Attachment 1.
(FACE SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 28/7/92

Principal Investigator M. John Albert

Trainee Investigator (if any) —

Application No. 92-023

Supporting Agency (if Non-ICDDR,B) —

Title of Study Role of Helicobacter pylori as a risk factor for cholera and a modifier of oral cholera vaccine efficacy

Project status:
(✓) New Study
( ) Continuation with change
( ) No change (do not fill out rest of 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) Minors or persons under guardianship — Yes No } NA
2. Does the study involve:
   (a) Physical risks to the subjects — Yes No
   (b) Social Risks — Yes No
   (c) Psychological risks to subjects — Yes No } NA
   (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, birth or other) — Yes No
   (b) Use of fetal tissue or abortus — Yes No } NA
   (c) Use of organs or body fluids — Yes No
4. Are subjects clearly informed about:
   (a) Nature and purposes of study — Yes No
   (b) Procedures to be followed including alternatives used — Yes No
   (c) Physical risks — Yes No
   (d) Sensitive questions — Yes No
   (e) Benefits to be derived — Yes No } NA
   (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 parent or guardian (if subjects are minors) — Yes No } NA
6. Will precautions be taken to protect anonymity of subjects — Yes No } NA
7. Check documents being submitted herewith to Committee:
   ___ Umbrella proposal - Initially submit an 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. Examples 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 Cttee. 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.

M. John Albert
Principal Investigator

Trainee

APPLICATION FOR PROJECT GRANT

1. PRINCIPAL INVESTIGATOR : M. John Albert[a]

2. OTHER INVESTIGATORS : Firdausi Qadri[a]
John D. Clemens[b]

[a]International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Dhaka, Bangladesh

[b]Division of Prevention Research, National Institute of Child Health and Human Development, Bethesda, MD, USA

3. TITLE OF PROJECT : Role of *Helicobacter pylori* infection as a risk factor for cholera and a modifier of oral cholera vaccine efficacy

4. STARTING DATE : As soon as the project is approved

5. DATE OF COMPLETION : Three months from starting date

6. TOTAL BUDGET REQUIRED : US$ 2,510

7. FUNDING SOURCE :

8. HEAD OF PROGRAMME : Professor R. Bradley Sack
Associate Director
Laboratory Sciences Division

9. AIMS OF THE PROJECT

a) General aims

Evaluation of the role of *Helicobacter pylori* infection as a risk factor for contracting cholera and a modifier of efficacy of oral cholera vaccine.

b) Specific aims

In 1985, a field-trial of two killed oral cholera vaccines, cholera toxin B subunit-killed whole cell (BS-WC) and killed whole cell only (WC), were conducted in a rural area of Bangladesh. As part of the trial, two serological surveys were carried out in the field-trial area for vibriocidal and anti-cholera toxin antibodies. Part of the sera were stored frozen. Since *H. pylori* infection results in hypochlorhydria [4] and hypochlorohydria is a known risk factor for cholera [18], the objectives are:

1) To find out the prevalence and level of anti-*H. pylori* antibodies in the sera by ELISA.

2) Correlate the serological data with development of cholera and vaccination status to find out whether:

   i) *H. pylori* infection as evidenced by serum antibodies predisposes individuals to cholera,

   ii) *H. pylori* infection as evidenced by serum antibodies affects the vaccine efficacy by an increased prevalence of cholera in vaccinees with evidence of *H. pylori* infection.

10. HYPOTHESES

Because *H. pylori* infection is capable of inducing acute and chronic hypochlorhydria, and because hypochlorhydria has been demonstrated to be a risk factor for cholera, we hypothesize that:

a) Infection by *H. pylori*, as evidenced by the presence of serum antibodies to *H. pylori*, predisposes individuals to increased risk of cholera.

b) Infection to *H. pylori* as evidenced by the presence of serum antibodies to *H. pylori*, diminishes the protection conferred by BS-WC and/or WC oral cholera vaccines.

11. SIGNIFICANCE

H. pylori infections are present worldwide but with a considerably higher prevalence and earlier onset of infection in developing than developed countries. *H. pylori* infection is a cause of hypochlorhydria, which is a risk factor for contracting cholera. If *H. pylori* infection predisposes individuals to an increased risk of cholera and decreases the protective efficacy of oral cholera vaccines, it will have implications for the control of cholera.

12. ETHICAL IMPLICATIONS

An antibody survey will be carried out using stored frozen sera obtained from individuals who took part in the killed oral cholera vaccine trial of 1985 in Matlab, Bangladesh.

13. BACKGROUND

Helicobacter (formerly *Campylobacter*) *pylori* is a curved- or spiral-shaped organism recently isolated from the gastric mucosa of humans [1]. It has now been firmly established that *H. pylori* is the causative agent of type B

gastritis [2,3]. Hypochlorhydria may accompany *H. pylori*-induced acute and chronic gastritis (symptomatic or asymptomatic) [4]. Though the causative role of this organism in the pathogenesis of other gastroduodenal diseases has not yet been fully established, there is a high level of association between *H. pylori* and the following disorders: chronic superficial gastritis, pre-pyloric gastric ulceration, duodenal ulceration, non-ulcer dyspepsia, malabsorption syndromes and gastric carcinoma [4].

