CSD 2002

International Centre for Diarrhoeal Disease Research, Bangladesh
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Memorandum

1 April 2002

To : Dr. G. H. Rabbani
Clinical Sciences Division

From: Professor Mahmudur Rahman
Chairman, Ethical Review Committee (ERC)

Sub : **Approval of protocol # 2002-003**

Thank you for your memo of 25th March 2002 attaching the modified version of the protocol# 2002-003 entitled "Clinical trial of L-Histidine in childhood Shigellosis". The modified version of the protocol is hereby approved upon your satisfactory addressing of the issues raised by the ERC in its meeting held on 6th March 2002.

You shall conduct the study according to the ERC-approved protocol; and shall be responsible for protecting the rights and welfare of the subjects and compliance with the applicable provisions of the ERC Guidelines. You shall also submit report(s) as required under the ERC Guidelines. Relevant excerpt of the ERC Guidelines is attached for your information and guidance.

I wish you all the success in running the above mentioned study.

copy: Acting Associate Director
Clinical Sciences Division

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CENTRE
FOR HEALTH AND
POPULATION RESEARCH

Memorandum

10 March 2002

To : Dr. G. H. Rabbani
Clinical Sciences Division

From: Professor Mahmudur Rahman
Chairman, Ethical Review Committee (ERC)

Sub : Protocol # 2002-003

Thank you for your protocol# 2002-003 entitled "Clinical trial of L-Histidine in childhood Shigellosis" ", which the ERC considered in its meeting held on 6th March 2002. After review and discussion, the Committee made the following observations on the protocol:

a) The description about the ERC was not considered to be part of the protocol. As such this part should be deleted.

b) The Committee expressed concerned whether 1-2 ml of blood would be sufficient for doing all the tests indicated in the protocol.

c) All items of para 4 of the ERC Face Sheet should be marked YES instead of NA. Item 2(d) both yes and no have been marked which should be corrected.

d) The data of should be shared with the ERC.

e) Bangla and English version of the consent forms are not identical. They should be similar (Item no. 5 in the English version is missing in the Bangla version. In cases of item 2-4, some information have been withheld in Bangla version). Further, the word `২৮৫' (item # 8 of the Bangla consent form) is not appropriate.

f) Information regarding funding (page 3 of the RRC application) should be correctly provided.

g) It was not clear to the Committee as to why the investigators plan to use ciprofloxacin though they have mentioned that nalidixic acid is the drug of choice in Bangladesh because it is sensitive to more than 90% of isolates (at para 6 of page 3).

h) The data of the first phase of the study should be submitted before the ERC for its consideration for approval of the second phase.

You are, therefore, advised to modify the protocol incorporating the above observations and submit the modified version of the protocol for consideration of the Chair.

Copy: Acting Associate Director
Clinical Sciences Division

Prof A K M Nurul Anwar
MBBS, MPhil, PhD, FCPS
Vice-Principal

IBRAHIM MEDICAL COLLEGE
An Institution of Diabetic Association of Bangladesh
Ibrahim Sarani, Segunbaghicha, Dhaka-1000.
Tel: 9567623

31 March 2002

Prof. Mahmudur Rahman
Chair, Ethical Review Committee, ICDDR-B

Sub: Revision of protocol # 2002-2003.

Thank you for your reference on the above subject.

I have gone through the responses of Dr. GH Robbani and the revision he has made in the revised protocol in response to comments made by ERC.

I understand he has satisfactorily explained and /or addressed all the issues raised by ERC.

You may kindly consider the approval of the proposal.

Thanking you

31/3/02

(Prof. A.K.M. Nurul Anwar)

To
Professor AKM Nurul Anwar.
Member, Ethical Review Committee

Sir,
It would be appreciated if you would kindly review whether the PI has addressed and incorporated all the observations of the Committee or not, in the modified version of the protocol.
The draft ERC minutes are attached for your reference.
Regards. Bejoy
25/3/2002

MEMORANDUM

25 March 2002

To: Professor Mahmudur Rahman
Chair, Ethical Review Committee

From: G H Rabbani, PI
Clinical Sciences Division

Subject: Revision of the Protocol # 2002-003

Thank you for your letter of 10 March 2002 along with the comments of the ERC. I have revised the protocol according to the comments of ERC and provided the following responses in relation to the specific comments made by the ERC.

- a). The ERC suggested that the description of ERC should be deleted from the protocol because it is not a "part" of the protocol itself.

  I am in full agreement with the comment of the ERC. However, the Thrasher Fund, USA which is the donor of the study requires that the protocol must provide evidence of ethical assurance and descriptions of IRB to satisfy the US-NIH guidelines for trial involving children. We have included this information because of the specific request by the Thrasher's reviewers. We would thus appreciate if ERC allows us to keep in the description of ERC in the protocol as required by the funding agency.

- b). We have checked the concern of the ERC whether 1-2 mL blood would be sufficient for all the tests. We found that this amount of blood which will be taken on 3 occasions during the hospitalization and would be just about right to carry out the tests since these will be done by a microassay system that requires very small amount of samples.

- c). As suggested, all entries under para 4 of the Face Sheet have marked YES in stead of NA. Item 2 (d) has been corrected.

- d). As suggested by ERC, the data of the safety trial in children will be shared with the ERC as soon as the data become available.

- e). The Bangla Consent Form has been corrected with specific changes suggested by ERC. Revised copy enclosed.

- f). We have now corrected the funding information on page 3 of the RRC Application Form.

- g). In response to the comments of ERC as to why we are prescribing ciprofloxacin in stead of nalidixic acid to treat shigellosis, we would like to mention that nalidixic is still a good drug for treating shigellosis caused by S. flexneri since most strains are sensitive to it. This is not true for S. dysenteriae, most of which are resistant to nalidixic acid but sensitive to cirprofloxacin. Since we will not know the type of the organism and its drug sensitivity at the time of admission when treatment has to be given, it is better to prescribe an antibiotic such as ciprofloxacin which is effective against both the organisms. Besides, ciprofloxacin is a newer drug, well-studied at ICDDR,B and is unlikely to become resistant soon; it is also active against a number of other enteric pathogens. This is why the reviewers from Thrasher Fund has recommended the use ciprofloxacin for this study in stead of nalidixic acid. All these have been described in the protocol.

- h). As suggested by the ERC, the results of the first phase of the study on safety trial will be submitted to ERC for its review and approval before beginning the second phase.

I would appreciate if you please approve the revised protocol in consideration of the above mentioned responses.

Thank you.

আর্ন্তজাতিক উদরাময় গবেষনা কেন্দ্র
মহাখালী, ঢাকা, বাংলাদেশ।

" রক্ত আমাশয় রোগে এল-হিস্টিডিন এর কার্যকারিতার উপর গবেষণা"

সম্মতি পত্র

নিম্নলিখিত তথ্যাবলী রোগীর পিতা বা প্রকৃত অভিভাবককে পড়ে শোনানো হবে

১। আপনার শিশু সম্ভবতঃ সিগেলা নামক এক ধরনের জীবাণু দ্বারা আক্রান্ত হয়ে রক্ত আমাশয়ে ভুগছে। এই রোগের চিকিৎসার জন্য কার্যকরী ওষুধের প্রয়োজন। দূর্ভাগ্যজনক যে, যে ওষুধের দ্বারা আগে চিকিৎসা করা হতো, সিগেলা জীবানু সেই ওষুধগুলির বিরূদ্ধে প্রতিরোধী হয়ে উঠেছে। সুতরাং আমরা একটি কার্যকরী সহায়ক চিকিৎসা ব্যবস্থা খুঁজে বের করার চেষ্টা করছি।

২। বর্তমান গবেষনায় আমরা শিশুদের সিগেলা দ্বারা সংক্রমিত আমাশয়ের চিকিৎসায় এল-হিষ্টিডিন নামক এক প্রকার আমিষ জাতীয় উপাদানের (এ্যামিনো এসিড) আরোগ্যকর ক্ষমতার মূল্যায়ন করছি। এল হিষ্টিডিন এক ধরনের এ্যামিনো এসিড যা' গরুরমাংস ও মুরগীর মাংস জাতীয় খাবারে থাকে। মানুষের বিভিন্ন অসুস্থতায় এই- এ্যামিনো এসিড চিকিৎসা হিসাবে ব্যবহৃত হয়েছে। যদিও খাবারের সংগে আমরা এল- হিষ্টিডিন খেয়ে থাকি, এ্যান্টিবায়োটিক (সিপ্রোফ্লোক্সাসিন) ওষুধের সংগে একত্রে রোগীকে দিলে এর কার্যকারিতা বৃদ্ধি করে কি না তা' আমরা পরীক্ষা করে দেখছি।

৩। আপনার শিশুকে হাসপাতালের গবেষণা ওয়ার্ডে ৭ দিন ভর্তি রাখা হবে। এই সময়ে আপনার শিশুকে অসুস্থতার জন্য প্রয়োজনীয় চিকিৎসা দেয়া হবে; যার মধ্যে থাকবে সাধারন খাবার, খাওয়ার স্যালাইন, শিরাপথের স্যালাইন (যদি লাগে) এবং ওষুধ (সিপ্রোফ্লোক্সাসিন ১৫ মিঃ গ্রাঃ/কেজি ওজন, ৮ ঘন্টা অন্তর)

৪। আপনার শিশুকে এল-হিষ্টিডিন চিকিৎসা দেয়া হবে কি হবে না তাহা দৈবচয়ন পদ্ধতিতে দেয়া হবে। অর্ধেক সম্ভাবনা আছে এল- হিষ্টিডিন চিকিৎসা পাবে এবং অর্ধেক সম্ভাবনা এল- হিষ্টিডিন ছাড়া চিকিৎসা পাবে। এই গবেষণা চলাকালে প্রতিদিন আপনার শিশুর পায়খানা, প্রশ্রাব এবং বমি মাপা হবে ও পরীক্ষার জন্য সংগ্রহ করা হবে। প্রত্যেকদিন ওজনসহ অন্যান্য রোগের লক্ষনাদি যেমন, তাপমাত্রা, নাড়ী, শ্বাস প্রশ্বাসের গতি মাপা হবে।

৫। চিকিৎসা চলাকালীন চিকিৎসা ও গবেষনার স্বার্থে আপনার সন্তানের বাহুর শিরা থেকে মোট ৩ বার (প্রথম, দ্বিতীয় ও চতুর্থ দিন) ১ থেকে ২ মিঃ লিঃ এর মত রক্ত সংগ্রহ করা হবে।

৬। যদি আপনি, আপনার শিশুকে এই গবেষনায় অন্তর্ভূক্ত করেন, তারপরও আপনি আপনার শিশুকে যে কোন সময় প্রত্যাহার করতে পারবেন। সেক্ষেত্রে আপনার কোন ক্ষতি হবে নাএবং আপনার শিশুর হাসপালের আদর্শ চিকিৎসা গ্রহনে 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 উপরিল্লিখিত সকল বিষয় বুঝেছেন, আপনার সকল প্রশ্নের সন্তোষজনক উত্তর আপনি পেয়েছেন এবং আপনি আপনার শিশুকে এই গষেণার অর্ন্তভূক্ত করতে চান, তবে আপনার সম্মতি স্বরূপ আপনি নীচে স্বাক্ষর করুন অথবা টিপসহি দিন।

গবেষকের স্বাক্ষর	স্বাক্ষীর স্বাক্ষর	পিতা/মাতা/অভিভাককের স্বাক্ষর/টিপসহি

রোগীর নাম _____ ভর্তি নং _____ তারিখ: _____

REVISED

Attachment 1 Date:

(FACE SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator: G-H. Rabbani

Application No. 2002 – 2003

Title of Study: CLINICAL TRIAL OF L-HISTIDINE IN CHILDHOOD SHIGELLOSIS

Trainee Investigator (if any): _____

Supporting Agency (if Non-ICDDR,B) Thrasher Fund

Project Status:

[✓] New Study

[ ] Continuation with change

[ ] No change (do not fill out rest of the form)

Circle the appropriate answer to each of the following (If Not Applicable write NA)

1. Source of Population:
   - (a) Ill subjects — (Yes) No
   - (b) Non-ill subjects — Yes (No)
   - (c) Minor or persons under guardianship — (Yes) No

2. Does the Study Involve:
   - (a) Physical risk to the subjects — Yes (No)
   - (b) Social risk — Yes (No)
   - (c) Psychological risks to subjects — Yes (No)
   - (d) Discomfort to subjects — Yes (No)
   - (e) Invasion of privacy — Yes (No)
   - (f) Disclosure of information damaging to subject or others — Yes (No)

3. Does the Study Involve:
   - (a) Use of records (hospital, medical, death or other) — (Yes) No
   - (b) Use of fetal tissue or abortus — Yes (No)
   - (c) Use of organs or body fluids — (Yes) No

4. Are Subjects Clearly Informed About:
   - (a) Nature and purposes of the study — (Yes) No
   - (b) Procedures to be followed including alternatives used — (Yes) No
   - (c) Physical risk — (Yes) No
   - (d) Sensitive questions — (Yes) No
   - (e) Benefits to be derived — (Yes) No
   - (f) Right to refuse to participate or to withdraw from study — (Yes) No
   - (g) Confidential handling of data — (Yes) No
   - (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — (Yes) No

5. Will Signed Consent Form be Required:
   - (a) From subjects — Yes (No)
   - (b) From parents or guardian (if subjects are minor) — (Yes) No

6. Will precautions be taken to protect anonymity of subjects — (Yes) No

7. Check documents being submitted herewith to Committee:
   - ✓ Umbrella proposal - Initially submit an with overview (all other requirements will be submitted with individual studies
   - ✓ Protocol (Required)
   - ✓ Abstract Summary (Required)
   - ✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw) (Required
   - ___ Informed consent form for subjects
   - ✓ Informed consent form for parent or guardian
   - ___ Procedure for maintaining confidentiality
   - ___ Questionnaire or interview schedule*

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary

1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy
2. Example of the type of specific questions to be asked in the sensitive areas
3. An indication as to when the questionnaire will be presented to the Committee for review

We agree to obtain approval of the Ethical Review Committee for any changes involving the rights and welfare of subjects before making such change.

G-H. Rabbani
Principal Investigator

_____
Trainee

ICDDR,B: Centre for Health & Population Research **RRC APPLICATION FORM**

RESEARCH PROTOCOL

Protocol No.: 2002– 003

FOR OFFICE USE ONLY

RRC Approval: Yes/ No Date:

ERC Approval: Yes/No Date:

AEEC Approval: Yes/No Date:

Project Title: Clinical Trial of L-Histidine in Childhood Shigellosis.

Theme: *(Check all that apply)*

- ☐ Nutrition
- ☐ Emerging and Re-emerging Infectious Diseases
- ☐ Population Dynamics
- ☐ Reproductive Health
- ☐ Vaccine evaluation
- ☐ Environmental Health
- ☐ Health Services
- ☐ Child Health
- ☑ Clinical Case Management
- ☐ Social and Behavioural Sciences

Key words: Shigellosis, L-Histidine, Ciprofloxacin

Principal Investigator: Dr. G. H. Rabbani, MD, PhD, FACG **Division:** CSD **Phone:** Ext.2321

Address: CSD, ICDDR, B
Mohakhali, Dhaka.

Email: rabbani@icddrb.org

Co-Principal Investigator(s): Prof.David A Sack, MD
Director, ICDDR, B

Co-Investigator(s): 1) Johny W Peterson, PhD
Professor, Department of Immunology and Microbiology, University of Texa
at Galveston, Texas, USA
2) Dr. G. B. NAIR, Head, LSD ICDDR,B

Student Investigator/Intern:

Collaborating Institute(s): University of Texas at Galveston, Texas, USA

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 5–60 months old children)
- ☐ Animal

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:

Total sample size: 225 ☐

Sub-group _____ ☐

1:1 randomization between Treatment and Control groups ☐

_____ ☐

_____ ☐

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

- ☐ Human exposure to radioactive agents?
- ☐ Fetal tissue or abortus?
- ☐ Investigational new device?
  (specify _____).
- ☑ Existing data available from Co-investigator
- ☐ Human exposure to infectious agents?
- ☐ Investigational new drug
- ☑ Existing data available via public archives/source
- ☐ Pathological or diagnostic clinical specimen only
- ☐ Observation of public behaviour
- ☑ New treatment regime (with established amino acid L-Histidine)

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 behaviour: sexual behaviour, 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 risk
- ☑ no more than minimal 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".

Project Application for ERC: 25 February 2002

Title: Clinical Trial of L-Histidine in Childhood Shigellosis

Principal Investigator/Program Director
Name and Address:

G. H. Rabbani, M.D., Ph.D, FACG
Scientist, Clinical Sciences Division
Head, Physiology Laboratory
ICDDR,B: Centre for Health and Population Research
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Co-Principal Investigator:
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Dr. G. B. Nair, PhD.
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Human Subjects:	**No**	**Yes**	**Pending**	**N/A**
Animal Welfare Assurance:	**Yes**	**Pending**	**N/A**	

Project Period: **Date from: March 2002 through February 2004**
Total Budget Request: **US$ 215,972**
Performance Site: ICDDR,B: Centre for Health and Population Research
GPO Box 128, Dhaka 1000,Bangladesh
Fax: 8802 8823116; Email: dsack@icddrb.org
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Applicant's Organization:
ICDDR,B: Centre for Health and Population Research
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Official Signatures of the Applicants:

We the undersigned, certify that the statements herein are true and complete to the best of our knowledge and that facilities are available for the proposed research. We accept the obligation to comply with the Thrasher Research Fund's Conditions of Grant and requirements for reporting in effect at the time of the award.

Print/Type name of principal investigator/ program director

Dr. G. H. Rabbani, M.D., Ph.D, FACG
Signature: Signed
Title: Scientist, ICDDR,B and Principal Investigator
Date: 29 April 2001

Prof. David A Sack, M.D.
Signature: Signed
Title: Director, ICDDR,B and Co-Principal Investigator
Date: 30 April 2001

Prof. J W Peterson, Ph.D.
Co-PI Signature: Signed
Title: Professor , Univ. of Texas Medical Branch at Galveston

Dr. G B Nair, PhD
Title: Head, Lab Sciences Division, ICDDR,B

Yes/No

☑ ☐ Is the proposal funded?

If yes, sponsor Name: Research Fund, Thrasher, USA

Yes/No

☑ ☐ Is the proposal being submitted for funding ?

If yes, name of funding agency: (1) _____

(2) _____

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?

Not Applicable

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

Dates of Proposed Period of Support	Cost Required for the Budget Period (S)				
(Day, Month, Year - DD/MM/YY)	*a.*	*1st Year*	*2nd Year*	*3rd Year*	*Other years*
Beginning date As soon as fund is availabe		83,958	77,538	41,704	15,983
End date 2 years 9 months from the beginning date	*b.*	*Direct Cost* 203,199		*Total Cost* 219,182	

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.

Dr. M.A. Salam
Name of the Division Director

Signature

31-01-2007
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: 29 January 2007

Name of Contact Person (if applicable)

Background and Significance

A. Hypothesis to be tested

We hypothesize that treatment with L-histidine, an essential amino acid possessing anti-inflammatory and antisecretory activity, will improve clinical and bacteriologic features of acute childhood shigellosis by specifically reducing inflammatory changes in the colonic mucosa of *Shigella*-infected patients.

Shigellosis remains a major cause of childhood morbidity and mortality in many developing countries, including Bangladesh. According to published reports, at least 140 million cases of shigellosis and almost 600,000 deaths due to shigellosis occur worldwide annually among children under the age of 5, primarily in developing countries. It is estimated that of approximately 3.8 million diarrhea-related deaths that occur worldwide in children annually (exclusive of China), 0.5 million are attributable to shigellosis.

Improved clinical management of dysentery caused by *Shigella* spp. necessitates a search for an effective and safe alternative adjunctive therapy, primarily because of the rapidly emerging drug-resistant shigellae. Our studies in response to this goal, have demonstrated that administration of L-histidine to rabbits can significantly improve clinical, histological, and bacteriological features of experimental shigellosis.. Among the findings were that L-histidine protected murine small intestine against tissue injury and loss of water and electrolytes following challenge with either *Salmonella typhimurium* or cholera toxin. In addition, L-histidine decreased the levels of pro-inflammatory cytokines (e.g., TNFα and IL-6) and diminished the biological activity of the pro-inflammatory eicosanoid, PGE_2. The imidazole ring of L-histidine was found to chemically react with PGE_2, thereby forming a covalent bond. In addition, the resulting PGE_2-imidazole adduct is a potent inhibitor of PGE_2 activity and effectively blocks PG synthesis by enzyme inhibition. Accordingly, because of these anti-inflammatory characteristics, L-histidine is likely to be a potential therapeutic agent in shigellosis.

B. Specific Aims of the Project

The primary aim of this study is to develop a safe, useful, and cost-effective treatment for acute childhood shigellosis using therapeutic agents other than antibiotics, such as L-histidine. Our objective is to diminish the effects of shigellosis by reducing the duration of illness, frequency of stools, fever, passage of blood and mucus in stool, by shortening the fecal excretion of shigellae and reducing the likelihood of complications such as dehydration, electrolyte abnormalities, protein loss, and hemolytic uremic syndrome.

A rationale approach of this study would be to consider as a secondary aim of reducing childhood mortality by developing a simple and useful treatment of shigellosis with L-histidine. If L-histidine proves efficacious, we will plan larger-scale studies with alternative formulations. For example, L-histidine could be added to oral rehydration solution (ORS), as is currently under study for cholera patients. Alternatively, for shigellosis, one could consider a simple added pharmaceutical to be given to patients, perhaps using ORS as a convenient vehicle for administration.

Project Background

Shigellosis: a major cause of worldwide childhood deaths and disability: The global burden of shigellosis has recently been estimated by a WHO expert group, which reviewed 9,240 reports published during the last 30 years (1966 to 1996) worldwide (1). WHO reported that the annual number of *Shigella* episodes throughout the world was 164.7 million, of which 163.2 million were in developing countries (with 1.1 million deaths) and 1.5 million in industrialized countries. A total of 69% of all episodes and 61% of all deaths attributable to shigellosis were in children under 5 years of age. In developing countries, the total number of diarrhoeal episodes was 487.5 million for infants of 0-11 months of age and 945 million for children 1-4 years old.

Mortality due to shigellosis among hospitalized children in Bangladesh, from 1974-88 was 13.9% for infants and 9.4% for 1-4-year-old children (2). Children under 5 years are responsible for 61% of all *Shigella*-related deaths (3). Although deaths due to shigellosis are rare in industrialized countries, morbidity could be substantial when outbreaks of shigellosis occur in custodial institutions and day-care centers. In France, 962 *Shigella* cases were reported between 1992 and 1997, (1.8 cases per 100, 000) (1). The rates for England and Wales were 3.3 cases/100,000; Israel, 130 cases/100,000; and in the USA, 6.5 cases/100,000 (1).

Shigellosis as a determinant of malnutrition: Although malnourished children are prone to develop shigellosis, reciprocally, the disease also induces malnutrition. *Shigella* infection is associated with anorexia and catabolism. The magnitude of fecal protein loss in shigellosis is much higher than in acute watery diarrheas; an adult may lose up to 500 ml of plasma in stool in a day during severe shigellosis (4-6). In children of developing countries, who are already in a marginal nutritional status, this loss of plasma protein is very detrimental.

Pathophysiology and treatment of shigellosis: The primary pathologic lesion in shigellosis is characterized by acute bacterial inflammation and destruction of colonic mucosa by the actively invading shigellae. The lesions are characterized by edema, infiltration of PMNs, macrophages, and other phagocytic cells into the lamina propria, development of vasculitis, and sometimes crypt abscess formation (7).

Effective treatment of shigellosis has been complicated by the recent emergence of multi-resistant strains (8, 9). These strains are resistant to ampicillin, co-trimoxazole and nalidixic acid, which until recently had been the drugs of choice for treating shigellosis. *In vitro* resistance to pivmecillinum (Selexid) has been only occasionally encountered. It is likely that, as with the other β-lactam agents, resistance will also develop to this agent with its increased use. Thus, there is a pressing need to identify other effective antimicrobial, as well as adjunctive therapies for shigellosis.

Current recommendations for prescribing an antimicrobial agent in the treatment of shigellosis depend upon the type/species of the organism that is prevalent in a particular region and their current sensitivities to different antimicrobial agents. In Bangladesh, where *S. Flexneri* is the most prevalent organism, nalidixic acid is the drug of choice because it is sensitive to more than 90% of isolates. The second drug of choice is pivmecillinum or selexid. *S. dysenteriae* type 1 is the second most frequently isolated organism which requires either ciprofloxacin or ceftriaxone for effective treatment; this strain causes the most severe infection and is resistant to most of the commonly prescribed antibiotics including nalidixic acid.

L-Histidine in the treatment of shigellosis: The project will enable us to evaluate the potential therapeutic value of L-histidine in protecting children against inflammatory diarrheal diseases (e.g., bacillary dysentery). While we suspect that benefits might accrue to patients infected with other microorganisms evoking an inflammatory response, this proposal will focus on shigellosis in children.

Preliminary studies have established that L-histidine is a potent antioxidant equally effective as N-acetyl-L-cysteine (10). L-Histidine's effectiveness as a scavenger of toxic free hydroxyl radicals and singlet oxygen is well documented (11-13). Importantly, L-histidine has been shown to protect murine small intestinal loops against tissue injury and loss of water and electrolytes caused by infection with *Salmonella typhimurium* (14). Moreover, histological examination of sections of small intestine challenged with *S. typhimurium* revealed impressive protection against structural damage and fluid loss (14).

Pharmacology of L-histidine: L-Histidine, like most other amino acids, has been extensively studied from a nutritional and metabolic point of view. The minimum daily requirement of L-histidine for human adults is 12 mg/kg, or 840 mg in a 70-kg adult. Normal plasma levels of L-histidine range from approximately 4 to 20 mg/l. The pharmacokinetics of L-histidine in humans is well described in the scientific literature. L-Histidine is rapidly absorbed, and peak plasma concentrations occur within 1 hour after both oral and intravenous administration. Clearance is similar following both routes of administration (15-17). The pathways of L-histidine metabolism are well characterized, involving oxidation and transamination.

A review of the literature over the last 40 years showed that oral or intravenous doses of 1 g/day or more of L-histidine have been administered to approximately 700 subjects, accounting for roughly 30,000 patient-days of exposure. The maximum oral dose reported was 64 g [18], while the maximum reported i.v. dose was 30 g in 30 minutes [19]. Very large doses of L-histidine (48 to 64 g/day for 2 to 4 days) resulted in minor neurological symptoms such as taste and smell distortion in scleroderma patients **and asymtomatic impairment of zinc absorption** (18,20). L-Histidine has been used as a diagnostic tool for folate deficiency and as a therapeutic agent in patients with rheumatoid arthritis and anemia. Studies in the rat and mouse showed no carcinogenic and teratogenic effects of L-histidine (21).

Dose and safety of L-Histidine in children:

Although the highest safe dose of L-histidine for children has not been absolutely determined, there are several reports of L-histidine trial in infants and children as described in the following Table. After a careful review of the available literature, along with our own clinical experience in Bangladeshi children with shigellosis, we propose to use the recommended dose of L-histidine of 15 mg/kg/2h (180 mg/kg/day). For example, in a 10-kg child, this will provide a daily dose of 1.8 g. For our cholera study involving 140 adults (just completed), we have given a maximum daily dose of 25 g L-histidine in a 47-kg adult, i.e., 532 mg/kg/day without documenting any side effects.

Moreover, L-Histidine has a very short half life in human (1.8 h) requiring frequent oral administration (2 hourly). Thus, histidine is unlikely to be accumulated in the body with normal renal function. The pathways involved in human histidine metabolism are well characterized, and the required enzymes and breakdown products have been described (36, 37).

During metabolism, histidine is mostly incorporated into protein or dipeptides, some portion is degraded to urocanate and glutamate involving specific enzymes in the liver. One intermediate product, formiminoglutamic acid (FIGLU) requires folate for its breakdown. Therefore, in folate-deficient patients, FIGLU accumulates in the urine after L-Histidine loading. This observation led to the use of 10-15 g oral loading doses of L-Histidine in pregnant women and other patients as a diagnostic test for folate deficiency, no side effects in mother or children were observed (38, 39). Behavioral and central nervous system effects attributed to large histidine doses have been

reported in rodents. However, physiologic effects attributable to histidine have not been produced in humans even with very large oral and intravenous doses of histidine.

Table: L-Histidine studies in children.

Authors	Journal	Dose of L-histidine (mg/kg/day)	Purpose of the study	Comments on safety
Zlotkin SH	Am J Clin Nutr, 48(2):330-334, 1988	124±34	Effect on urinary Zn^{++} excretion in 23 newborns	No side effects reported
Zlotkin SH	J Pediatr, 114(5):859-864, 1989	95 vs.165	Effect on urinary Zn^{++} excretion in 14 newborn infants	No side effects reported, considered safe
Anakura M	Hokkaido Igaku Zasshi, 56(1):1-15, 1981.	30-35 and 40-50	Therapy of histidinemia in newborn infants.	No side effects reported, mental retardation and growth failure were not found.
Bellone J et al.	J Ped End Metab 1996; 9:523-31.	500 mg/kg iv infusion, single dose	Effects of amino acids on normal, short stature children, 5-14 yr.	No side effects observed.

One of our investigator (Prof. JW Peterson) has personally contacted Dr. Zlotkin who studied L-Histidine in children and published two papers in late 1980s. Dr. Zlotkin did not observe side effects in children and new born babies in his studies with L-Histidine. He is also willing to provide more detail comments on this topic if requested.

2. Safety evaluation review:

The ICDDR,B has an independent **Ethical Review Committee (ERC)** comprising outside members and rigid terms of reference for protection of human subjects in biomedical research. ICDDR,B ERC has been given recognition as an IRB by the US Federal Government through its Federal Wide Awareness programme, the registration number for ICDDR,B is FWA00001468. This federal approval ensures that ICDDR,B ERC applies all ethical guidelines for human research as approved by the US Federal Government and NIH. The ERC is (i) a fully independent body comprising non-institutional members, (ii) has access to the trial codes of all clinical drug trial studies having specific terms of references for their open examination, (iii) has the authority to spot examination of the trial and ask for explanation from the investigators and

take actions for irregularities including termination of the trial. A copy of the ethical guidelines and review procedures of ICDDR,B ERC has been enclosed for information of the reviewers.

Safety Evaluation Committee
This committee will comprise the following members:

Prof. M. R. Khan, Pediatrician: Chair
Prof. Misbah Uddin, Pharmacologist, IPGMR: Member
Dr. Abdus Salam, Chief Physician, ICDDR,B: Member

L-Histidine comes in the form of a white, soluble, pyrogen-free powder: the amount of L-histidine that will be required for a child for each day of treatment will be mixed with a vanilla flavored rice ORS and given to patients at a dose of 1-2 teaspoons (5-10 ml) every 2 h. L-histidine tastes very mildly salty, when mixed with rice ORS which has a similar taste, and flavored with vanilla, the final preparation will be identical in taste, consistency, and color with the control preparation of rice ORS similarly flavored, but without added L-histidine. A pharmacist according to a computer-generated randomization list will prepare these preparations during patient enrollment. The test (drug) and control preparations will be coded as Treatment-A and Treatment-B, the list will be kept confidentially in the custody of the pharmacist until the study is completed and the data have been analyzed.

Supportive Preliminary Data

Preliminary observations of L-histidine: Mechanism of action: Histidine has long been recognized as a scavenger of hydroxyl radicals (22) and of singlet oxygen (delta form of $^1O^2$). L-Histidine interacts with toxic oxygen species through two distinct mechanisms : (1) by interfering with the redox reactions involving metal ions that produce the hydroxyl radical and (2) by direct interactions of the imidazole ring of L-histidine with singlet oxygen.

Hydroxyl radical and singlet oxygen (O_2): *In vitro* "spin trap" studies using Fenton-type chemistry to generate the hydroxyl radical, that is, reaction of Fe^{2+} complexes of ADP or ATP and hydrogen peroxide to generate the hydroxyl radical, showed that, when added to the iron-containing reactions prior to the addition of hydrogen peroxide, L-histidine was among the most effective scavengers among 29 biological compounds tested. L-Histidine was found to strongly inhibit the formation of lipid peroxidation products when it was exposed to Fe^{3+} before the iron was added to other reaction components.

L-Histidine is generally recognized as the most active of the amino acids at scavenging singlet oxygen, with rate constants for reaction with singlet oxygen roughly 2- to 3-fold higher than those for L-tryptophan and 5-fold higher than those for L-methionine (23). L-Histidine's reaction with singlet oxygen has been calculated to be in the range of 4×10^7 to 1×10^8 $M^{-1}S^{-1}$ (10, 24). The imidazole ring of L-histidine has been shown to be responsible for the antioxidant activity of several biologically important dipeptides, including carnosine (β-alanyl-L-histidine), anserine (β-alanyl-3-methyl-L-histidine), and homocarnosine (aminobutyryl-L-histidine) (11, 12, 13).

Observations on mechanisms of action: Our recent study (25) showed that L-histidine reacted with imidazole and PGE_2 forming PGE_2-imidazole or PGE_2-histidine adducts, and imidazole catalyzed the formation of a Michael adduct between C11 of 11-deoxy-$\Delta^{10}PGE_2$ and the *tau* nitrogen in the imidazole ring of L-histidine. Both adducts inhibited CT-induced fluid loss and reduced cAMP accumulation in the CT-challenged mouse intestinal loops. The protection provided by PGE_2-imidazole, PGE_2-histidine, and L-histidine against fluid loss could provide a basis for therapy against diarrhoea.

L-Histidine as an anti-inflammatory agent: L-Histidine clearly has an anti-inflammatory effect at high local concentrations that is due at least in part to its singlet oxygen and hydroxyl radical scavenging characteristics. Our work using three models of gastrointestinal conditions, data from the literature, and some unpublished studies clearly indicate potential cytoprotective effects of L-histidine.

Inflammation and L-histidine: In a mouse model of infectious diarrhea due to *Salmonella typhimurium*, Peterson *et al.* (26) showed that L-histidine (175 mM) significantly reduced intestinal inflammatory and secretory responses. L-histidine also reduced oxygen species in the intestinal lumen during infection (26). L-histidine was shown to effectively reduce LPS-mediated oxidation of an oxidative probe that fluoresced in response to oxidative stress. In a rat model of inflammatory bowel disease, Keshavarzian *et al.* (personal communication) showed significant protective effects of L-histidine assessed by histology and myeloperoxidase activity. Miller *et al.* (personal communication) has shown that L-histidine significantly protected against indomethacin-induced gastritis in mouse.

Effects of L-histidine in experimental shigellosis: Recent experimental studies were initiated by the PI (Dr. G. H. Rabbani) with a rabbit colonic model challenged with *Shigella flexneri* 2a. Preliminary results reported at the last FASEB meeting in San Diego, CA (April 2000) offer promise that L-histidine, indeed, may also protect against tissue injury from experimental shigellosis (27). These experiments were followed by further investigations determining the anti-inflammatory effects of L-histidine in the rabbit shigellosis model. The final results indicate that IP administration of 3.8% L-histidine significantly improved clinical, histopathologic and bacteriologic characteristics of shigellosis. L-Histidine

significantly ($p<0.05$) reduced colonic inflammation, as determined by decreased MPO activity, blood, mucus, and number of PMNs in the colonic tissue and stools. The animal gained body weight and the number of viable *Shigellae* recovered from the colonic lumen and tissue were significantly ($p<0.05$) lower in the L-histidine-treated rabbit than controls. These results were presented at the Experimental Biology meeting in Orlando, FL this year, 2001 (28).

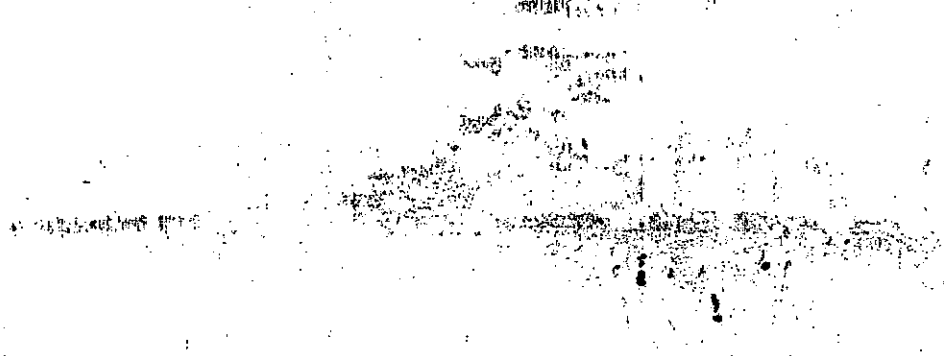
Histidine-supplemented rice-ORS reduces diarrheal stool in cholera patients: A double-blind, randomized study (Abstract will be presented by Dr. G. H. Rabbani at the DDW Confc, May 2002: San Francisco).

Since L-Histidine, an amino acid inhibits cholera toxin stimulated intestinal secretion of fluids and electrolytes in animals, we have evaluated its therapeutic effects in reducing fluid loss in patients with cholera.

In a double-blind trial, 126 adults with *Vibrio cholerae* infection, L-Histidine was administered orally *ad libitum* mixed with a rice-based ORS (CeraLyte-90) at a concentration of 2.5 g/L to 62 patients; 64 received the same ORS without L-Histidine (controls). All received ciprofloxacin, 500 mg 12 h for 72 h. Output of stool, urine, and vomit and intake of ORS, water, and iv fluids were determined 8 hourly for 72 h. Stool bacteriology, serum electrolytes, hematologic profiles, and duration of illness were also determined.

Pretreatment characteristics including age, gender, body weight, and severity and duration of illness were comparable among the patients in both groups. The mean body weight was 47.6 kg and each adult produced a mean stool of 45.5 mL/kg/4h before treatment. L-Histidine significantly ($p<0.05$) and consistently reduced stool volume during 32 to 64 h of treatment compared to the control group; mL/kg, 32-48h: 11.5 ± 6.9 vs. 18.8 ± 16.0; 40-48h: 6.7 ± 4.4 vs. 11.5 ± 9.7; and 56-64h: 6.3 ± 5.8 vs. 7.8 ± 4.1. During the initial 0-24h, the stool volume in the histidine group was 12-20% less, but these differences were not statistically significant. An overall stool reduction of 22% was observed during the entire course of the illness. There was a significant ($p<0.05$) reduction of unscheduled intravenous infusion in the histidine group compared to controls (mL/kg: 0-24h: 82.5 ± 44.4 vs 158.6 ± 72.2, $p<0.01$; 24-48h: 41.6 ± 40.4 vs. 52.5 ± 22.1, $p>0.05$). The ORS intake was consistently less in the histidine group during the treatment period, but the difference is significant only during 48-72h (mL/kg: 0-24h: 214 ± 67 vs. 219 ± 75, 24-48h: 108 ± 55 vs. 126 ± 78; 48-72h: 41 ± 25 vs. 64 ± 49, $p=0.05$). Total duration of illness was also significantly shorter in the L-Histidine group (hours, mean±SEM: 42.7 ± 1.7 vs. 47.0 ± 1.8, $p<0.05$). No side effects were observed in these patients.

We conclude that L-Histidine reduces stool volume in cholera and could be a useful and safe therapeutic adjunct to increase success rate of ORS and antibiotic therapy in cholera.



Experimental Design and Methodology

This study will be carried out in two phases:

1.First Phase: A preliminary safety trial in 20 children.

2. Second Phase: Main double-blind clinical trial involving 205 patients.

First Phase: Safety Trial

Objectives and Background

A full clinical trial to test the efficacy of L-Histidine in the treatment of childhood shigellosis is going to be carried out at the ICDDR,B in Bangladesh involving 205 children aged 5 to 60 months. This proposal has recently been reviewed by Thrasher Fund, USA and a conditional approval for funding has been considered. However, it has been suggested by the reviewers that a preliminary safety evaluation of the proposed L-Histidine dose should be carried out and analyzed before starting the main study. Therefore, the primary objective of the study described under this addendum is to provide information relating to the safety of L-Histidine in children, 5-60 months old. If no untoward effects were observed in the preliminary study, the main trial could be started.

Methods

The methods for safety evaluation will be same as those described for evaluation of children in the principal study. Therefore, children will be selected, randomized, treated, and evaluated using the same definitions and clinical conditions described in the principal protocol.

However, the distribution of 20 children to different treatment (dose) groups will be as follows:

Batch 1: N=5 children will get L-Histidine at a dose of 96 mg/kg/day.
Batch 2: N=5 children will get L-Histidne at a dose of 165 mg/kg/day
Batch 3: N=5 children will get L-Histidine at a dose of 180 mg/kg/day (recommended)
Batch 4: N=5 children will get L-Hisdtidine at a dose of 250 mg/kg/day

Treatment will be given for 7 days and the next higher dose will be studied if no untoward effects were observed in the lower dose.

Safety evaluation will be done by examining **clinical and laboratory** parameters as described in detail in the principal proposal. Briefly they are:

Clinical cure: A patient will be considered to be clinically cured if on day 5 no frank blood and mucus are observed in the stool, and if no watery stool, no more than 3 stools, and no fever (rectal temperature > 37.8 C) are recorded.

Clinical treatment failure: A patient will be considered to be a clinical treatment failure if on day 5 there are more than 3 stools, (watery or soft), presence of blood/mucus in more than one stool, presence of fever (rectal temperature >37.8 C.

Marked improvement: On day 5, no frank bloody/ mucoid stool, one or zero watery stool, <5 stools.

Clinical Safety Evaluation

All patients enrolled in the study will be followed every day with a list of clinical sign/symptoms to identify any unpleasant incident or untoward reaction due to treatment. These will include nausea, vomiting, headache, fever, skin rash, palpitation, tachycardia, bradycardia, hurried respiration, dyspnea, eye symptoms, tremor, sweating, and/or paresthesia. Laboratory evaluations of hepatic, renal, hematologic, urinary, and immune functions will be evaluated on admission, day 2, and day 7 (discharge). Any untoward reaction observed or reported by the patients or guardians or the attending nursing staff will be immediately brought to the attention of the Principal Investigator for further evaluation and action. In the event of severe reaction, the treatment may be discontinued, and appropriate management initiated. In the morning and evening rounds, all records of the patient will be checked and confirmed by the investigators.

Definition of severe reaction

A severe reaction after administration will include: development of shock, severe bradycardia (≤55/min), hypothermia (≤36 C), severe hypotension, central cyanosis, severe dyspnoea, severe vomiting, gen. convulsion, sudden jaundice, urinary suppression, severe skin eruption, raised serum creatinine and liver enzymes, and bone marrow depression. Any unusual clinical manifestation recognized by the physician, nursing, or attending staff will be attended to for proper evaluation.

In addition, an independent **Safety Evaluation Review** will be performed by an institutional review committee not participating in the study. This group of physicians, headed by a Chair, will make a monthly review of the safety profile of the patients under study by specifically examining their clinical records, data sheet, safety evaluation reports, doctors' notes, and nursing records. In addition, they will make spot visits to patients in the study ward and make sure that the safety procedures are followed and ethical standards are maintained. Their observations and suggestions will be communicated to the Ethical Review Committee of the ICDDR,B for necessary actions.

Safety Evaluation Committee

This committee will consist of the following members:

Prof. M. R.Khan (Chair), Pediatrician
Prof. Misbah Uddin Ahmed (Member), Head of Pharmacology, IPGMR
Dr. Abdus Salam, Acting Associate Director, ICDDR,B

Laboratory Evaluation of Safety Parameters

1. L-Histidine levels in blood/serum: will be determined at days 0, 2, and 4.
2. Number of WBCs and RBCs in stool microscopic examination during the treatment period.
8. Renal functions: Na, K, Cl, HCO3, TCO2, Creatinine, protein
9. Hepatic functions: Bilirubin, SGOT, SGPT, Alk Phos.
10. EKG in clinically suspected cases.

Organization of the trial

The data will be analyzed in a stepwise fashion at the completion of each dose group. Both clinical and laboratory parameters will be examined and evaluated and discussed with all investigators. The data set

will also be shared with Thrasher Fund or its delegated reviewers for comments and approval before starting the main study.

Second Phase: Double-blind clinical trial in 205 patients

After successful completion of the first phase of the trial establishing the safety of the dose of L-Histidine in children, the main part of the double-blind trial involving 205 children will be started.

Study design and sample size: Double-blind study: This study will be a double-blind, controlled clinical trial involving 225 children, both boys and girls, aged 6 - 60 months, with *Shigella* dysentery who will be hospitalized for 7 days in a research ward of Dhaka Hospital of ICDDR,B. As recommended by the reviewers, the first 20 children will be evaluated to establish the safety of L-Histidine in children. All children will receive syrup ciprofloxacin, 15 mg/kg 8 hourly for 7 days. They will be randomly assigned to either L-histidine or placebo treatment.

Sample size calculations

In a recent study, Salam *et al.* (29) found 80% and 65% clinical improvement comparing ciprofloxacin and pivmecillinam, respectively with a 5-day course of treatment of shigellosis in a similar group of Bangladeshi children. However, these differences were not statistically significant (p=0.10).

As pointed out by the reviewers, we have calculated the sample size giving details of the formulae used (Table). We considered several options and found that we can still observe the effects of L-histidine compared to those from ciprofloxacin alone by increasing the sample size. If we consider that ciprofloxacin cures 80% of shigellosis patients in 5 days, as reported by Salam et al. (29) and if an addition of L-histidine in the treatment plan further improves the cure rate to 95%, making a 15% difference, then we need 57 patients in each group as shown in the Table.

If the clinical success rate for standard treatment (ciprofloxacin) is less than 80% or even lower, i.e., 70%; in such a situation, we will need a total of 205 patients to detect similar differences in success rates, 15% (Table). We consider this as a reasonable assumption based on our long clinical experience of treating shigellosis in this population. Considering the practical management aspects, duration of study, and the cost involvement, we would suggest that we study a total 205 patients which will satisfy all these criteria at an acceptable level.

Table: Sample size determination with cost and duration.

Expected difference	Clinical success by standard treatment	Clinical success by new treatment	Sample size (n) in each group	Sample size (n) in two groups	Total Sample size with 10% for design effects and dropouts.	Total Cost	Duration (Years)
15%	80%	95%	57	114	125	US$ 161,496	2.0
15%	70%	85%	93	186	205	US$ 203,200	2.5

One sided tests, statistical power 80%, $\alpha = 0.05$, $\beta = 0.2$

Calculations:

Ciprofloxacin is the standard in this case and 80% of all patients will show improvement (i.e., 20% will show no improvement) (Salam *et al.*, Lancet, 1998, Ref 29).

It is decided that if L-histidine treatment is able to further improve 15% (i.e., 95% of all patients will show improvement and 5% no improvement), then we would like to be 80% certain that this is detected as statistically significant at the 5% level.

Now,
P_1 = percentage of successes expected from ciprofloxacin treatment
P_2 = percentage of successes expected from L-histidine treatment (which should be different from P_1), $\alpha = 0.05$
$1-\beta$ = the degree of certainty that the difference $P_1 - P_2$, if present, would be detected ($1-\beta = 0.80$).
Accordingly, $P_1 = 80\%$, $P_2 = 95\%$, $\alpha = 0.05$, $\beta = 0.2$

Therefore, for one sided tests

$$n = \frac{P_1 \times (100 - P_1) + P_2 \times (100 - P_2)}{(P_{c2} - P_1)^2} \times f(\alpha, \beta)$$

$$n = \frac{80 \times 20 + 95 \times 5}{(95-80)^2} \times 6.2$$

$= 9.2 \times 6.2$
$= 57$

Therefore, we need 57 patients in each group.

Similarly calculated, we will require 93 patients in each group to detect 15% difference between the standard (70%) and the new treatment (85%) with 80% power at 0.05% significance level. Thus we will need a total of 205 patients including design effects/drop outs (10%).

Selection criteria:

1. Age: 6-60 months.
2. Both boys and girls will be included.
3. Stool characteristics: Frankly bloody or bloody mucoid on inspection.
4. Duration of dysentery: ≤ 72 hours.

Exclusion criteria:

1. Failure to obtain parental consent.
2. Prior treatment with an antimicrobial and/ or antidiarrhoeal agent (loperamide, metronidazole).
3. Co-infection with erythrophagocytic trophozoite of *Entamoeba histolytica.*
4. Severe malnutrition (weight for age ≤ 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, and renal failure.
6. Patients with severe anorexia, constant vomiting or unable to tolerate oral medication will be excluded.

Other tests such as 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]. If *S. dysenteriae* type 1 is isolated, CBC will be obtained on day 4 for clinical reasons.

L-Histidine blood levels will be determined at days 0, 2, and 4.

Laboratory studies: Performed by Dr. J.W. Peterson, University of Texas Medical Branch at Galveston, Texas (Core Laboratory Protein Chemistry)

1. L-Histidine levels in blood/serum – Serum samples will be stored frozen and shipped to UTMB for the purpose of determining blood levels of free histidine achieved following oral L-histidine administration as described previously (25). The sera will be diluted 1:10 and 20 µl will be pipetted into Waters Pico Tag Ultrafiltration devices before centrifugation at 2000 xg for 1-2 hr. A 40-µl aliquot from each sample will be placed in an ABI (Applied Biosystem) 420 Derivatizer with an on-line 130 A Separation System and a 920 A Data Analysis Module for amino acid analysis. All samples will be corrected to nmoles of amino acid/ml of human serum. The UTMB Protein Chemistry Core Facility performed similar analyses in this manner on animal sera for earlier studies!

2. Reduced glutathione stool and serum – Depletion of natural tissue antioxidant potential is reflected by a decrease in reduced glutathione levels (14). The glutathione (GSH) assay (14) will be performed on 1.0-ml samples of stool filtrate and serum. Briefly, the GHS-400 method (OXIS International Inc., Portland, OR) is based on the formation of thioesters between SH-containing substances and 4-chloro-1-methyl-7-trifluoromethyl-quinolinium methasulfate and mercaptans. In alkaline conditions, the substitution product obtained with GSH is converted into a chromphoric thione with maximal absorbance at 400 nm. GHS determinations of patient specimens will enable us to measure the oxidative stress occurring in the inflamed intestinal tissue among patients treated with L-histidine and control patients receiving standard therapy. The blood specimen that will be drawn for CBC and histidine level in the morning of day 0, day 2, and day 4 will also be used for GSH measurement. Blood will be collected every morning (9-10 AM).

3. Myeloperoxidase (MPO) activity in stool – The extent of intestinal inflammation in the patients also will be assessed by measuring the levels of myeloperoxidase in the intestinal fluids of patients with and without antioxidant treatment. Increased levels of myeloperoxidase will be measured by standard procedures described previously and should reflect the extent of infiltration of polymorphonuclear neutrophils (PMNs). Intestinal fluids will be collected from the anus of the patients using a wide mouth bottle and stored frozen (-70°C) until they can be clarified by centrifugation (10,000 xg), and assayed for myeloperoxidase activity. No rectal tube will be used. L-Histidine treatment is expected to reduce the number of PMNs infiltrating the intestine, which would be reflected by a concomitant decrease in myeloperoxidase activity. For this test stool will be collected from anus using a wide mouth bottle. No intubation is needed.

4. **Determination of eicosanoid levels in stool** – In addition to exhibiting increases in cytokine levels, inflamed tissues contain increased amounts of pro-inflammatory eicosanoids, which are products of the cyclooxygenase and lipoxygenase pathways. Stool samples will be collected from patient using the same collection method described MPO activity above. We will use commercial ELISA assays for measuring levels of leukotrienes LTB_4 and LTC_4, as well as PGE_2 and $PGF_{2\alpha}$. Our past investigations have used as reagents supplied by PerSeptive Diagnostics. For this test stool will be collected from anus using a wide mouth bottle. No intubation is needed.

Measurement of cytokine levels in stool to be performed at ICDDR, Bangladesh

It has been shown that during cellular invasion, *Shigellae* activate the inflammatory cells in the mucosa, which may lead to a regulated expression of a number of proinflammatory cytokines (34). These cytokines mediate and amplify the signals from the invading shigellae to initiate the inflammatory process (8). It has been shown that severe inflammation was associated with increased production of IL-1, IL-6,

IFN-γ and TNFα (34). Raqib *et al.* (34) also reported that patients with shigellosis have higher numbers of cytokine-producing cells for IL-1α, IL-1β, IL-1α, TNF*a*, IL-6, IL-8, IL-4, IL-10, interferon-γ, TNF-β, and transforming growth factor β1-3. Similar observations in shigellosis patients in Bangladesh have been reported by Salam *et al* (35). Thus, it appears that cytokines may play an important role in the pathogenesis of shigellosis, thereby providing a useful marker in assessing the severity of colonic mucosal inflammation in shigellosis. Accordingly, we are planning to measure some of these cytokines (IL-4, IL-4, IL-5, IL-10, IFNγ, TNFα, IL-8), which can be useful indicators of assessing the anti-inflammatory effects of L-histidine on the colonic mucosa of shigellosis children.

For the measurement of cytokines, fecal specimens will be collected from patients' anus using wide mouth bottle, no rectal tube will be used.

Evaluation of treatment effects:

A. Primary response variables: Fecal Hb, lactofferin, MPO, fecal leucocytes.

- Fever: Time to last fever (>37.8°C)
- Number of WBCs and RBCs in stool microscopic examination during the treatment period.
- Isolation of *Shigella* spp. in stool culture during the treatment period.

B. Secondary Response Variables

- Straining/tenesmus: Presence of straining/tenesmus on every study day, and time to last straining/tenesmus.
- 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.

L-histidine treatment and blinding procedures

L-Histidine will be administered orally after admitting a child into the study. The dose will be calculated on the basis of body weight (15 mg/kg orally every 2 hours) and will be given by mixing with WHO ORS. Each day the pharmacist will prepare the supply of L-Histidine for each child by dissolving L-Histidine powder (tasteless, white) at a concentration of 150 mg/5 mL WHO ORS, this solution is stable at room temperature for 24-48 hours. For each child, 5-10 mL dose will be given orally with spoon every 2 h. When L-Histidine powder is mixed with either WHO or rice-ORS there is no change in colour, taste, or consistency.

On admission, each child will be given a study enrollment ID number and a coded treatment allocation number which will be written in a piece of paper and preserved in an opaque, sealed envelope. The treatment may be coded as A for treatment and B for control. The envelopes will be opened only by the pharmacist at the time of allocating treatment to a new entry. This way treatment allocation will be concealed from the clinical observers and patients. No clinical staff members related to the study will have access to the code numbers.

Drug Accountability

The Principal Investigator must ensure that all drug supplies are kept in a locked area with access to the study drug limited to appropriate study personnel. The Principal Investigator must maintain accurate records of the receipt of all drug shipments from the Sponsor, including date received, amount received and the disposition of all study medication. Current dispensing records will also be maintained on the appropriate case report form for each study subject. This case report form will indicate the date and the number of tablets administered by the Dispensing Pharmacist/Nurse. At the end of the study, all medication must be accounted for. Any unused study drug medication will be inventoried by the Dispensing Pharmacist and retrieved by the Sponsor, or disposed of by the dispensing Pharmacist in accordance with local regulations and recorded in the appropriate case report form.

Definitions of clinical outcome:

Clinical cure: A patient will be considered to be clinically cured if on day 5 no frank blood and mucus are observed in the stool, and if no watery stool, no more than 3 stools, and no fever (rectal temperature > 37.8 C) are recorded.

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

Clinical treatment failure: A patient will be considered to be a clinical treatment failure if on day 5 there are more than 3 stools, (watery or soft), presence of blood/mucus in more than one stool, presence of fever (rectal temperature >37.8 C.

Marked improvement: On day 5, no frank bloody/ mucoid stool, one or zero watery stool, <5 stools.

Management of treatment failure and complications: Patients who fail to respond by five days to treatment, or develop complications will be transferred to the General Ward or Special Care Unit of the Dhaka 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 the 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 to the study from 8:30 am to 5 pm.

Data analysis: The SPSS (windows version 7) program will be used to analyze the data. 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 a 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. **Kaplan-Meyer survival analysis** will be carried out to assess clinical success in the two treatment groups. A p value of < 0.05 will be considered significant. Dropout patients (after randomization and before completion of the study) will also be included in the analysis, and the data of those patients will be analyzed as long as they maintain the protocolized management.

Facilities available

Site: The Research Ward of the Dhaka Hospital of ICDDR,B will be used for hospitalization of the study patients. The facility has been 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 (capital of Bangladesh), where a majority of patients contract shigellosis each year; study subjects will be selected from this patient population.

For this study the total number of patients (n=225) have to be recruited within a specified period of 30 months. Since the number shigella children coming to ICDDRB hospital has been declining, it would be wise to plan for extending the study to other hospital or treatment centers. In view of this, there are three places that offer opportunities for collaboration, these are: Kamlapur slum community, Matlab Hospital of ICDDRB, and Infectious Disease Hospital in Calcutta, India. We have already made arrangements that depending upon the availability of patients in Dhaka, additional treatment centers can be opened in those places with short notices. If multicentre trial can be organized successfully, the study protocol can be competed within the stipulated time.

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). These laboratories are competent and regularly used for all protocols.

Safety Evaluation:

All patients enrolled in the study will be followed every day with a list of clinical sign/symptoms to identify any unpleasant incident or untoward reaction due to treatment. These will include nausea, vomiting, headache, fever, skin rash, palpitation, tachycardia, bradycardia, hurried respiration, dyspnea, eye symptoms, tremor, sweating, and/or paresthesia. **Laboratory evaluations of hepatic, renal, and hematologic functions will be evaluated on admission, day 2, and day 7 (discharge).** Any untoward reaction observed or reported by the patients or guardians or the attending nursing staff will be immediately brought to the attention of the Principal Investigator for further evaluation and action. In the event of severe reaction, the treatment may be discontinued, and appropriate management initiated. In the morning and evening rounds, all records of the patient will be checked and confirmed by the investigators.

In addition, an independent Safety Evaluation Review will be performed by an institutional review committee not participating in the study. This group of physicians, headed by a Chair, will make a monthly review of the safety profile of the patients under study by specifically examining their clinical records, data sheet, safety evaluation reports, doctors' notes, and nursing records. In addition, they will make spot visits to patients in the study ward and make sure that the safety procedures are followed and ethical standards are maintained. Their observations and suggestions will be communicated to the Ethical Review Committee of the ICDDR,B for necessary actions.

আর্ন্তজাতিক উদরাময় গবেষনা কেন্দ্র
মহাখালী, ঢাকা, বাংলাদেশ।

" রক্ত আমাশয় এল-হিস্টিডিন এর কার্যকারিতার উপর গবেষণা"

সম্মতি পত্র

নিম্নলিখিত তথ্যাবলী রোগীর পিতা বা প্রকৃত অভিভাবককে পড়ে শোনানো হবে:-

১। আপনার শিশু সম্ভবতঃ সিগেলা নামক এক ধরনের জীবাণু দ্বারা আক্রান্ত হয়ে আমাশয়ে ভুগছে। এই রোগের চিকিৎসার জন্য কার্যকরী ওষুধের প্রয়োজন। দূর্ভাগ্যজনক যে, যে ওষুধের দ্বারা আগে চিকিৎসা করা হতো, সিগেলা জীবানু সেই ওষুধগুলির বিরূদ্ধে প্রতিরোধী হয়ে উঠেছে। সুতারাং আমরা একটি কার্যকরী সহায়ক চিকিৎসা ব্যবস্থা খুঁজে বের করার চেষ্টা করছি।

২। বর্তমান গবেষনায় আমরা শিশুদের সিগেলা দ্বারা সংক্রমিত আমাশয়ের চিকিৎসায় এল-হিষ্টিডিন নামক এ্যামিনো এসিডের আরোগ্যকর ক্ষমতার মূল্যায়ন করছি।

৩। আপনার শিশুকে হাসপাতালের গবেষণা ওয়ার্ডে ৭ দিন ভর্তি রাখা হবে। এই সময়ে আপনার শিশুকে অসুস্থতার জন্য আদর্শ চিকিৎসা দেয়া হবে: যার মধ্যে থাকবে সাধারন খাবার, খাওয়ার স্যালাইন, শিরাপথের স্যালাইন (যদি লাগে) এবং ওষুধ (সিপ্রোফ্লোক্সসিন)

৪। আপনার শিশুকে এল-হিষ্টিডিন চিকিৎসা দেয়া হবে কি হবে না তাহা দৈবচয়ন পদ্ধতিতে দেয়া হবে।

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

৬। যদি আপনি, আপনার শিশুকে এই গবেষনায় অন্তর্ভূক্ত করেন, তারপরও আপনি আপনার শিশুকে যে কোন সময় প্রত্যাহার করতে পারবেন। সেক্ষেত্রে আপনার কোন ক্ষতি হবে নাএবং আপনার শিশুর হাসপালের আদর্শ চিকিৎসা গ্রহনে 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 গযেণার অর্ন্তভূক্ত করতে চান, তবে আপনার সম্মতি স্বরূপ আপনি নীচে স্বাক্ষর করুন অথবা টিপসহি দিন।

গবেষকের স্বাক্ষর স্বাক্ষীর স্বাক্ষর পিতা/মাতা/অভিভাককের স্বাক্ষর/টিপসহি

রোগীর নাম ভর্তি নং তারিখ:

VOLUNTARY CONSENT FORM

The following information will be read aloud to parents or the legal guardians of patients in local language (*Bangla*).

1. Your child is suffering from invasive diarrhea / dysentery (bloody stool) due to infection with a germ named *Shigella*. Management of this disease requires the use of effective drugs. Unfortunately the *Shigella* germs have become resistant to most of the antibiotic drugs once useful in its treatment. **Therefore, we are attempting to find effective adjunctive therapy that might enhance the therapeutic effects of standard antibiotic treatment in shigellosis.**

2. In the present study we are evaluating the therapeutic effects of L-histidine in the management of *shigellosis* in children. L-Histidine is an amino acid, normally present in food such as beef and poultry and has been therapeutically used in man for treatment different disorders. Although, L-histidine is normally consumed in food, we are investigating **whether L-histidine can enhance the therapeutic effects of antibiotics (ciprofloxacin) when given together (ciprofloxacin + L-histidine).**

3. Your child will be admitted into the hospital study ward for 7 days. During this period the child will be given the standard treatment for the illness, which may include normal diet, ORS, iv fluid, and drugs (e.g. ciprofloxacin 15 mg/kg body weight 8 hourly).

4. Your child will be randomly assigned to any of the two different treatment groups and there is an equal chance that the child will get L-histidine or placebo (inactive material). During the study, all stools, urine, and vomit will be collected and their volume measured daily. Daily body weights will be measured and other clinical parameters (temperature, pulse, respiration etc) will be recorded.

5. **During the course of treatment, 3 blood tests (1-2 mL from an arm vein on day 0, day 2, and day 4) will be done on your child for clinical management and study purposes.**

6.If you have your child admitted into the study, you will still possess the right to withdraw the child from the study at any point in time and still get the normal treatment at 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
Date: _____

Signature of Parent/Guardian
Date: _____

Patient's Name _____ Adm # _____ Date _____

Ethical Assurance for Protection of Human Rights:

This study will enroll children, aged 6 - 60 months. One-half of them will be provided with L-histidine and the remaining half will receive placebo. The potential for adverse effects is very low. 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*-infected patients. Finally, informed parental consent will be obtained prior to recruitment of each subject.

The study has been reviewed by the Ethical Review Committee (ERC) and Research Review Committee (RRC) of the ICDDR,B. Moreover, a local and independent safety monitoring body will be set up to review the records of the patients during the study.

Dissemination and use of findings:

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 journals.

Future outreach plan:

In the proposed study, L-histidine will be evaluated in *Shigella*-infected children in a hospital setting. However, our long-term objective is to develop a simple, effective, and safe treatment of acute shigellosis using L-histidine and ORS that can be successfully introduced into the community in Bangladesh and elsewhere as a life saving public health tool. Therefore, further plans are underway to examine whether L-histidine supplemented ORS is useful in the community management of *Shigella* dysentery with comparable efficacy. This will be a logical step to follow, assuming that L-histidine produces the expected results in our hospital-based study. Future studies can be accomplished in a larger community-based trial in the Matlab Field Station, where ICCDR,B has maintained a large rural population under a Demographic Surveillance System for many years. Such studies will be useful to address questions like cultural acceptability, tolerance, service delivery issues, cost-effectiveness, and impact evaluation. Negotiations have been started with the Calcutta-based Institute of Cholera and Enteric Diseases in India to examine the possibility of a multicentre trial of L-histidine and ORS in two different populations with similar problems. We believe that the results of these trials would be applicable to other Asian, African, and Latin American countries, because of the similarity of disease pattern in the targeted groups of children and prevalence of drug-resistant shigellae. Moreover, all these regions are characterized by poverty, overcrowding, and poor sanitation, where bacillary dysentery is a common disease.

Technological issues relating to production, storage, distribution, and consumption at the household level would need to be carefully considered. Although the preparation of L-histidine-based ORS does not require sophisticated technology, there are several options that can be considered. For small-scale home management, L-histidine can be mixed with ORS just before use. For hospital management, the amino acid can be added to large volumes of ORS, or it can be supplied as a prepackaged commercial product. Since shigellosis patients do not require large volumes of ORS (because of less fluid loss compared to cholera), most cases can be managed by frequent drinking

of small quantities of ORS containing L-histidine. Thus ORS is desirable, not essential; but it provides a good vehicle for administering L-histidine while providing some fluid, electrolytes, and calories that may be required for the sick child .

Other issues such as cost, storage, distribution channels, etc. need to be considered. However, with the use of WHO and UNICEF ORS introduced almost 30 years ago, most of these problems have been standardized and they have ceased to be major obstacles now. At this stage introduction of another improved ORS would not face as much difficulty as would have occurred in pre-ORS days. With regard to developing a plan for its production, our initiatives with a manufacturer have been very encouraging. However, its long-term application nationally and internationally would require a broader interest, preferably among other industries, governments, and international development agencies including UN organizations. A positive role of an individual government's primary care program and the Integrated Management of Childhood Illnesses (IMCI) may be very useful.

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Collaborative Arrangements

Reasons for international collaboration:

This study will be performed at the ICDDR,B, Dhaka, Bangladesh in collaboration with Prof. J W Peterson, PhD, at the University of Texas at Galveston, USA. This collaboration is important for the study because the original work on L-histidine, which provided the molecular basis of its therapeutic actions, was performed by Prof. J W Peterson, who has maintained an interest of more than 30 years' duration in the pathogenesis of enteric infections and their pharmacologic interruption. Prof. Peterson is a coinvestigator on this study. He has contributed significantly to the development of this project from the beginning. Under this collaborative arrangement, The University of Texas Medical Branch at Galveston will provide laboratory support by performing tests on clinical samples from the study patients, particularly those tests for which laboratory facilities are not available at Dhaka. Arrangements have been made and procedures developed for shipping clinical materials from Dhaka to Galveston, Texas. A list of tests that will be performed at the University of Texas Medical Branch were described in the Methods Section of this proposal; briefly these are:

- Measurement of L- histidine blood levels during the treatment period.
- Assessment of reduced glutathione in liquid stool filtrate and serum and measurement of myeloperoxidase levels in the intestinal fluids of patients with and without L-histidine treatment, will serve to indicate the level of oxidative stress due to acute inflammation in the intestine of the patients.
- Measurement of levels of myeloperoxidase by standard procedures described previously; theseshould reflect the extent of infiltration of polymorphonuclear neutrophils (PMNs) in intestinal tissues.
- Determination of eicosanoid levels in sera and intestinal fluids. In addition to increases in cytokine levels, inflamed tissues contain increased amounts of pro-inflammatory eicosanoids, which are products of the cyclooxygenase and lipoxygenase pathways.
- Assay by commercial ELISA assays for leukotrienes LTB_4 and LTC_4, as well as PGE_2 and $PGF_{2\alpha}$. Our past investigations have used reagents supplied by PerSeptive Diagnostics.

Another objective of the this collaboration is to provide technical assistance to train laboratory personnel at Dhaka to set up some of these tests at the ICDDR,B laboratory that can be developed with the available lab facilities. To organize this program, a trip to Dhaka from Texas for Prof. Peterson has been proposed in the budget of this study. A copy of a collaborating letter from Prof. Peterson is enclosed at the end.

The corresponding address of Prof. J W Peterson is given below.

Johnny W. Peterson, PhD
Samuel Baron Distinguished Professor
Department of Microbiology and Immunology
WHO Collaborating Center for Tropical Diseases
University of Texas Medical Branch
3.170 Medical Research Building
301 University Boulevard
Galveston, TX 77555-1070; Tel: (409) 772-4910
Fax: (409) 747-6869; Email: johnny.peterson@utmb.edu

Revised budget with total number of patients, n=225

First Year Budget

Principal Investigator/Program Director: Dr. G.H. Rabbani

Detailed Budget for First 12-Month Budget Period		From	Through

Personnel		Time/Effort		US $ Amount Requested (omit cents)		
Name	Project position Title	%	Hours per week	Salary	Fringe benefits	Totals
G.H. Rabbani, MD, PhD	Principal Investigator	20	8	6,000/m	included	18,000
David Sack, MD	Co-PI	1	0.5	No cost	Included	
J.W. Peterson, PhD	Co-PI	5	--	No salary cost		
G.B. Nair, PhD	Co-PI	20	8	2,500/m		6,000
Physician (PhD level)	Co-PI/Project Officer/Other	50	20	1,365/m	Included	5,938
Hospital Attendant x 2	Project worker	100	40	174/m	included	4,698
Data Entry Technician	Project staff	100	40	230/m	included	2,760
					included	
Subtotals						37,396

Consultant(s) Costs

Name	Institutional affiliation	Salary	Fringe benefits	Totals
J. W. Peterson, PhD	Professor Univ. of Texas, Galveston	No Salary cost – Lab. cost only (see contractual cost below)		

Contractual costs	**itemize**	
Special Lab. test at Prof. J.W. Peterson's Lab., Galveston, TX (sample will be air shipped from Dhaka to Galveston, TX)		11,324
Supplies	**Itemize**	
	Drugs/Antibiotics/Reagents	633
	Hosp. supplies (glassware, bucket, gloves etc.)	633
	Lab. supplies	380
	Misc. supplies & accessories	1,077
Other Expenses	**Itemize**	
	Lab. tests (stool exam., microbiology culture, biochemistry assays, X-rays etc.)	14,567
	Repair, rent, communication, utilities, printing etc.	1,267
Travel Specify and justify all travel		
Domestic: Local transport for project staff and patients (optional)		300
Foreign: For PI, one return trip to USA for data presentation at convention and discussion study progress and review of Lab. work at Galveston, USA.		4,000
For Co-PI (Prof. J.W. Peterson), one return trip to Dhaka to discuss and review study progress and Lab. work and training.		4,000
Patient Care Costs		
Inpatient: Hospital bed cost @$25/ day x 75 pts x 7 days		16,625
Outpatient: Nil		
	Subtotal:	92,202
Indirect Costs Indirect costs may not exceed seven (7) percent of the total grant, excluding equipment allocations.		6,454
Equipment Itemize Nil		00
	Total:	98,656

2nd Year Budget

Principal Investigator/Program Director: Dr. G.H. Rabbani

Detailed Budget for Second 12-Month Budget Period				From	Through
Personnel		**Time/Effort**		**US $ Amount Requested (omit cents)**	
Name	Project position Title	%	Hours per week	Salary	Fringe benefits	Totals
G.H. Rabbani, MD, PhD	Principal Investigator	20	8	6,000/m	included	14,400
David Sack, MD	Co-PI	1	0.5	No cost	Included	
J.W. Peterson, PhD	Co-PI	5	--	No salary cost		
G.B. Nair, PhD	Co-PI	20	8	2,500/m	Included	6,000
Physician (PhD level)	Co-PI/Project Officer/Other	50	20	1,365/m	included	4,914
Hospital Attendant x 2	Project worker	100	40	174/m	included	4,176
Data Entry Technician	Project staff	100	40	230/m	included	2,760
			Subtotals			32,250

Consultant(s) Costs				
Name	Institutional affiliation	Salary	Fringe benefits	Totals
J. W. Peterson, PhD	Professor, Univ. of Texas, Galveston	No Salary cost - Lab. cost only (see contractual cost below)		

Contractual costs **Itemize**		
Special Lab. test at Prof. J.W. Peterson's Lab., Galveston, TX (sample will be air shipped from Dhaka to Galveston, TX)		8,940
Supplies **Itemize**		
	Drugs/Antibiotics/Reagents	500
	Hosp. supplies (glassware, bucket, gloves etc.)	500
	Lab. supplies	300
	Misc. supplies & accessories	850
Other Expenses Itemize		
Lab. tests (stool exam., microbiology culture, biochemistry assays, X-rays etc.)		9,500
Repair, rent, communication, utilities, printing etc.		1,000
Travel Specify and justify all travel		
Domestic: Local transport for project staff and patients (optional)		300
Foreign: For PI, one return trip to USA for data presentation at convention and discussion study progress and review of Lab. work at Galveston, USA.		4,000
Patient Care Costs		
Inpatient : Hospital bed cost @$25/ day x 75 pts x 7 days		13,125
Outpatient: Nil		
	Subtotal:	71,265
Indirect Costs Indirect costs may not exceed seven (7) percent of the total grant, excluding equipment allocations.		4,989
Equipment Itemize Nil		00
	Total:	76,254

3rd Year Budget (6months)

Principal Investigator/Program Director: Dr. G.H. Rabbani

Detailed Budget for last 6-Month Budget Period					**From**	**Through**
Personnel		**Time/Effort**		**US $ Amount Requested (omit cents)**		
Name	Project position Title	%	Hours per week	Salary	Fringe benefits	Totals
G.H. Rabbani, MD, PhD	Principal Investigator	20	8	6,000/m	included	7,200
David Sack, MD	Co-PI	1	0.5	No cost	Included	
J.W. Peterson, PhD	Co-PI	5	--	No salary cost		
G.B. Nair, PhD	Co-PI	20	10	2,500/m	included	3,000
Physician (PhD level)	Co-PI/Project Officer/Other	50	20	1,365/m	included	2,457
Hospital Attendant x 2	Project worker	100	40	174/m	included	2,088
Data Entry Technician	Project staff	100	40	230/m	included	1,380
			Subtotals			16,125

Consultant(s) Costs				
Name	Institutional affiliation	Salary	Fringe benefits	Totals
J. W. Peterson, PhD	Professor, Univ. of Texas, Galveston	No Salary cost - Lab. cost only (see contractual cost below)		

Contractual costs	**itemize**	
Special Lab. test at Prof. J.W. Peterson's Lab., Galveston, TX (sample will be air shipped from Dhaka to Galveston, TX)		5,960
Supplies	**Itemize**	
	Drugs/Antibiotics/Reagents Hosp. supplies (glassware, bucket, gloves etc.) Lab. supplies Misc. supplies & accessories	333
Other Expenses	itemize	
Lab. tests (stool exam., microbiology culture, biochemistry assays, X-rays etc.)		6,333
Repair, rent, communication, utilities, printing etc.		
Travel Specify and justify all travel		
Domestic: Local transport for project staff and patients (optional)		
Foreign: For PI, one return trip to USA for data presentation at convention and discussion study progress and review of Lab. work at Galveston, USA.		
Patient Care Costs		
Inpatient : Hospital bed cost @$25/ day x 55 pts x 7 days		9,625
Outpatient: Nil		
	Subtotal:	38,376
Indirect Costs Indirect costs may not exceed seven (7) percent of the total grant, excluding equipment allocations.		2,686
Equipment Itemize Nil		00
	Total:	41,062

Total Budget for two and half years

Principal investigator/Program Director:	Dr. G.H. Rabbani

Budget for Entire Proposed Project Period

Budget category totals		First budget period (12 months)	Additional years support requested	
			Second (12 months)	Third (6 months)
Personnel salary and fringe benefits (applicant organization only)		37,396	32,250	16,125
Consultant costs		00	00	00
Contractual costs		11,324	8,940	5,960
Supplies		2,723	2,150	333
Other expenses		15,834	10,500	6,333
Travel	Domestic	300	300	00
	Foreign	8,000	4,000	00
Patient care costs	Inpatient	16,625	13,125	9,625
	Outpatient	00	00	00
Total direct costs		92,202	71,265	38,376
Total indirect costs		6,454	4,989	2,686
Equipment		00	00	00
Total by year		**98,656**	**76,254**	**41,062**
Total for entire proposed project period (Also enter on page 1)				**$ 215,972**

Budget Justifications

Describe the specific functions of all personnel and consultants. Justify costs for all equipment, travel, and contractual arrangements. Include the percentage of anticipated recurring annual increases. Justify yearly increases in all categories. (Use additional pages if necessary.) *A letter confirming the need for principal investigator or co-investigator salary support must be included.* This letter should be signed by an appropriate official indicating the current level of institutional financial support for the investigator.

Budget Justification:

Personnel cost: We have budgeted an amount of $37,396 as personnel cost for the first year of the project, $32,250 for the second year, and $ 16,125 for the last 6 months (total 215,972). This includes salaries of only local investigators and project staff including the PI. However, there are no salary costs for expatriate investigators including Dr. David Sack and JW Peterson. The cost of the P.I.'s salary from this grant proposal does not exceed 20% of the total grant costs per year. The cost of the additional 3-month dosing/safety study in 20 children that was recommended by the Call Panel is included in year one.

The hospital bed cost for 225 patients is $ 39,375 in two years and six months; this is the standard current cost of ICDDR,B hospital @$25/day/pt including nursing, cleaning, diet, and utility services.

The lab costs for necessary investigations including microbiology, biochemistry, clinical, and other tests including repair, printing, communication etc. are $15,834 for the first year, $10,500 for the second year and $ 6,333 for the last 6 months; we think these are justified at the present rate of costs of ICDDR,B services. The first year budget includes the additional costs for the dosing/safety study recommended by the Call Panel. For specialized lab test at Prof. JW Peterson's lab in Texas we have budgeted a total amount of $26,224, which is justified according to the rates provided by his laboratory.

For International travel of PI and Co-PI Prof. J.W. Peterson, we have budgeted three trips ($12,000) in two years between US and Bangladesh; this is justified because direct involvement will be required to review progress of the study, maintain quality control of lab and clinical data between Dhaka and Texas, and to present results in international meetings.

Other expenses including miscellaneous supplies, local transport, sample shipment, etc. are reasonable and self-explanatory.

Other Support

Sources of current funding for each investigator:

1. **Dr. G. H. Rabbani, M.D., Ph.D., Principal Investigator**

i. Studies on nitric oxide and reactive oxygen species in experimental shigellosis (on going): Funded by USAID/Washington. Amount $38,000; June 2000-May 2002; Time allocation 30%.

ii. Evaluation of plant polyphenol in a rabbit model of secretory diarrhoea (on going): Funded by Tomen Corporation, Japan. Amount $36,000; November 2000-December 2001; Time allocation 20%.

iii. Clinical evaluation of L-histidine supplemented rice ORS in patients with cholera (on going): Funded by CATO Research, USA, Amount $78,000; Oct 1999-Dec 2001; Time allocation 30%.

iv. Rice-based green banana in the treatment of persistent diarrhoea in children (on going): Funded by USAID/Washington; Amount $70,000; May 2001-April 2003; Time allocation 20%.

2. **Prof. David Sack, M.D., Co-Investigator,**

Receives no direct research funding; employed as Director, ICDDR,B. Role in this project as no cost investigator/consultant.

3. **Johnny W Peterson, PhD, University of Texas at Galveston, Co-Investigator**

Active
RO1 AI121463-11A2 (Peterson) 05/01/01-04/30/0225%
NIH/NIAID $150,000/annum
Molecular mode of action of bacterial enterotoxins

The objective of the research is to clarify the cause-effect relationships of cyclic AMP and arachidonic acid metabolites along with serotonin as mediators of the physiological effects of cholera toxin on the small intestine.

Pending
(Peterson) 12/01/01-11/30/06 40%
NIH/NIAID $200,000/annum
Modulation of Secretory Diarrheas

The objective is to examine whether eicosanoid-imidazole covalent adducts have the potential to interrupt the pathogenic mechanism of secretory diarrheal diseases.

Explanations of International Activities

This study aims to test the efficacy of L-histidine, an amino acid, in the treatment of acute shigellosis in children in Bangladesh. The clinical study will be performed at the Dhaka Hospital of the ICDDR,B: Centre for Health and Population Research in Bangladesh. The reasons for conducting the study in Bangladesh are as follows:

1. Although shigellosis is now a worldwide disease, the number of deaths and disabilities are highest in the developing countries, including Bangladesh.

2 ICDDR,B, an international research center located in Bangladesh, has been recognized worldwide as a center of excellence for research in public health and infectious diseases since the early sixties. The Centre provides unique clinical and laboratory facilities for evaluation of therapeutic agents for shigellosis; moreover the investigators have significant experience and necessary skills and ability to successfully perform such studies.

3 By status, ICDDR,B is an international center, duly recognized by the Government of Bangladesh and run by the funds provided by international donor communities including USAID, DFID (UK), World Bank, and many other governments, UN agencies, and private foundations. ICDDR,B is non political and philanthropic.

4 ICDDR,B in Bangladesh provides unique opportunities in terms of cost-effectiveness of performing such studies. Patient management, laboratory investigations, clinical evaluation, project management, and data analysis can be done in the most economic way.

5 ICDDR,B maintains high ethical standards of biomedical research and excellent clinical practice.

6 ICDDR,B in Bangladesh has very good research collaboration with many research centers, laboratories, and universities around the world.

7 The progress and results of the study can be reviewed and experience shared by any collaborating institute in other countries.

8 ICDDR,B has established a functional research collaboration with the University of Texas Medical Branch at Galveston during the development of this project.

9 Since ICDDR,B is the only institute of its kind in the world, it would be difficult to find a better alternative site that could guarantee a greater probability of success.

10 If the study determines that L-histidine improves treatment of shigellosis, its further development and promotion as a public health tool for child survival can be undertaken by ICDDR,B in its community-based Child Health Programs in rural Matlab.

11 As has been shown in the past, the results of the Bangladesh study can be reproduced and implemented in other countries with similar problems.

12 The above-mentioned reasons would provide sufficient justifications for conducting the proposed study at ICDDR,B Bangladesh.

Biographical Sketch

Dr. G.H. Rabbani: 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.

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

1983 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 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.

4. 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.

5. Rabbani GH, Albert MJ, Islam M, Alam K. Short-chain fatty acids inhibit cholera toxin induced fluid and electrolyte secretion in the rabbit colon *in vivo*. Dig Dis Sci 1999; 44:1547-1553.

6. Rabbani GH. The search for better oral rehydration solution for cholera. Editorial. N Eng J Med 2000; 342, No. 5.

7. Rabbani GH, Sack DA, Peterson JW. L-Histidine improves colitis in experimental shigellosis in rabbit. Abstract in Experimental Biology (FASEB) 2000. San Diego, CA, April 15-18, 2000.

7. Rabbani GH, Teka T, Zaman B, Fuchs G. Green banana and pectin in the dietary management of persistent Diarrhoea in children. Gastroenterology 2001; 121:554-560.

8. Rabbani GH, Teka T, Zaman B, Fuchs G. Green banana and pectin improve intestinal permeability in Bangladeshi children with persistent diarrhoea. Abstract in Gastroenterology, 2000.

9. Rabbani GH, Islam S, Fuchs G. Elevated nitric oxide concentrations in patients with cholera and shigellosis. Am J Gastroenterology, 2000 (Accepted).

10. Rabbani GH, JW Peterson, D Sack: Antiinflammatory activity of L-histidine in a rabbit model of Colitis due to infection with *S. flexneri* 2a. Abstract in FASEB Journal, Orlando, FL, 1-4 May, 2001.

Biographical Sketch

NAME: David A. Sack	Date of Birth:
Title: Director, ICDDRB,B, Centre for Health and Population Research and Professor, Department of International Health, Johns Hopkins University	30 Nov 1943

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Lewis and Clark College, Portland Oregon	BS	1961-65	Natural Science
University of Oregon Medical School, Portland, Oregon	MD	1964-68	Medicine
University of Iowa School of Medicine, Iowa City, Iowa		1968-70 & 1971-73	Internship & Residency Internal Medicine
Johns Hopkins University School of Medicine, Baltimore		1974-75	Fellowship, Infectious Diseases

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. DO NOT EXCEED TWO PAGES.

PROFESSIONAL EXPERIENCE

1999 Director, ICDDRB, Centre for Health and Population Research, Dhaka, Bangladesh

1994-1999 Head of the Johns Hopkins Vaccine Testing Unit, and Professor, Department of International Health, (with joint appointment in Department of Epidemiology) The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland 21205. Joint appointment: Department of Medicine, Division of Infectious Diseases, The Johns Hopkins School of Medicine

1993-1994 Coordinator for Control of Diarrheal Disease Projects for BASICS, Rosslyn VA

1991-92 Medical Officer for USAID sponsored PRITECH project, with emphasis on assistance with cholera control

1985-1994 Associate Professor, Department of International Health, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland 21205

1984-1987 Associate Director of ICDDR,B and Head of Division of Epidemiology and Laboratory Sciences, International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

1982-1985 Associate Professor, Division of Geographic Medicine, Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

1977-1980 Scientist, International Centre for Diarrhoeal Disease Research, Bangladesh (formerly Cholera Research Laboratory) Dhaka, Bangladesh

1977-1982 Assistant Professor, Division of Geographic Medicine and Division of Infectious Disease, The Johns Hopkins University School of Medicine, Baltimore, Maryland

1976 Instructor, Infectious Disease Division, The Johns Hopkins University School of Medicine, Baltimore, Maryland

1971 Volunteer Physician, Lubumbourg (Kananga), Zaire, Africa

1969-1971 Public Health Service, Director Indian Health Center, Lame Deer, Montana

ADVISORY PANELS

1985-1989	Member, World Health Organization Scientific Working Group on Immunology and Vaccine Development for the WHO Global Program on Diarrhoeal Diseases, Geneva
1990	Organizing Committee of an international symposium in Gothenburg Sweden on May 28-29, 1990: "New vaccines against enteric infections: Prospects for public health benefits in developing countries."
1992-1994	Advisor to Virus Research Institute on vaccine development, Cambridge, Massachusetts
1993-1996	Head, Task force on Cereal Based ORS for the International Child Health Foundation
1995-present	Member, Data safety and monitoring committee for the U.S. Army oral *E. coli* vaccine trials in Egypt, Boston
1999-present	Member, Data safety and monitoring committee for the ARIVAC trial of pneumococcal vaccine in Philippines

1985-1989	Member, World Health Organization Scientific Working Group on Immunology and Vaccine Development for the WHO Global Program on Diarrhoeal Diseases, Geneva
1990	Organizing Committee of an international symposium in Gothenburg Sweden on May 28-29, 1990: "New vaccines against enteric infections: Prospects for public health benefits in developing countries."
1992-1994	Advisor to Virus Research Institute on vaccine development, Cambridge, Massachusetts
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1995-present	Member, Data safety and monitoring committee for the U.S. Army oral *E. coli* vaccine trials in Egypt, Boston
1999-present	Member, Data safety and monitoring committee for the ARIVAC trial of pneumococcal vaccine in Philippines

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BIBLIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
Johnny W. Peterson	**Professor**

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.*)

INSTITUTION AND LOCATION	DEGREE (*if applicable*)	YEAR(s)	FIELD OF STUDY
University of Texas at Arlington, TX	B.S.	1967	Biology/Chemistry
Univer. of North Texas, Denton, TX	M.S.	1969	Microbiology/Biocl
Univer. of Texas Southwestern Medical, Dallas	Ph.D.	1972	Microbiology/Biocl

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

APPOINTMENTS: Depart. of Microbiol. & Immunol., Univ. TX Med. Branch, Galveston, TX, Assist. Prof., 1972-77; Assoc. Prof., 1977-82; Professor, 1982-2001; Vice-Chair, 1997-2000; Samuel Baron Distinguished Professor, 2001-2006.

HONORS: President's Fellowship Award, ASM, 1971; O.B. Williams Award, 1971; S.E. Sulkin Award, 1972; President, Texas Branch ASM, 1981-82; Texas Branch ASM Distinguished Service Award, 1990; NIH Bacteriology-Mycology Study Section, 1982-87; Distinguished Service Award for Research, UTMB Graduate School of Biomedical Sciences, 1991.

MEMBERSHIP IN SCIENTIFIC SOCIETIES: American Society for Microbiology; Texas Branch of American Society for Microbiology; American Academy for Microbiology; American Gastroenterological Association

RESEARCH PROJECTS ONGOING OR COMPLETED DURING THE LAST 3 YEARS:

Title: "Virulence factors in the pathogenesis of salmonellosis"
Principal Investigator: Johnny W. Peterson, Ph.D.
Agency: National Institute of Allergy and Infectious Disease
Type: R01 (AI18401,Year 15) Period: 09/01/95 - 07/31/99
Summary: The objective was to define the role of *Salmonella* enterotoxin (Stn) as a virulence factor in the intestinal phase of *Salmonella* infection.

Title: "Molecular mode of action of bacterial enterotoxins"
Principal Investigator: Johnny W. Peterson, Ph.D.
Agency: National Institute of Allergy and Infectious Disease
Type: R01 (AI121463-11) Period: 05/01/96-04/30/00 and R21(AI21463) Period: 05/01/01-04/30/02
Summary: The objective is to clarify the cause-effect relationships of cyclic AMP and arachidonic acid metabolites as mediators of the physiological effects of cholera-toxin on the small intestine.

PUBLICATIONS (selected):

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Lay Abstract

Background and Objectives:

Shigellosis is characterized by dysenteric illness with fecal blood and mucus and is a major cause of childhood deaths and disability in both developing and developed countries of the world. Published reports indicate that at least 140 million cases of shigellosis, and almost 600,000 deaths due to shigellosis occur worldwide annually among children younger than 5 years of age, primarily in developing countries. It is estimated that of approximately 3.8 million diarrhea-related deaths that occur worldwide in children annually (exclusive of China), 0.5 million are attributable to shigellosis. Better management of dysentery caused by *Shigella* necessitates the search for effective and safe alternative adjunctive therapy, primarily because of the development of bacterial resistance to most commonly used anti-infective drugs against shigellae. In view of this, L-histidine, a normal constituent of food, could be a potential therapeutic agent because of its protective anti-inflammatory effects on the intestines, particularly the colon, since shigellosis is predominantly a colonic disease. In a recent study in rabbits, we have demonstrated that L-histidine significantly improved the clinical symptoms and intestinal damage caused by these bacteria in a rabbit model. L-Histidine has been shown to protect against intestinal damage and the loss of water and salts due to challenge with another bacterium (*Salmonella typhimurium* and cholera toxin. L-Histidine and its products decrease the levels of chemical agents in the body that mediate the tissue-damaging effect of the bacterial infection. Acting through a complex chemical mechanism, L-histidine is able to reduce and reverse the intestinal damage produced by the bacteria and thus improve the severity of the disease.

Methods: We, therefore, propose a clinical study with the primary objective of evaluating the therapeutic efficacy of L-histidine in the clinical management of children with shigellosis. One hundred fifty children, aged 6-60 months, with *Shigella* dysentery of <72 hours duration will be hospitalized for 7 days in a study ward at ICDDR,B. Routine clinical care will be provided. All children will receive ciprofloxacin, 15 mg/kg every 8 hours (standard therapy) for 7 days. They will be randomly allocated to either L-histidine (n=75) or placebo (n=75) treatment groups. L-Histidine will be given by mouth in a dose of 75 mg/kg every 2 hours mixed into a rice-based, vanilla flavored ORS. The control children will be given rice-based ORS without added L-histidine. A detailed clinical history will be obtained, and a thorough physical examination will be performed. Vital signs will be recorded every 8 hours. 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 two treatment groups. Breast-fed children will continue to breastfeed and the amount will be recorded by test weighing. The effects of L-histidine treatment will be evaluated by specific indicators, including resolution of fever and fecal blood, decrease in number of fecal leucocytes, fecal RBCs, fecal Hb, lactoferrin, fecal MPO, and quantitative bacterial counts using streptomycin containing medium. Secondary indicators of treatment success will include clinical and bacteriologic cure rates based on clinical characteristics (fever, tenesmus, leucocytosis, cramps, fecal blood, fecal mucus, number of bowel movements per day) and duration of fecal excretion of shigellae.

Rationale: If the study demonstrates efficacy of L-histidine as an adjunct in the clinical management of shigellosis, it would improve the treatment of shigellosis by providing a non-antibiotic, safe, and effective adjunct treatment. This treatment can also be introduced as a home-treatment in the community, preferably in combination with ORS. This treatment would provide an important public health tool in the management of diarrhoeal diseases for child survival strategy.

Abstract Summary for ERC

Background and Objectives:

Shigellosis remains a major cause of childhood morbidity and mortality in many developing countries,- including Bangladesh. Published reports reflect that at least 140 million cases of shigellosis, and almost 600,000 deaths due to shigellosis occur worldwide annually among children under the age of 5 years, primarily in developing countries. It is estimated that of approximately 3.8 million diarrhea-related deaths that occur worldwide in children annually (exclusive of China), 0.5 million are attributable to shigellosis. Better management of bacillary dysentery caused by *Shigella* spp necessitates a search for effective and safe alternative adjuvant therapy, primarily because of the rapidly emerging drug-resistant shigellae. In view of this, L-histidine could be a potential therapeutic agent because of its anti-inflammatory effects on the intestines, particularly the colon, since shigellosis is predominantly a colonic disease. In a recent study we demonstrated that L-histidine significantly improved clinical, histological, and bacteriological features of experimental shigellosis in a rabbit model. L-Histidine has been shown to protect against tissue damage and the loss of water and electrolytes upon challenge with both*Salmonella typhimurium* and cholera toxin. L-Histidine and its imidazole derivatives decrease the levels of proinflammatory cytokines (e.g., TNFα and IL-6) and diminish the biological activity of the proinflammatory eicosanoids PGE_2 and LTB_4. In the case of eicosanoids, it was demonstrated that the imidazole group of L-histidine chemically reacts with PGE_2, forming a covalent bond. Additional research has demonstrated that the resulting PGE_2-imidazole adduct is a potent inhibitor of PGE_2 activity and effectively blocks cholera toxin-induced intestinal fluid loss.

Methods: We, therefore, propose a double-blind, controlled clinical trial with the primary objective of evaluating the therapeutic efficacy of L-histidine in the management of children with shigellosis. One hundred fifty children aged 6-60 months, with *Shigella* dysentery of ≤48 hours duration will be hospitalized for 7 days in a study ward at ICDDR,B. Routine clinical care will be provided. All children will receive ciprofloxacin, 15 mg/kg every 8 hours for 7 days. They will be randomized into either L-histidine (n=75) or placebo (n=75) treatment groups. L-Histidine will be given by mouth at a dose of 75 mg/kg every 2 hours. L-Histidine will be administered orally by mixing it with a rice-based, vanilla flavoured ORS. The children in the control group will be given rice ORS without added L-histidine. A detailed clinical history will be obtained, and a thorough physical examination will be performed. Vital signs will be recorded every 8 hours. 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 two treatment groups. Breast-fed children will continue to breastfeed and the amount will be recorded by test weighing. The effects of L-histidine treatment will be evaluated by specific indicators including: resolution of fever and fecal blood, decrease in number of fecal leucocytes, fecal RBCs, fecal Hb, lactoferrin, fecal MPO, and quantitative bacterial count using Streptomycin containing medium. Secondary outcome variables would include clinical and bacteriologic cure rates based on clinical characteristics (fever, tenesmus, leucocytosis, cramps, fecal blood, fecal mucus, number of motions per day) and duration of fecal excretion of shigellae.

Rationale: If the study demonstrates efficacy of L-histidine as an adjunct in the management of shigellosis, it will improve the treatment of shigellosis by using a non-antibiotic, safe, and effective treatment, and thus, reduce child mortality in the long run. This treatment can also be introduced as a home-treatment in the community, preferably in combination with ORS. This treatment would provide an important public health tool in the management of diarrhoeal diseases for child survival strategy.