PHSD 2002

International Centre for Diarrhoeal Disease Research, Bangladesh
CENTRE FOR HEALTH AND POPULATION RESEARCH
Mail : ICDDR, B, GPO Box 128, Dhaka-1000, Bangladesh
Phone : 880-2-8811751-60, Telex : 642486 ICDD BJ
Fax : 880-2-8823116, 8812530, 8811568, 8826050, 9885657, 8811686, 8812529
Cable : Cholera Dhaka

Memorandum

19 January 2003

To : Dr. Kh. Zahid Hasan
Principal Investigator of protocol # 2002-013
Public Health Sciences Division

From: Professor Mahmudur Rahman
Chairperson, ERC

Sub : **Approval of protocol # 2002-013**

Thank you for your memo dated 15th January 2003 with the modified version of your protocol # 2002-013 entitled "Longitudinal study of events associated with *H. pylori* acquisition in Bangladeshi children". The modified version of your protocol is hereby approved upon your satisfactory addressing of the issues raised by the ERC in its meeting held on 29th May 2002.

You shall conduct the study in accordance with the ERC-approved protocol; and shall be responsible for protecting the rights and welfare of the subjects and compliance with the applicable provisions of ERC Guidelines. You shall also submit report(s) as required under ERC Guidelines. Relevant excerpt of ERC Guidelines and '*Annual/Completion Report for Research Protocol involving Human Subjects*' are attached for your information and guidance.

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

Thank you.

Copy: Associate Director
Public Health Sciences Division

International Centre for Diarrhoeal Disease Research, Bangladesh
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Cable : Cholera Dhaka

15 January 2003

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

FROM : Dr. Kh. Zahid Hasan
Public Health Science Division

SUB : Protocol # 2002-013

As per you letter dated 3 June 2002, I have corrected the face sheet of the protocol # 2002-013 entitled " Longitudinal study of events associated with *H. pylori* acquisition in Bangladeshi children" and submitting to you for your consideration.

Thank you

Attachment 1

Date:

(FACE SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator: K. Z. Hasan

Application No. 2002 - 013

Title of Study: Longitudinal study of events associated with H. pylori acquisition in Bangladeshi children

Trainee Investigator (if any): _____

Supporting Agency (if Non-ICDDR,B) _____

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 N/A
   - (b) Procedures to be followed including alternatives used — Yes No N/A
   - (c) Physical risk — Yes No N/A
   - (d) Sensitive questions — Yes No N/A
   - (e) Benefits to be derived — Yes No NA
   - (f) Right to refuse to participate or to withdraw from study — Yes No N/A
   - (g) Confidential handling of data — Yes No NA
   - (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — Yes No NA

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 — NA 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.

Principal Investigator

Trainee

International Centre for Diarrhoeal Disease Research, Bangladesh
CENTRE FOR HEALTH AND POPULATION RESEARCH
Mail : ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh
Phone : (880-2) 8826318 / 8810117, PABX: 8811751-60
Fax : 880-2-8823116, 8826050, 8811568, 8811686, Cable: Cholera Dhaka
Email: analam@icddrb.org or ted@icddrb.org or brsaha@icddrb.org

MEMORANDUM

3 June 2002

To : Dr. Kh. Z. Hasan
Public Health Sciences Division

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

Sub : Protocol # 2002-013

Thank you for your protocol # **2002-013** entitled "Longitudinal study of events associated with *H. pylori* acquisition in Bangladeshi children", which the ERC considered in its meeting held on 29th May 2002. After review and discussion, the Committee made the following observations on the protocol:

a) The full title of the protocol should be mentioned on the ERC Face Sheet.

b) On the ERC Face Sheet, item # 3(a) –3(c) should marked YES instead of no.

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

Thank you.

cc: Associate Director
Public Health Sciences Division

Attachment I

Date:

(FACE SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator: K. Z. Hasan

Application No. 2002 - 013

Title of Study: Longitudinal study

Trainee Investigator (if any): _____

Supporting Agency (if Non-ICDDR,B) _____

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)
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   - (c) Minor or persons under guardianship — Yes (No)

2. Does the Study Involve:
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   - (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)

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   - (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 N/A
   - (b) Procedures to be followed including alternatives used — Yes No N/A
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   - (d) Sensitive questions — Yes No N/A
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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 — N/A 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
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* 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
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3. An indication as to when the questionnaire will be presented to the Committee for review

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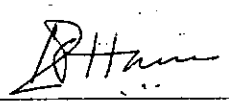

Principal Investigator

Trainee

RESEARCH PROTOCOL

Protocol No. 2002 - 013

FOR OFFICE USE ONLY
RRC Approval: Yes/ No Date:
ERC Approval: Yes/No Date:
AEEC Approval: Yes/No Date:

Project Title: Longitudinal study of events associated with *H. pylori* acquisition in Bangladeshi children

Theme: *(Check all that apply)*

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

Key words: ***H.pylori*** , environmental factors associated with acquisition, child development

Relevance of the protocol: *Helicobacter pylori* infection is probably the commonest infection worldwide, and is linked with the development of peptic ulcers and gastric cancers, among other things. *H. pylori* is highly prevalent in developing countries, including Bangladesh, and acquisition starts early in life. However, little is known about its transmission, the risk factors for its spread or the early consequences. This study will measure the acquisition rate of *H. pylori* in the first 2 years of life, and will examine its association with family size, crowding, birth weight, and birth order. This study will also examine the time course and evolution during the acute phase as well as the early chronic phase of *H. pylori* infection in infancy and early childhood. In addition, this study will assess the impact of *H. pylori* infection on childhood growth and development as well as incidence of diarrhoeal diseases. Thus, this protocol will address important issues on the epidemiology of *H. pylori* infection in childhood and will help in defining effective control strategies.

Principal Investigator: K. Z. Hasan, ICDDR,B **Division : PHSD** **Phone: 8811751-60/2211**

Co-Principal Investigator(s): P. K. Bardhan, ICDDR,B

Co- Investigator(s): **SA Saker, ICDDR,B**
Guillermo I Perez-Perez, New York University School of Medicine
Martin J. Blaser, NYUSM
R.B. Sack, JHU

Collaborating Institute(s): Division of Infectious Disease, New York University School of Medicine
International Health, JHU, Bloomberg School of Public Health, Baltimore

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

Gender
- X Male
- X Females

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

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

Revised on: 11 March 2002

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

- ☐ Dhaka Hospital
- ☐ Matlab Hospital
- ☐ Mirsarai
- ☐ Patyia

- ☐ Matlab DSS area
- ☐ Matlab non-DSS area
- X Mirzapur
- ☐ Dhaka Community
- ☐ Chakaria
- ☐ Abhoynagar
- ☐ 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
- x Longitudinal Study (cohort or follow-up)
- ☐ Record Review
- ☐ Prophylactic trial
- ☐ Surveillance / monitoring
- x Others: Laboratory investigation of collected samples and Analysis of existing data

Targeted Population *(Check all that apply)*:

- X No ethnic selection (Bangladeshi)
- ☐ Bangalee
- ☐ Tribal groups
- ☐ Expatriates
- ☐ Immigrants
- ☐ Refugee

Consent Process *(Check all that apply)*:

- ☐ Written
- ☐ Oral
- X None
- ☐ Bengali language
- ☐ English language

Proposed Sample size: Total sample size: 250 children from birth to 24 months of age

Sub-group _____ ☐ _____ ☐

_____ ☐ _____ ☐

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

Yes/No

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

- ☐ x a. place the subject at risk of criminal or civil liability?
- ☐ x 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
- x 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".

Yes/No

Yes/No

x ☐ Is the proposal funded?

If yes, sponsor Name: Thrasher foundation

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?

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

Dates of Proposed Period of Support	**Cost Required for the Budget Period ($)**			
(Day, Month, Year - DD/MM/YY)	*a. Ist Year*	*2nd Year*	*3rd Year*	*Other years*
Beginning date_July 1, 2002	30,700	15,430		
End date___February 29, 2004	*b. Direct Cost :* 43,472	*Total Cost :* 46,130		

Approval of the Project by the Division Director of the Applicant

The above-mentioned project ~~has~~ not been discussed and reviewed at the Division level ~~as well~~ or by the external reviewers. ~~The protocol has been revised according to the reviewer's comments and is approved.~~ Is a follow-up and use of a previous study. Forwarded to RRC,

Prof. Lars Ake Persson		1/5 2002
Name of the Division Director	Signature	Date of Approval

Certification by the Principal Investigator

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

Signature of PI _____

Date: 30/4/2002

Name of Contact Person (if applicable)

_____

Project Application-Thrasher Research Fund

Title (Provide a descriptive title rather than a general title.)

Longitudinal study of events associated with *H. pylori* acquisition in Bangladeshi children

Principal Investigator
Specify one person who is responsible to the Thrasher Research Fund for
for scientific or technical work of the project This individual is responsible for grant-related correspondence and expenditures.

Name and address
Perez-Perez Guillermo I
6027W VAMC NYUniversity
423 East 23th. Street
New York NY 10010
Telephone 212-252-7169 Fax 212-252-7167
E-mail perezg02@med.nyu.edu

Co-principal Investigator(s)
List additional individuals who are responsible to the Thrasher Research Fund for
scientific or technical work of the project.

Names and institution(s)
Martin J. Blaser NYUSM
Zahid H International Center for
Bardhan PK Diarrhoeal Disease Research
Sarker SA Dhaka, Bangladesh

Human Subjects If human subjects are involved, complete the Protection of Human Subjects form (page 13).
Form status
No X Yes X Pending N/A

Animal Welfare Assurance If animals are involved, please provide proper documentation of compliance with the laws
Form status
X No Yes Pending X N/

Project Period The entire project may not exceed 3 years The project start date will be determined in con ultation with Thrasher Research Fund Staff.
Approximate start date April 2002 Approximate end date March 2004

Total Budget Request
$ 207,314.00

Performance Site(s) Indicate where work described in the "Experimental Design and Methodology" section will be performed Please provide specific organization
names and complete addresses, including zip code/postal code, telephone number, tax number, and e-mail.

Organization name and address
Division of Infectious Diseases
New York University School of Medicine
6027 W VAMC
423 East 23th. Street
New York, NY 10010

Organization name and address
ICDDRB; Centre for Health & Population Research
Dhaka, Bangladesh
Mohakhali
Dhaka 1212, Bangladesh

Supervising Institution Name the one organization that will be legally and financially responsible and accountable for the use and disposition of any funds awarded on the basis of this application.
Supervising Institution contact information (name, address, phone, and fax)
New York University School of Medice Phone: 212-263-8822
550 First Avenue Fax: 212-263-8201
New York NY 10016

Official Signatures for Supervising Institution Provide those signatures that apply to your organization.
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 will comply with the Thrasher Research Fund's Conditions of Grant and requirements for reporting that are in effect at the time of the award.

Print/Type name of principal investigator	Title
Signature	Date
Print/Type name of department chairman, if applicable	Title
Signature	Date
Print/Type name of official from supervising institution	Title
Signature	Date

Abstract Summary

Infection with the bacterium *Helicobacter pylori* is very common in developing countries such as Bangladesh, where almost 80% of the adult population is infected, but even more important is that around the half of all the children younger than 10 years are already infected with this organism. It is known that the infection is acquired mainly in childhood but the exact mechanisms involved in the transmission and establishment of persistent infection are unknown. Furthermore, there is no information of the clinical consequences of *H. pylori* acquisition, nor of the complications that the infection may bring with it. This proposal aims to follow the progress of a group of Bangladeshi newborn babies through the first 2 years of life to determine when *H. pylori* is acquired and under what circumstances it persists. The study will be conducted retrospectively in a birth-cohort in Mirzapur, originally recruited in 1993-94. Samples (blood, breast milk, and stool) already collected and stored will be analysed, generating information about status of *H. pylori* infection, type and intensity of specific immune response, and gastric functional response. These information will be correlated with data already collected (socio-economic status, family size and birth order, nutritional anthropometry, feeding pattern, morbidity sutveillance for diarrhoea and acute respiratory infection). In addition, whether the presence of *H. pylori* affects the general health and natural development of children or has any influence on the development of other infectious diseases like diarrhoea will be determined. Thus, this protocol will provide important information on the epidemiology of *H. pylori* infection in childhood, helping in defining effective control strategies.

Project Background and Significance

A. Hypothesis(es) to be Tested

1. *H. pylori* acquisition in developing countries occurs after the first year of life and continues during the first decade of life. Such early acquisition of *H. pylori* is associated with large family size, crowding, and birth order.

2. The acquisition of *H. pylori* represents a major disturbance in the gastric mucosa of the colonized child, and gastric colonization with *H. pylori* has a major influence on the health and normal development of children. The effects of early acquisition of H. *pylori* is manifested in lower body weight and height and higher incidence of diarrhoea, particularly during the second year of life.

B. Specific Aim(s) of the Project

1. **To define the timing of *H. pylori* acquisition and the host and environmental factors associated with acquisition.** We aim to describe the timing, prevalence and predisposing factors for *H. pylori* infection through study of a cohort of children in Bangladesh.

2. **To study the physiological and immunological consequences of acquisition of *H. pylori* at**

different ages and its impact on child development. Specially, we aim to define the time course and evolution during the acute phase as well as in the early chronic stage of *H. pylori* colonization (the first 2 years) of the following parameters: a) gastric acid response (gastrin) to acquisition of *H. pylori*, b) serum markers of disordered gastric physiology (e.g. pepsinogen I and II) and c) the humoral immune response to relevant *H. pylori* antigens. We aim here to assess the impact of *H. pylori* carriage on childhood growth and incidence of diarrheal diseases.

C. Background and Project Significance

Gastric colonization by the gram negative bacterium *Helicobacter pylori* was demonstrated in 1983 (1) and its now one of the most important bacterial infections in humans (2). *H. pylori* infection causes most cases of peptic ulceration and is a major risk factor for gastric adenocarcinoma (3,4). These diseases create an enormous burden of morbidity and mortality, especially in developing countries where the prevalence of *H. pylori* among adults is about 80% (5,6). Although diseases associated with *H. pylori* infections occur mainly during adulthood, *H. pylori* usually is acquired in childhood, and in the absence of specific treatment frequently persists for life (2,7). Thus, the source of the infection, its mode of transmission and its risk factors can be most meaningfully studied in childhood. Events surrounding childhood infection may influence disease expression in adulthood; for example, birth order is a determinant of gastric cancer risk among infected individuals (8). It has been postulated that the onset of infection may cause an acute vomiting or diarrheal illness (9,10) that could be the source of transmission of *H. pylori* to other susceptible individuals.

Although *H. pylori* colonization causes disease in adults, it is unclear what effects, if any, it has in childhood (11,12). Acute infection in adults is known to cause temporary gastric achlorhydria, and this could lead to further health disadvantages in children. For example, by abolishing the gastric acid barrier, *H. pylori* infection might increase the risk for acquiring other infectious agents via the gastrointestinal tract. Furthermore, chronic infection with its accompanying inflammation may also be deleterious, and it has been suggested that this may stunt growth. On the other hand, *H. pylori* infection could have health advantages in childhood. For example, the non-specific immune response (13), the antibacterial peptides produced by *H. pylori* (14), and the lowered gastric pH induced by chronic infection in some people could reduce the incidence or the severity of diseases acquired through the gastrointestinal tract (15).

The question of whether *H. pylori* infection is harmful, neutral, or beneficial to young children is critical, especially in developing countries where it is so prevalent, and where other gastrointestinal infections are hyperendemic. Development of *H. pylori* vaccines is progressing, and combined antibiotic therapies offer effective treatments. There is reason to believe that eradication of childhood infection by either prevention or treatment would virtually abolish peptic ulceration from a community and significantly reduce the incidence of gastric carcinoma. However, before applying strategies to eradicate *H. pylori*, it is vital to know whether *H. pylori* infection has any benefits in children, or whether it has any specific disadvantages, which make it important to treat infection early rather than screen and treat adolescents or young adults. From retrospective analyses, there is controversy over whether the presence of *H. pylori* increases (16,17) or decreases (15) the risk or exerts not risk of

childhood diarrhea.

There are thus a number of other specific points that need to be addressed to determine when and how to manage *H. pylori* infection in children. Specifically, we need to know: 1. When children acquire the organism and how frequently the infection becomes persistent (so intervention can be timed appropriately), 2. From whom children acquire the organism and what determines their risk (so the infection cycle can be broken), and 3. Whether events in childhood determine expression of disease in later life and if so which events are important (so treatment can be targeted to those who need it).

Previously, it has been difficult to study the crucial period of *H. pylori* acquisition in childhood. However, now most non-invasive tests show accurately whether *H. pylori* is present or not. Such assays include the isotope urea breath and urine tests (18-20), the stool antigen test and serological antibody-based tests (21-23). Other new tests are less sensitive but allow culture of the bacterium; these include stool culture and the string culture test (24-26).

D. Supportive Preliminary Data

D1. Validation of a serologic test for the diagnosis of *H. pylori* infection in Developing countries. In 1987, our group was one of the first in the world to develop accurate serologic assays for determination of *H. pylori* status (22,28). We also sought to use such methods for studies in developing countries (29). Given the paucity of knowledge about *H. pylori* infection and the immune response in pediatric populations (especially in developing countries), we have validated the serological for *H. pylori* whole cell extracts, as well as to the major virulence factor CagA. The immunoassays for serodiagnosis were developed using either an antigen preparation with native *H. pylori* strains isolated from Mexican patients' (30) or using the antigen preparation with the 5 Western H. pylori strains (28). We first defined the cutoff values by studying the serum antibody response of 30 *H. pylori*-negative children. The assay then was validated in 20 children with biopsy-confirmed *H. pylori* infection and 30 uninfected children in whom the absence of infection was determined by biopsy urease test, histologic examination and culture. The whole-cell extract ELISA had a sensitivity of 85% and specificity of 87%. The assay was then used in children with recurrent abdominal pain (RAP) and in healthy control children. We demonstrated that *H. pylori* prevalence was higher in the symptomatic pediatric group than in the control group (65% vs. 48% p=0.009). Importantly, our results indicated that we could detect the presence of *H. pylori* without subjecting the patients to endoscopy.

D2. Comparison of invasive and non-invasive methods for the diagnosis of *H. pylori* infection in children. Since acquisition of *H. pylori* occurs mainly during childhood, it is important to have reliable diagnostic methods in this age group. We studied 59 children in Mexico City to validate invasive and non-invasive tests for the diagnosis of *H. pylori* infection in children (31). The urea breath test has been used by our group in Bangladesh to assess the prevalence of *H. pylori* infection in children (32,33). These results confirm that it is possible to document the presence of *H. pylori* by non-invasive tests in children. The high specificity of both assays and the sensitivity of the urea breath test and specificity of serology will allow us to establish precise *H. pylori* status.

D3. Performance of community-based seroepidemiologic studies of *H. pylori* infection. We performed a nation-wide community-based survey of *H. pylori* infection in Mexico, and identified socioeconomic and demographic risk factors for infection. We demonstrated that by the age of one year, 20% of the children are infected and the prevalence of infection increased to 50% by age 10 years. The results also showed that infection is highly prevalent in Mexico and occurs early in life. Crowding and low socioeconomic class were the main risk factors for infection (6). From this study, we can estimate that *H. pylori* acquisition rates in children under 5 years of age is as high in Mexico as is observed in other developing countries (29,35). In particular, prevalence of H. pylori is 60% in Bangladeshi children by 5 years of age (36). Since the epidemiology of *H. pylori* in Bangladesh is similar to Mexico, thus Bangladesh offers conditions in which to study *H. pylori* acquisition, risk factors for acquisition, and consequences of infection, using a relatively small sample size. The similarity of the epidemiology of *H. pylori* in urban and rural Bangladesh will allow us to work in an urban setting with consequent easing of strategic issues, such as patient accrual and the processing of samples.

D4 . Study of *H. pylori* acquisition in a birth cohort of Native American children. *H. pylori* is chiefly acquired in childhood, but knowledge of the exact timing of acquisition and the host responses are not well understood.Longitudinal studies in children might permit new insights into these questions. We have recently assessed transient and persistent colonization by *H. pylori* in Apache children (37). We studied two groups of Native American children from the White Mountain Apache reservation in Arizona. Among 48 available mothers of the birth-cohort children, 77.1% showed evidence of *H. pylori*-positively. This result is similar to that found (76%) in 25 women of childbearing age from a nearby community. As expected, in children, maternal antibodies progressively decline by 6 months. The antibody levels were below the threshold for both whole cell and CagA ELISAs by six month of age. These results suggested that it is possible to document seroconversion in children older than 9 months independently of the mother's *H. pylori* status. Among the 44 birth-cohort children followed by more than 12 months, 24 (54.5%) seroconverted. However, 8 (18.2%) of the 24 seroconverted children were only transient, and 16 (36.4%) were persistent. Evaluation of CagA antibodies increased sensitivity of *H. pylori* detection and their rise often preceded *H. pylori* antibodies. These data indicate that *H. pylori* acquisition is common and often transient in this population, especially in the second year of life

D5. Serological responses to *H. pylori* and pepsinogen levels in a birth cohort of Native American and Bangladeshi children. We have recently studied a birth cohort of Native American (Apache) children in Arizona and observed that acquisition of *H. pylori* occurs mainly after the first year of life, when both transient and persistent infections are observed (37) Furthermore, we demonstrated that children who showed persistent seroconversion to *H. pylori* developed significant changes in serum pepsinogen I compared with those that did not seroconvert (38). These findings in a population where *H. pylori* is highly endemic suggest that acquisition of *H. pylori* occurs most commonly after the age of one year and that the acute phase of infection affects gastric secretory physiology that may involved pepsinogen I and II and gastrin levels. We have confirmed the increased levels of serum pepsinogen II in H. pylori infected children in Bangladesh (33).

Experimental Design and Methodology

Specific aim 1. To define the timing of *H. pylori* acquisition and the host and environmental factors associated with acquisition.

Rationale. We aim to describe the timing for *H. pylori* colonization in Bangladeshi children. Our hypothesis is that infection occurs mainly in early childhood probably after the first year of life, mainly in the second year of life [as we have documented in developing (Native American) populations in the USA (37)], and to identify the risk factors for acquiring the organism. We will evaluate the potential role of risk factors associated to the family members, such as crowding, number of sibblings, birth order, *H. pylori* status of the mother, usage of antimicrobials, etc, in the transmission of this organism, we will assess the effect of socioeconomic and demographic variables pertaining to the acquisition of infection and potential bacterial factors.