H. pylori infection can be diagnosed by a variety of techniques: examination of gastric biopsy material obtained during gastroduodenal endoscopy, by histological staining for the presence of gastritis and *H. pylori*, culture of biopsy material for recovery of the bacterium, a rapid urease test using biopsy material, a urea breath test using ^{13}C and ^{14}C, and finally, serology [5-8].

A variety of serological tests have been developed for the diagnosis of *H. pylori* infection. These include agglutination tests, complement fixation tests, and most recently ELISA [9-11]. Several investigators have used ELISA for diagnostic and epidemiological studies with tremendous success because its sensitivity and specificity are exceedingly high (>90%) [8,10,12]. Indeed, it has been found that serology is the most accurate method of diagnosing *H. pylori* infection, since *H. pylori* gastritis may be patchy, and biopsy may fail to sample the affected portion of the gastric mucosa [4]. Moreover, titres of serum anti-*H. pylori* antibodies remain chronically elevated if the infection is not usually eradicated by specific chemotherapy, since untreated infections are not eradicated by natural immune responses. Thus, measurements of serum antibodies serve as markers of active infections and response to therapy [13,14]. Finally, the simplicity of serology coupled with its low

cost and ability to handle a large number of samples, makes it the ideal test for large scale epidemiological studies.

Serological tests have permitted studies on the acquisition of *H. pylori* antibodies (and hence infection) by populations in both developing and developed countries. Serum antibody surveys have suggested that prevalence progressively increases with age. In developed countries, it is uncommon for children to be colonized, whereas approximately 50% of adults are colonized by the age of 60. In developing countries, colonization occurs during childhood and prevalence continues to rise in adult age groups to levels well above those observed in populations from developed countries. For example, in developed countries like Australia, Netherlands and France, less than 20% of children below 10 years of age have antibodies to *H. pylori* [4], whereas in developing countries like Thailand, Vietnam, Papua New Guinea, Peru, Algeria and Ivory Coast [15,16], between 40 and 70% of children at 10 years of age have antibodies. The cumulative prevalence continues to increase up to between 70 and 90% by the age of 40 [4]. In some countries, antibody levels decrease in the elderly [15,16]. However, no serological study has yet been undertaken in Bangladesh to determine the prevalence of infection in this population. By analogy with populations from other developing countries, it is expected that the prevalence of *H. pylori* infection in this population will be high, particularly in the young age groups.

Cholera is endemic in Bangladesh. In 1985, ICDDR,B conducted a trial of killed oral cholera vaccines in Matlab, a riverine rural area of Bangladesh, endemic for cholera. In this trial, the efficacies of BS-WC and WC were assessed in relation to a placebo (*E. coli* K-12) among children and adult females. During the first year of the trial, community-based serological

surveys of Matlab residents were conducted in August-September, 1985; November-December, 1985; and March-April, 1986. These surveys were done to enable comparisons of acute-phase sera from cholera cases with sera from community controls for titres of vibriocidal and IgG anti-cholera toxin antibodies, so that the role of these antibodies as determinants of the risk of cholera and as modifiers of vaccine efficacy could be evaluated [17]. Leftover sera from these surveys are still available. Since acute and chronic gastritis caused by *H. pylori* results in hypochlorhydria [4] and hypochlorhydria has been recognized as a risk factor for cholera in Bangladesh [18], it is probable that antecedent or active *H. pylori* infection might predispose individuals to increased risk of cholera and possibly also reduce the efficacy of oral cholera vaccines. Availability of leftover sera presents us with a unique opportunity to study the prevalence and level of serum antibodies to *H. pylori* and correlate the serological data with the risk of cholera and vaccine efficacy.

14. METHODS

The proposed research would adopt a case-control approach, and would focus on the role of active infection by *H. pylori* indicated by the presence of IgG anti-*H. pylori* serum antibodies, as determinants of a risk of cholera and a modifier of oral cholera vaccine efficacy. Specifically, the role of active *H. pylori* infection in increasing the risk of cholera would be ascertained by comparing the proportion of sera having IgG anti-*H. pylori* antibodies in: (a) cholera cases (using acute-phase sera); and (b) community controls who are frequency-matched to the cases on the basis of age and calender interval of selection. Cases and controls will be selected from subjects assigned to the *E. coli* K-12 placebo group. A higher proportion of sera exhibiting such

antibodies in cases than in controls (after controlling for confounding variables - all sera under analysis had measurements of vibriocidal and IgG anti-cholera toxin antibodies done, and corresponding blood specimens had been ABO blood-grouped; data on several demographic variables associated with the risk of cholera, such as, age, sex, religion, proximity of residence to a river or to a bazaar, etc. are available). For the analysis of risk, we will compare 290 cases of cholera with 580 controls in the placebo group (1:2 frequency-matched). Assuming conservatively that the prevalences of seropositivity for IgG ani-*H. pylori* antibodies is 45% in the control group (based on the known age-distribution of this group and the age-related pattern of seropositivity in other developing countries), this sample will detect a relative risk of $\geq$1.5 at $P<0.05$ (2-tailed) with 80% power.

