reducing diarrhea, increasing SCFA, and hastening bacteriologic recovery in childhood shigellosis.

Strictly speaking, this is a trial of green banana in management of shigellosis, and the title of the project should reflect this. If green banana is indeed found to be effective, it will remain to be proved that it acts through SCFA formation. Banana is also a source of other biologically active molecules, which could potentially contribute to any observed effect.

The study is well designed with regard to dietary interventions, study numbers, collection of data, inclusion and exclusion criteria. Keeping proper account of intake would be important. Since feces will not be quantitatively collected for SCFA, it appears that only SCFA concentrations would be measured. This may be fallacious, since SCFA concentrations would anyway rise as a result of resolution of watery diarrhea. I.e., as stool water decreases (during recovery) SCFA concentrations will rise. In other words, this will only tell us that green banana works, not that it works through SCFA production. Total SCFA output in the feces, although it has its own drawbacks, will provide a slightly better measure of SCFA production.