Methods. In this proposal, we plan to retrospectively study a cohort of 250 children from families in Mirzapur, Bangladesh from birth until the age of two years. The children enrolled all were born between September 1993 and October 1994, and the data collected during that period was used for the doctoral deissertation of the PI (34). The criteria for inclusion in the cohort included the following: Mothers who were in the third trimester of a low-risk, normal pregnancy and without underlying diseases were enrolled. Their children were followed-up by a study pediatrician and field workers which was a strong incentive for mothers to permit their children to participate. [A twice a week and every six months visits by a field worker to get information of any use of medications including antibiotics, the presence of diarrheal illness, and acute respiratory illnesses, as well as other intercurrent infections was performed for each child]. Anthropometrics measurements were performed every month in each child and a detailed nutritional data was recorded.

Serum samples of the index cases from the birth-cohort were collected at birth (cord-blood) and every six months thereafter for a period of 24 months; in total 525 blood samples have been stored. In addition, during the first two years of life monthly stool specimens were obtained from each child, amounting to 1726 diarrhoeal stool samples and 5707 non-diarrhoeal stool samples. We also collected monthly breast milk samples from nursing mothers. All samples have been kept frozen at –20°C at the ICDDR in Dhaka .

A. Collection of demographic and socioeconomic data. At entry into the study, a detailed census was used to gather demographic, socioeconomic and medical data from the family and all household members. Field workers visited homes every six months. At that time, questionnaires were completed. These surveys will include questions on family composition, parents' income, education and occupation, diet, illnesses, immunization, medication, and contact with other children including regular care in a nursery environment and behaviors related to hygiene. Field workers visits also were used to measure height and weight and to gather serum, breast milk and stool samples. In addition, visits twice a week by a field worker were used to get information specifically on diarrheal and respiratory illness.

B. Monitoring of infection in index children. *H. pylori* acquisition in index children will be monitored by serology and stool antigen detection test using serum and stool samples obtained at 0, 6, 12, 18, and 24 months. Additionally, index children will be tested (stool antigen test) during

or immediately after, and again at one month after episodes of vomiting or diarrhea to determine whether the symptomatic episode represents an initial acquisition of *H. pylori*.

B. Humoral immune response. We will monitor the development of specific antibodies to *H. pylori* proteins in relation to the acquisition of infection. Antibody development will be assessed in serum. The serologic assays will continue every six months until the end of the follow-up in both the index child becomes positive for *H. pylori* infection and in the *H. pylori* negative children. With this follow-up program we will be able to assess whether the timing, pattern, or intensity of the antibody response associated with *H. pylori* acquisition is age-related. Determination of class-specific (22) and antigen-specific (23,) antibodies may enable the development of new approaches for non-invasive diagnosis of *H. pylori* colonization in children. In addition, our follow-up will allow us to assess whether colonization with *H. pylori* is a transient or a persistent event, and whether persistence correlates with a positive stool antigen detection test. The serologic assays will be performed every six months until the end of the follow-up period. The serologic assays will be performed in Dr. Perez-Perez's lab at the NYUSM. We intend to demonstrate that the lack of *H. pylori* colonization correlates with the lack of changes in the humoral and physiological parameters that we are proposing to assess here (see below).

Definitions. We have established definitions of *H. pylori* positivity and negativity using serum samples from children who are not colonized with *H. pylori*. In addition, we will define *H. pylori* status based on the stool antigen detection test. Based on these definitions, we will be able to calculate the annual rates of acquisition and loss for the study population.

C. Antigen stool detection assay. Stool samples were collected from the birth cohort group monthly for all the study period (24stool samples per child) and since recently a new test has been developed, that apparently has a higher sensitivity and specificity for the diagnosis of *H. pylori* (39,40), we hope to determine the presence of *H. pylori* by this method. The advantage of this test is that it permits demonstrating active infection, and its results can be correlated with the serological results. However, this test will be validated (for Bangladeshi infant and young children population)by testing on stored stool samples from children on whom *H. pylori* status is known through ^{13}C-urea breath testing. These tests will be performed in the ICDDR,B.

All the stool samples will be tested in Dhaka at ICDDR,B to detect *H. Pylori* infection by using ELISA, (H. pylori antigen test). Blood samples will be shipped to the lab of Dr. Perez for detection of H. Pylori IgG, IgM, IgA, serum gastrin and pepsinogens I & II.

D. Sample size estimation. A sample population of 250 newborns children participated in the study. Assuming that 40% of the 250 children will develop infection during the two-year follow-up period (37) and that 20% will be lost during follow-up (lost subjects contributing on average half of the person-time and half of the expected cases), 240 person-years of infection-free follow-up and 80 cases of infection are expected, resulting in an incidence rate of 40%/year with an exposure prevalence of 50%. A rate ratio of 2.0 would imply an incidence rate among the unexposed of 8.1%. Using the exact Poisson distribution, the expected number of cases will yield 90% power for detecting a rate ratio of 2.0 or greater at a two-tailed 5% significance level. Higher exposure prevalence, higher incidence rate or lower loss-to follow-up rate will increase the power.

Analysis. The data obtained from the above studies will be collected and used to test our hypothesis that *H. pylori* acquisition occurs after the first year of life and that the transmission is mainly from infected mothers. Analysis of the timing of the infection in relation to family data, including: birth order, growth status, breastfeeding, evidence of maternal antibodies, and type of diet will be performed. Particular attention will be given to the moment when a child changes from an only liquid diet, to a liquid and solid diet. For this analysis, the incidence rate of *H. pylori* infection will be calculated as number of cases/number of person-years at risk. For the calculation of person-years at risk, the onset of each new infection will be assumed to occur at the midpoint of the preceding interval. To examine the effect of socioeconomic indicators, household composition and diet on *H. pylori* acquisition (incidence of new infections), three outcomes will be used; never infected during follow-up; the number of infection episodes; and time to first infection. For the dichotomous outcome, never infected during the follow-up, the logistic regression model will be applied. For the count outcome, number of infection episodes, the generalized linear model with the Poisson distribution, logarithmic link and duration of the observation as the offset variable will be applied. For the time to first infection, the Cox proportional hazards model will be used. To investigate the effects of study factors on persistence of infection, the Cox proportional hazards model will be used to examine the relationship between study factors and the duration from the onset of the first infection to the time of clearance or the end of the observation. All socioeconomic variables, household composition variables and dietary variables collected in this study will be used as possible covariates to identify factors that independently influence the outcomes of interest. The virulence markers of infection will be used as variables in the previously and subsequently described analyses assessing determinants of infection timing, and persistence, effects on immunological markers, on gastric physiology, and clinical effects.

Expected results and alternative approaches. We expect to define the age-specific incidence, which will confirm our hypothesis that *H. pylori* transmission occurs early in life and usually occurs in the family setting. In addition, we hope to identify the major risk factors involved in the transmission of this organism.

Specific Aim 2. To study the physiological and immunological consequences of acquisition of *H. pylori* at different ages and the impact on child development.

Rationale. Previous reports have suggested that the age at which *H. pylori* is acquired influences the long-term outcome of infection (42), as is the case for other infectious diseases such as varicella-zoster virus and hepatitis B (43). Likely differentiating points include early immunological and physiological
events. Our working hypothesis is that children who acquire *H. pylori* infection at an older age (2-4 years) have more intense changes, both in the immune response and in physiological events, compared to children who acquire the organism at younger age (<2 years). We hypothesize that *H. pylori* positive children detected in this project will differ in their physiologic parameters from comparably aged children with no evidence of *H. pylori* infection, and those with transient acquisition. There is also great interest in testing the hypothesis that the presence of *H. pylori* in children impacts their normal growth and development (44,45). In particular, we plan to examine if the presence of *H. pylori* is beneficial to children in the prevention of other infectious diseases, such as diarrheal illnesses (13-15). We expect to demonstrate that those children colonized with *H. pylori* have a lower incidence of diarrheal diseases than the ones without colonization.

Methods. In this second aim, our main goal is to establish which factors contribute to persists of *H. pylori* colonization. We will also assess the difference in phisiological changes that might ocuured as result of the *H. pylori* infection in the gastic mucosa. Furthermore, we hope to identify *H. pylori* antigens that are specifically recognized by those individuals with persistence *H. pylori* colonization.

Physiological consequences of the infection. In adults, the level of acid production in the stomach is a major determinant of disease outcome, but why some adults develop a hypochlorhydric response and others a hyperchlorhydric response to *H. pylori* is unknown. We hypothesize that events surrounding childhood infection may be a major factor, but there is little information on *H. pylori*-specific effects on gastric physiology in childhood. It is possible that what have been thought in the past to be normal age-related developments are in fact consequences of *H. pylori* infection. As indicated above, serum samples were taken every six months during the first two years of life. Using these samples, we will compare pepsinogens I and II, and gastrin levels as markers of change in gastric secretory physiology among *H. pylori*-positive and *H. pylori*-negative children during the follow-up period, as we reported in recent studies (35,46). These tests will be performed by Dr. Perez-Perez.

Immunological consequences of the infection. The level of antibody response to *H. pylori* antigens is different among colonized individuals. However, the ELISA technique does not allow us for assessment of the qualitative differences among positive serum samples. In this proposal we will compare the number and the type of *H. pylori* antigens recognized by children with either persistent or transient colonization using western blot techniques. The combination of immunoblotting and ELISA methods is amost efficient means of detecting serum antibodies to *H. pylori* antigens (47). We will also determine if the profile of antigens varies during follow-up in the same colonized patient using the methodologies previously reported (28). These tests will be performed in Dr. Perez-Perez's lab.

General consequences of the infection. We plan to estimate the effects of *H. pylori* infection and it's timing of acquisition on child health, including growth and the occurrence and severity of other infectious diseases. The index child's weight and height were measured and recorded every six months during the first two years of life. The weight to height ratio will be used as a measure of nutritional status (48); height-for-age will be used as a measure of stunted growth (48). These measures (defined

as percentiles on international growth curves) will be calculated using the Epi Info Nutritional Anthropometry program (49). Field workers obtained weight and height measurements using standard anthropometric protocols (50) under the supervision of a pediatrician. The nature of this cohort study and the detailed data that was collected by questionnaire will allow comprehensive multivariate analysis (comparison of *H. pylori* infection with all the variables collected in the questionnaire) to estimate the effects of *H. pylori* infection on the health of the children.

Analysis. Our hypothesis that during the acute phase of the *H. pylori* infection, major changes both at the physiological and immunological level occurred, and that the age at which *H. pylori* is acquired has an important bearing on the long-term outcome (8), will be tested with the data collected from this specific aim. Linear regression modeling will be used to estimate the effect of age and other factors on indicators of the immune response such as IgA, IgG, and IgM immune response to whole cell preparation and IgG immune response to CagA, and physiological parameters that are measured on a continuous scale. Of particular interest will be the effect of age on the level of the humoral immune response in children who become infected. Multiple regression models will also be used to estimate the effect of environmental factors, host factors (including growth status) and strain factors (including strain genotypes and number of infecting strains) on early physiological events (for continuous outcome variables, linear regression models will be used; for dichotomous outcome variables, logistic regression models will be applied). Regression models will also be used to estimate the effect of infection characteristics of the infected strain on the age at which the infection is acquired. The impact of the infection on children's growth will be analyzed by comparing height and weight between those who become infected and those who remain free of infection, after adjusting for socioeconomic status and other relevant covariates. The analysis will be performed using linear regression models to estimate the effect of infection status on the child's rate of growth.

Expected results and alternative approaches. We do not anticipate any major problems in the testing of the immunological response to the specific *H. pylori* antigens. To overcome the lack of sensitivity and specificity of the ELISA methods in young children, we will use immunoblotting techniques that appear to be useful for the diagnosis of *H. pylori* in children (51). We have enough experience in these techniques to have a high level of confidence. We also have accumulated experience in determination of the pepsinogen and gastrin levels in serum. We expect to document differences in anthropometrical measurements between the group of colonized and non-colonized children as indicators of the potential impact of *H. pylori* on health status. If no differences are observed, an alternative indicator of the effects of *H. pylori* colonization will be our data on differences in the morbidity of infectious diseases between colonized and non-colonized children.

Collaborative Arrangements

This study will be conducted according to collaborative arrangements between the New York University School of Medicine and the ICDDR in Dahka, Bangladesh

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Detailed Budget

Title of the project: Longitudinal study of events associated with H. pylori acquisition in Bangladeshi children

PI: Dr. Zahid Hasan

Duration: 1 year 8 months

Personnel salaries

Name	Project position	% effort	Duration in month	1st 12 month	2nd 8 months	Total (in US $)
Dr. Zahid Hasan	Principal Invest.	15%	12	2,748	1,924	4,672
Dr. P.K. Bardhan	Co-PI	20%	12	3,422	2,694	6,116
Dr. S.A. Sarker	Co-Investigator	5%	12	900	-	900
Mr.Badrul	Sample Coordinator	20%	12	2,000	-	2,000
To be named	Lab. Res. Officer	100%	12	4,981	3,653	8,634
To be named	Data Entry Tech.	20%	12	1,200	-	1,200
Sub-total:				**15,251**	**8,271**	**23,522**

Supplies & Materials			
Antigen stool detection kits	6,000	5,000	11,000
Labware & chemicals	500	500	1,000
Sub-total:	**6,500**	**5,500**	**12,000**
Other expenses			
Shipment/communication	1,500	500	2,000
Sub-total:	**1,500**	**500**	**2,000**
Domestic Travel			
Domestic transportation of the samples to the airport etc.	300	150	450
Sub-total:	**300**	**150**	**450**
Total Direct Cost	**23,551**	**14,421**	**37,972**
Indirect Cost (7% of total grant, excluding equipment cost)	**1,649**	**1,009**	**2,658**
Equipment			
Computer with accessories for data base	1,500	-	1,500
ELISA Reader	4,000	-	4,000
Sub-total:	**5,500**	**-**	**5,500**
Total Project Cost:	**30,700**	**15,430**	**46,130**

30/4/200

Md. Bozlur Rahman
Manager, Budget & Costing
ICDDR,B: Centre for
Health & Population
Mohakhali, Dhaka-1212
Bangladesh

March 7, 2002

Julie Busse, MS
Research Manager
Thrasher Research Fund
16 E. South Temple Street, 3rd Floor
Salt Lake City, UT 84150-6910

Dear Ms. Busse:

We have read the summary of the conference call on December 20, 2001 regarding our proposal entitled "Longitudinal study of events associated with *Helicobacter pylori* acquisition in Bangladeshi children." We have made some modifications according to the points raised during discussion, and we are now submitting a revised proposal that follows the recommendations of the panel of experts and focuses exclusively on the first phase.

Our answers to the points the reviewers' raised during the discussion are as follows:

a. ***Using serological assays is not a strong approach.***
We understand there is concern over the use of serology proposed in the methodology of this study (see response to reviewer #3, December 17, 2001). Our previous work has demonstrated that the serological assessment of *H. pylori* antibodies in sequential serum samples of the same patient tested the same day, even in the case of children, provides a more accurate result than a single serum sample test.

b. The set of archived samples is very valuable
We completely agree with the reviewers. The possibility of having this type of material from a well defined population is invaluable. We feel that this is one of the major strengths of this proposal.

c. Being able to compare fecal samples obtained about the time of acquisition of H. pylori could yield useful information.
We believe that the use of a stool antigen detection test in our proposal would yield useful information. The stool antigen detection test can be performed with both fresh and stool samples from storages. In addition, the test has the same sensitivity and specificity whether diarrhea stools or normal stools are used.

Summary of Conference Call for Perez-Perez proposal. December 20. 2001

Summary statements:

This is a research group which is experienced in dealing with H. pylori, linking with a major organization an a developing country. This is an understudied area, and potentially a really important project, but it has methodological problems. The proposed project will use samples collected 7-8 years ago: at that time the current assay technologies were not available. The section proposing to use the archived samples could be really useful, especially if the results can be correlated with clinical conditions. There are problems with the second part of the proposed project activities.

At first reading, it seems like an exciting proposal, but there are huge methodological problems. The PI may not get new information from the retrospective work. We know that in the birth to two age group the tests may not be valid. As written, the retrospective portion of the proposal simply may not work. Ideally, data is needed from birth through age 3-5. Because data is missing from age 2 to 8, the two aims of the proposal cannot be related.

It was hard to understand the PI's proposed work for the second portion: easier to understand what they intend to do with the retrospective work. Useful information may be gained from the retrospective work, but maybe not from the proposed follow-up. Can PCR be used for determining the timing of acquisition of H. pylori, as well as information regarding persistence of infection?

Points raised during discussion

- Using serological assays is not a strong approach.
- However, this set of archived samples is very valuable.
- Being able to compare fecal samples obtained about the time of acquisition of H. pylori could yield useful information.
- A significant portion of the children described in the PI's upcoming publication of work on the White Mountain Apache reservation seemed to be positive for H. pylori at one point, and negative at another. Is this the result of a transient infection, or a non-specific test?
- Lots of studies have shown a change from positive to negative to positive for H. pylori. It is not known if this represents clearance.
- The reviewers may simply not be agreeing with the PI regarding which questions are the most interesting, with regard to what the archived samples could be used for.
- Would Western blot, added to the testing of the archived samples, be useful?
- The PI's own papers admit that the proposed techniques may be weak.
- The string test is not likely to be feasible in children.
- There is more pediatric H. pylori work than the PI seems to be aware of.
- If the archived samples are used wisely, the results could have implications in the US and globally, not just in Bangladesh.

Recommendation:
Submit a revised proposal focusing on the stored specimens: the call committee cannot recommend support for the second phase of the proposed work. Use the approach of "What don't we know, how can we get answers?" Use the best techniques available. Perhaps a broader approach, such as using Western blot to look for antibodies to more antigens, will yield more useful information.

The possibility of comparing fecal samples obtained before, around, and after the time of acquisition of *H. pylori* in a child and correlating this with his/her serological response to the *H. pylori* antigen preparations would help us to understand the colonization process.

d. *A significant proportion of the children described in the work on the White Mountain reservation seemed to be positive for H. pylori at one point and negative at another. Is this the result of a transient infection or a non-specific test?*

Spontaneous clearance or change from positive to negative results in tests such as the urea breath test or serological assays have been reported, particularly in young children ( ). In most of those reports there is an agreement that this phenomenon represents a transient infection and not the result of a non-specific test. Our overall conclusion in the Apache study is that at least 33% of children who were colonized with *H. pylori*, particularly in the first five years of life, represented a transient infection.

e. *A lot of studies have shown a change from positive to negative to positive for H. pylori. It is not known if this represents clearance.*

To fully confirm clearance of *H. pylori*, whether in a previously colonized child with a negative *H. pylori* test, or in a colonized adult after antimicrobial therapy requires an endoscopy of the upper gastrointestinal tract, which probably will be justified in the adult patient but not in the child. Once again, we have to depend on other tests that indirectly confirm the persistence of *H. pylori*.

f. *The reviewers may not agree with the PI regarding which questions are more interesting, with regard to what the archived samples could be used for.*

It is possible that investigators who have access to archived samples have other priorities of what is important in the *H. pylori* research field. We have stated our rationale and described our hypothesis. We feel that the specific aims that we have proposed may help to learn more about the natural history of *H. pylori* and identify relevant points in the transmission for this organism.

g. *Would western blot, added to the testing of the archived samples, be useful?*

Western blot serological technique has the advantage of making it possible to determine relative immunogenicity with regard to individual antigens present in a whole preparation of *H. pylori*. Therefore, the qualitative properties of this assay are useful. We have now included in our proposal the use of western blot to compare the number and the type of antigen components that are recognized for children with transient colonization and for those children with persistent colonization. This point is now included in the proposal.

h. *The PI's own papers admit that the proposed techniques may be weak.*

We agree with the panel of reviewers that our proposed techniques may be weak, but this assumption is true for almost any technique described to demonstrate the presence of *H. pylori* in pediatric populations. At the very least this proposal intends to establish more realistic threshold values in the serological tests, and to correlate those results with other methods such as the stool antigen detection test and stool PCR for *H. pylori* detection.

i. *The string-test is not likely to be feasible in children.*

This point is irrelevant since we have now eliminated the second phase of the original proposal.

j. *There is more pediatric H. pylori work than the PI seems to be aware of.*
We have reviewed the most current literature and included in the proposal only those papers related to pediatric *H. pylori* research that are relevant to this study.

k. *If the archived samples are used wisely, the results could have implications in the U.S. and globally, not just in Bangladesh.*
We fully agree with this point. We are seeking support from The Thrasher Fundation. In part, because they have a serious commitment to bring the benefits of medical research to the rest of the world.

We hope that this revised proposal includes most of the recommendations of the reviewers' panel and answer those points raised during the discussion of the previous proposal. In addition, we keep a specific budget for the ICDDR, B since this type of collaborative efforts it is always desirable and we want more participation by the local institution. We believe that Thrasher also likes as much work to be done at the local site as possible and our collaborators at the ICDDR, B have an excellent record for this type of work.

We have included a new time line and a new budget that reflects the changes in the proposal. We believe that these new modifications will allow us to achieve the aims of our proposal.

Thank you for considering this work.

Yours sincerely,

Guillermo Perez-Perez, D.Sc.
Associate Professor of Medicine and Microbiology
Department of Medicine and Microbiology
Institute for Urban and Global Health, NYU.

GP-P/rp
Enclosures

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Guillermo I. Perez-Perez	Associate 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
Escuela Nacional de Ciencias Biologicas, IPN, Mexico	BSc	1972	Bacteriology
Escuela Nacional de Ciencias Biologicas, IPN, Mexico	MSc	1982	Microbiology
Escuela Nacional de Ciencias Biologicas, IPN, Mexico	DSc	1985	Medical Microbiology

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 Positions:

1973-1976	Lecturer and Head, Department of Microbiology, UAP, Puebla, Mexico.
1976-1979	Microbiologist, Control and Production Departments, INH, Ministry of Health, Mexico.
1977-1979	Visiting lecturer, Department of Microbiology, UAP, Puebla, Mexico.
1979-1981	Microbiologist, Intestinal Bacteriology Lab, Hospital Infantil, Mexico.
1981	Microbiologist, Enterobacteriaceae Laboratory, ISET, Ministry of Health, Mexico.
1982-1985	Head of Bacteriology Department, ISET, Ministry of Health, Mexico.
1985-1986	Post Doctoral Fellow, Infectious Disease Section, Univ Colorado School of Medicine, Denver, CO
1986-1987	Instructor, Division of Infectious Diseases, Department of Medicine, University of Colorado School of Medicine, Denver, Colorado.
1987-1989	Assistant Professor, Division of Infectious Disease, Department of Medicine, University of Colorado School of Medicine, Denver, Colorado.
1989-1993	Research Assistant Professor, Division of Infectious Diseases, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee
1993-2000	Research Associate Professor, Division of Infectious Diseases, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee
2000-present	Associate Professor of Medicine, Division of Infectious Diseases, Department of Medicine, New York University School of Medicine, New York, New York

Selected Publications (from 100 reviewed articles)

Perez-Perez GI, and Blaser MJ. Conservation and diversity of *Campylobacter pyloridis* major antigens. Infect Immun 55:1256-1263, 1987.

Perez-Perez GI, Dworkin BM, Chodos JE, and Blaser MJ. *Campylobacter pylori* antibodies in humans. Ann Intern Med 109:11-17, 1988.

Dunn BE, Perez-Perez GI, and Blaser MJ. Characterization of *Campylobacter pylori* proteins. Two-dimensional gel electrophoresis and immunoblotting. Infect Immun 57:1825-1833, 1989.

Dooley CP, Cohen H, Fitzgibbons PL, Bauer M, Appleman MD, Perez-Perez GI, and Blaser MJ. Prevalence of *Helicobacter pylori* infection and histologic gastritis in asymptomatic persons. N Engl J Med 321:1562-1566, 1989.

Perez-Perez GI, Bodhidatta L, Wongsrichanalai J, Taylor DN, Baze WB, Dunn BE, Echeverria PD, and Blaser MJ. Seroprevalence of *Helicobacter pylori* infections in Thailand. J Infect Dis 161:1237-1241,1990.

Drumm B, Perez-Perez GI, Blaser MJ, and Sherman P. Intrafamilial clustering of *Helicobacter pylori* infection. N Engl J Med 322:359-363, 1990.

Dunn BE, Campbell GP, Perez-Perez GI, and Blaser MJ. Purification and characterization of *Helicobacter pylori* urease. J Biol Chem 265:9464-9469, 1990.

Perez-Perez GI, Witkin SS, Decker MD, and Blaser MJ. The seroprevalence of *Helicobacter pylori* infection in couples. J Clin Microbiol 29:642-644, 1991.

Cover TL, Puryear W, Perez-Perez GI, and Blaser MJ. Effect of urease on HeLa cll vacuolation induced by *Helicobacter pylori* cytotoxin. Infect Immun 59:1264-1270, 1991.

Perez-Perez GI, Marrie T, Inouye H, Shimoyama T, Marshall G, Meiklejohn G, and Blaser MJ. The effect of age and occupation on the seroprevalence of *Helicobacter pylori* infection Can J Infect Dis 3:134-138, 1992.

Nomura A, Stemmermann GN, Chyou P-H, Kato I, Perez-Perez GI, and Blaser MJ. *Helicobacter pylori* infection and gastric carcinoma in a population of Japanese-Americans in Hawaii. N Engl J Med 325:1132-1136, 1991.

Mai UEH, Perez-Perez GI, Allen JB, Wahl SM, Blaser MJ, and Smith PD. Surface proteins from *Helicobacter pylori* exhibit chemotactic activity of human leukocytes and are present in gastric mucosa. J Exp Med 175: 517-525, 1992.

Parsonnett J, Blaser MJ, Perez-Perez GI, Hargrett-Beam N, and Tauxe RV. Symptoms and risk factors associated with *Helicobacter pylori* infection n a cohort of epidemiologists. Gastrointerol 102:41-46, 1992.

Perez-Perez GI, Marrie T, Inouye H, Shimoyama T, Marshall G, Meiklejohn G, and Blaser MJ. The effect of age and occupation on the seroprevalence of *Helicobacter pylori* infection Can J Infect Dis 3:134-138, 1992.

Perez-Perez GI, Olivares AZ, Cover TL, and Blaser MJ. Characteristics of *Helicobacter pylori* strains selected for urease deficiency. Infect Immun 60:3658-3663, 1992.

Carmel R, Perez-Perz GI, Blaser MJ. *Helicobacter pylori* infection and food-cobalamin malabsorption. Digest Dis Sci 39:309-314, 1994.

Perez-Perez GI, Gower CB, Blaser MJ. Effects of cations on *Helicobacter pylori* urease activity, release, and stability. Infect Immun 62:299-302, 1994.

Perez-Perez GI, Brown WR, Dunn BD, Cover TL, Cao P, Blaser MJ. Correlation between gastric mucosal inflammation and serum immune responses in *Helicobacter pylori* infection. Clin Diagnos Lab Immunol 1:325-329, 1994.

Nomura A, Stemmerman GN, Chyou P-H, Perez-Perez GI, Blaser MJ. *Helicobacter pylori* infection and the risk for duodenal and gastric ulceration. Ann Intern Med 120:977-981, 1994.

Perez-Perez GI, Sheperd VL, Morrow JD, Blaser MJ. Activation of human THP-1 cells and rat bone marrow-derived macrophages by *Helicobacter pylori* lipopolysaccharide. Infect Immun 63:183-1187, 1995.

Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmermann GH, Nomura A. Infection with *H. pylori* strains possessing *cagA* associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Research 55:3222-2115, 1995.

Perez-Perez GI, Thiberge JM, Labigne A, Blaser MJ. Relationship of immune response to heat-shock protein A and characteristics of *Helicobacter pylori*-infected patients. J Infect Dis 174:1046-1050, 1996.

Perez-Perez GI, Bhat N, Gaensbauer J, Fraser A, Taylor DN, Kuipers EJ, Zhang L, You WC, Blaser MJ. Country- specific constancy by age in *cagA*$^+$ proportion of *Helicobacter pylori* infections. Int J. Cancer 72:453-456,1997.

Perez-Perez GI. Role of *Helicobacter pylori* infection in the development of pernicious anemia. Clin Infect Dis. 25:1020-1022, 1997.

Perez-Perez GI, Cutler A, Blaser MJ. Value of serology as a noninvasive method for evaluating the efficacy of treatment of *Helicobacter pylori* infection. Clin Infect. Dis. 25: 1038-1043, 1997.

Hook-Nikanne J, Perez-Perez GI, Blaser MJ. Antigen characterization of *Helicobacter pylori* strains from different parts of the world. Clin Diagn Lab Immunol 4:592-597, 1997.

Torres J, Leal-Herrera Y, Perez-Perez GI, Gomez A, Camorlinga-Ponce M, Cedillo-Ribera R, Tapia-Conyer R, Munoz O. A community-based seroepidemiological study of *Helicobacter pylori* infection in Mexico. J Infect Dis 178:1089-1094, 1998.

Camorlinga-Ponce M, Torres J, Perez-Perez GI, Leal-Herrera Y, Gonzalez-Ortiz B, Madrazo de la Garza A, Gomez A, Munoz O. Validation of a serologic test for the diagnosis of *Helicobacter pylori* infection and the immune response to urease and CagA in children. Am J Gastroenterol 93: 1264-1270, 1998.

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Everhart JE, Kruszon-Moran D, Perez-Perez GI, Tralka TS, McQuillan G. Seroprevalence and ethnic differences in *Helicobacter pylori* infection among adults in the United States. J Infect Dis, 2000;181:1359-1363.

Perez-Perez GI, Peek RM, Legath AJ, Heine PR, Graff LB. The role of CagA status in gastric and extragastric complications of *Helicobacter pylori*. J Physiol Pharmacol 1999; 50:833-845.

Yañez P, Madrazo-de la Garza A, Perez-Perez GI, Cabrera L, Muñoz O, Torres J. Comparison of invasive and non-invasive methods for the diagnosis and evaluation of eradication of *Helicobacter pylori* infection in children.Arch Med Res.2000;31:1-7.

Ando T, Perez-Perez GI, Kusugami K, Ohsuga M, Bloch KE, Blaser MJ. Anti-cagA IgG responses correlate with IL-8 induction in human gastric mucosa biopsy culture. Clin Diagn Laboratory Immunology 2000;7:803-809.

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CURRICULUM VITAE OF K Z HASAN

1. SURNAME: HASAN DATE OF BIRTH: January 05, 1948
FORENAMES: KHUNDKAR ZAHID NATIONALITY: Bangladeshi

2. DEGREES, DIPLOMAS etc. (Subject, class, university and dates)

Medicine, Surgery, Obs. & Gynae.	MBBS	Chittagong Medical College, Bangladesh	1972
Public Health and Epidemiology	MPH	Johns Hopkins University, U.S.A	1977
Public Health and Epidemiology	DrPH	Univ. of Alabama at Birmingham, U.S.A	2000

3. CURRENT POST (with dates)

Title:	Associate Scientist
Department:	Child Health Programme, Public Health Sciences Division, ICDDR,B
Date of appointment:	29 July 1993
Expected termination:	Not applicable

4. CONTRACT OF EMPLOYMENT: International Centre for Diarrhoeal Diseases Research, Bangladesh (ICDDR,B)

5. LAST 3 POSTS HELD (with dates)

- Associate Scientist, PHSD, ICDDR,B — 1993 to present
- Assistant Scientist, ICDDR,B — 1980-1985, 1988-1993
- Physician/Specialist/Epidemiologist- a joint program of ICDDR,B and Ministry of Health, Kingdom Saudi Arabia — 1985-1988

6. RECENT PUBLICATIONS

Unicomb LE, Podder G, Gentsch JR, Woods PA, **Hasan KZ**, Faruque AS, Albert MJ, Glass RI. Evidence of high-frequency genomic reassortment of group A rotavirus strains in Bangladesh: emergence of type G9 in 1995. J Clin Microbiol. 37(6): 1885-91, 1999 Jun.

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Hasan KZ and Hossain MA. (1991). A comparative study on the use of transport media and direct plating for isolation of Shigella spp. in the rural context of Bangladesh. Bangladesh Journal of Microbiology.

Hossain MA, Albert ML and **Hasan KZ** (1990) Epidemiology of shigellosis in Teknaf, a coastal area of Bangladesh: a 10-year survey. Epidemiolo. Infect.105(1): 41-50.

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Briend A, **Hasan KZ**, Aziz KMA, Hoque BA. (1989). Are diarrhea control programs likely to reduce childhood malnutrition ? Observations from rural Bangladesh. Lancet 5,319-322.

CURRICULAM VITE OF SHAFIQUL A. SARKER.

1.: Education

Rajshahi University	MBBS 1976	Medicine
University of Basel, Switzerland	MD 1984	Internal Medicine

2. RESEARCH OR PROFESSIONAL EXPERIENCE

Medical Officer	ICDDR,B	1982-87
Assistant Scientist/ Sr. Medical Officer	ICDDR, B	1987-92
Associate Scientist	ICDDR, B	1992 –2000
Scientist	ICDDR, B	2000-till date

3. **PUBLICASTIONS**

Casswall T, **Sarker SA**. Wadström T, Albert MJ, Fuchs GJ, Bergström M, Björck L, Hammarstrom L. Treatment of *Helicobacter pylori* infection in infants in rural Bangladesh with hyperimmune bovine colostrum:a pilot study. *liment Pharmaceutical Ther* 1998;12:563-68.

Sarker SA, Casswall TH, Mahalanabis D, Alam NH, Albert MJ, Fuchs G, Hammarström L. Successful treatment of rotavirus diarrhoea in children with immunoglobulin from immunized bovine colostrum. Pediatr Infect Dis J 998;71:1149-54.

Casswall T, Nilsson HO, Bergström M, Aleljung P, Wadström T, Dahlström AK, Albert MJ, **Sarker SA.** Evaluation of serology, ^{13}C-urea breath test and PCR of stool to detect *Helicobater pylori* in Bangladeshi children. J pediatr Gastroenterol Nutr 1999;28:31-6.

Khaled MA & **Sarker SA**. Changes of oxidant and antioxidant status in humans due to *H. pylori* infection. Nutr Res 1998;18:1463-1468.

Rahaman MM, Mahalanabis D, **Sarker SA**, Bardhan PK, Hildebrand P, Beglinger C, Gyr K. *Helicobacter pylori* infection in infants and young children and diarrhoea morbidity. J Trop Med Hyg 1998;71:283-7.

Name: Pradip Kumar Bardhan

Academic Qualifications

M.B.B.S.	Dhaka University, Bangladesh	1975
M.D.	Basle University, Switzerland	1990

Current Position: Associate Scientist/Gastroenterologist, ICDDR,B

Recent Publications

1: Hildebrand P, Bardhan P, Rossi L, Parvin S, Rahman A, Arefin MS, Hasan M, Ahmad MM, Glatz-Krieger K, Terracciano L, Bauerfeind P, Beglinger C, Gyr N, Khan AK. Recrudescence and reinfection with Helicobacter pylori after eradication therapy in Bangladeshi adults. Gastroenterology. 2001 Oct;121(4):792-8.

2: Alam NH, Meier R, Schneider H, Sarker SA, Bardhan PK, Mahalanabis D, Fuchs GJ, Gyr N. Partially hydrolyzed guar gum-supplemented oral rehydration solution in the treatment of acute diarrhea in children. J Pediatr Gastroenterol Nutr. 2000 Nov;31(5):503-7.

3: Bardhan PK, Feger A, Kogon M, Muller J, Gillessen D, Beglinger C, Gyr N. Urinary choloyl-PABA excretion in diagnosing small intestinal bacterial overgrowth: evaluation of a new noninvasive method. Dig Dis Sci. 2000 Mar;45(3):474-9.

4: Bardhan PK, Beltinger J, Beltinger RW, Hossain A, Mahalanabis D, Gyr K. Screening of patients with acute infectious diarrhoea: evaluation of clinical features, faecal microscopy, and faecal occult blood testing. Scand J Gastroenterol. 2000 Jan;35(1):54-60.

5: Wenneras C, Qadri F, Bardhan PK, Sack RB, Svennerholm AM. Intestinal immune responses in patients infected with enterotoxigenic Escherichia coli and in vaccinees. Infect Immun. 1999 Dec;67(12):6234-41.

6: Bardhan PK, Sarker SA, Mahalanabis D, Rahman MM, Hildebrand P, Beglinger C, Fuchs G, Gyr N. Helicobacter pylori infection in infants and children of Bangladesh. Schweiz Rundsch Med Prax. 1998 Dec 24;87(51-52):1814-6.