Correspondingly, the role of antecedent *H. pylori* infection in modifying the efficacy of the BS-WC and WC vaccines would also be ascertained by comparing the proportion of sera having IgG anti-*H. pylori* antibodies in: (a) cholera cases (using acute-phase sera); and (b) community controls who are frequency-matched to the cases on the basis of age and calender interval of selection. However, in this comparison, cases and controls will be taken from recipients of two or three doses of BS-WC or WC, and cases and controls will be further matched on the basis of which vaccine had been received, and the number of doses ingested. The analysis of two or three doses of vaccine as opposed to only three doses, is compelled by data indicating that the efficacy of two doses of vaccine was similar to that of three doses. A higher proportion of sera exhibiting such antibodies in cases than in controls (after controlling for confounding variables) would provide evidence suggesting that infection by *H. pylori* places a vaccinated individual at increased risk for vaccine failure, thereby reducing vaccine efficacy. In this analysis, we will compare

145 cases of cholera with 556 controls who received two or three doses of BS-WC or WC (1:4 frequency match). Assuming conservatively that the prevalence of seropositivity of IgG anti-*H. pylori* antibodies is 40% in the control group (based on the known age-distribution of this group, and the age-related pattern of seropositivity in other developing countries), this sample size will detect a relative risk of $\geq$1.75 at $P<0.05$ (2-tailed test) with 80% power.

For both assessments, cases will comprise cholera episodes detected during treatment centre surveillance between July 1, 1985 and June 30, 1986, and controls will be selected from persons bled in one of the community-based surveys. For the purposes of matching case and controls, the calender interval of selection will be partitioned into July 1, 1985 to October 15, 1985 (for the first serosurvey); October 16, 1985 to January 31, 1986 (for the second serosurvey); and February 1, 1986 to June 30, 1986 (for the third serosurvey).

Detection of anti-*H. pylori* IgG antibodies

The presence and level of antibodies will be measured by a commercially available ELISA kit, PYLORI STAT (Whitaker Bioproducts, Walkersville, MD, USA) using a single serum dilution according to manufacturer's instructions.

15. REFERENCES

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Clemens JD, van Loon FPL, Sack DA *et al.* 1991. Field trial of oral cholera vaccines in Bangladesh: serum vibrocidal and antitoxic antibodies as markers of the risk of cholera. J Infect Dis 163:1235-1242.

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16. TASKS OF INVESTIGATORS

M. John Albert and Firdausi Qadri	Supervise antibody studies
John D. Clemens	As PI of the oral cholera vaccine trial project of 1985, he has overall knowledge of this project. He will help with the analysis and interpretation of data.

17. BUDGET

a)	Cost ELISA kit	...	US$ 1,500
b)	Freight charge	...	100
c)	Salary of a technician for 3 months		610
d)	Communication, publication, etc.		200
e)	Miscellaneous	...	100
Total			US$ 2.510

MJA:mh/J&A:HPYLOR.PRT

সম্মতি পত্র

আন্তর্জাতিক উদরাময় গবেষনা কেন্দ্রের ঢাকা হাসপাতালে যে সব রোগী আসেন, তাদের মধ্যে প্রতি পাঁচশতম রোগীর বিস্তারিত তথ্য গ্রহন করা হয়।

ডাক্তারের পরীক্ষার পর আপনাকে কিছু প্রশ্ন করা হবে। অসুস্থতার কারনে আপনার হাসপাতালে ভর্তির প্রয়োজন হলে, সুস্থ হবার পর আপনাকে প্রশ্ন করা হবে। রোগজীবানু পরীক্ষার জন্য আপনার/আপনার শিশুর সামান্য পরিমান মল/মল দ্বার থেকে মাথায় তুলা লাগানো কাঠি দিয়ে মল নেওয়া হবে।

এতে আপনার/আপনার শিশুর কোন প্রকার ক্ষতি হবে না।

আপনার/আপনার শিশুর সুচিকিৎসার ব্যবস্থা করা হবে এবং ব্যক্তিগত তথ্যাদি গোপন রাখা হবে। যে কোন সময় এ গবেষনা কার্যক্রম থেকে আপনি আপনার সম্মতি প্রত্যাহার করতে পারবেন এবং তাতে আপনি/আপনার শিশু এ হাসপাতালের প্রচলিত সুচিকিৎসা থেকে বঞ্চিত হবেন না।

আপনি এ গবেষনায় অংশগ্রহনে রাজী থাকলে দয়া করে নীচে আপনার স্বাক্ষর/টিপসই দিন।

গবেষকের/স্বাস্থ্য কর্মীর স্বাক্ষর:

তারিখ:

রোগীর/অভিভাবকের স্বাক্ষর/টিপ

তারিখ: