hment 1.

Date 5.7.98

REVIEW BOARD ON THE USE OF HUMAN SUBJECTS, ICDDR,B.

ipal Investigator DR. ANWARUL HUQ Trainee Investigator (if any) _____

:ation No. 98-013 Supporting Agency (if Non-ICDDR,B) NIH

of Study A simple water filtration & cholera intervention. Project status:

( ) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

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

urce of Population:

) Ill subjects — Yes No
) Non-ill subjects — (Yes) No
) Minors or persons under guardianship — Yes No

es the study involve:

) Physical risks to the subjects — Yes (No)
) Social Risks — Yes (No)
) Psychological risks to subjects — Yes (No)
Discomfort to subjects — Yes (No)
Invasion of privacy — Yes (No)
Disclosure of information damaging to subject or others — Yes (No)

s the study involve:

Use of records, (hospital, medical, death, birth or other) — (Yes) No
Use of fetal tissue or abortus — Yes (No)
Use of organs or body fluids — Yes (No)

subjects clearly informed about:

Nature and purposes of study — (Yes) No
Procedures to be followed including alternatives used — Yes No
Physical risks — Yes (No)
Sensitive questions — Yes (No)
Benefits to be derived — (Yes) No
Right to refuse to participate or to withdraw from study — (Yes) No
Confidential handling of data — (Yes) No
Compensation &/or treatment where there are risks or privacy is involved in any particular pro... — (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)
6. Will precautions be taken to protect anonymity of subjects — Yes No
7. Check documents being submitted herewith to Board:
   ___ 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 Board for review.

to obtain approval of the Review Board on the Use of Human Subj... the right ... subjects before making such change.

U. S. Islam
pal Investigator

Trainee

Attachment 1a.

INFORMATION TO INCLUDE IN ABSTRACT SUMMARY

The Board will not consider any application which does not include an abstract summary. The abstract should summarize the purpose of the study, the methods and procedures to be used, by addressing each of the following items. If an item is not applicable, please note accordingly:

1. Describe the requirements for a subject population and explain the rationale for using in this population special groups such as children, or groups whose ability to give voluntary informed consent may be in question.

2. Describe and assess any potential risks - physical, psychological, social, legal or other - and assess the likelihood and seriousness of such risks. If methods of research create potential risks, describe other methods, if any, that were considered and why they will not be used.

3. Describe procedures for protecting against or minimizing potential risks and an assessment of their likely effectiveness.

4. Include a description of the methods for safeguarding confidentiality or protecting anonymity.

5. When there are potential risks to the subject, or the privacy of the individual may be involved, the investigator is required to obtain a signed informed consent statement from the subject. For minors, informed consent must be obtained from the authorized legal guardian or parent of the subject. Describe consent procedures to be followed including how and where informed consent will be obtained.

   (a) If signed consent will not be obtained, explain why this requirement should be waived and provide an alternative procedure.

   (b) If information is to be withheld from a subject, justify this course of action.

   (c) If there is a potential risk to the subject or privacy of the individual is involved in any particular procedure include a statement in the consent form stating whether or not compensation and/or treatment will be available.

6. If study involves an interview, describe where and in what context the interview will take place. State approximate length of time required for the interview.

7. Assess the potential benefits to be gained by the individual subject as well as the benefits which may accrue to society in general as a result of the planned work. Indicate how the benefits outweigh the risks.

8. State if the activity requires the use of records (hospital, medical, birth, death or other), organs, tissues, body fluids, the fetus or the abortus.

The statement to the subject should include information specified in items 2, 3, 4, 5(c) and 7, as well as indicating the approximate time required for participation in the activity.

CHECK-LIST FOR SUBMISSION OF PROPOSALS TO THE RESEARCH REVIEW COMMITTEE (RRC)

[Please tick (✓) the appropriate box]

1. Has the proposal been reviewed, discussed and cleared at the Division level ?

   Yes ☐

   No ☑

   If 'No', please clarify the reasons: This is an external protocol.

2. Has the proposal been peer-reviewed externally ?

   Yes ☑

   No ☐

   If the answer is 'NO', please explain the reasons: _____

3. Has the proposal scope to address gender issues ?

   Yes ☐

   No ☑

   If the answer is 'YES', have these been adequately incorporated in the proposal. Please indicate: _____

4. Has a funding source been identified ?

   Yes ☑

   No ☐

   If the answer is 'YES', please indicate the name of the donor: National Institute of Health (NIH), USA.

5. Whether the proposal is a collaborative one ?

Yes ☑

No ☐

If the answer is 'YES', the type of collaboration, name and address of the institution and name of the collaborating investigator be indicated:

International, Professor R.R. Colwell, Dr. Anwar ul Hug, University of Maryland biotechnology Institute, 4321 Hartwick Rd, Suite 550, College Park, Maryland 20740.

6. Has the budget been cleared by Finance Division ?

Yes ☐ As approved by NIH

No ☐

If the answer is 'NO', reasons thereof be indicated: _____

7. Does the study involve any procedure employing hazardous materials, or equipments ?

Yes ☐

No ☑

If 'YES', fill the necessary form.

5.7.98
Date

for M. S. Reddy
Signature of the
Principal Investigator

APPLICATION FOR PROJECT GRANT

TITLE:	A simple water filtration fo cholera intervention
PRINCIPAL INVESTIGATORS:	Dr. Rita Colwell University of Maryland Biotechnology Institute 4321 Hartwick Road, Suit 500 College Park, MD 20742, USA E-mail:colwell@umbi.umd.edu
CO PI:	Dr. Anwar Huq University of Maryland, College Park Dr. Parick Vaughan Community Health Division, ICDDR,B Dr. Md. Sirajul Islam Laboratory Sciences Division, ICDDR,B Dr. Estelle Russek-Cohen University of Maryland, College Park
Investigators:	Dr. K.M.A. Aziz Community Health Division, ICDDR,B Dr. M. Yunus Community Health Division, ICDDR,B
RESEARCH LOCATION:	ICDDR,B , Bangladesh & UMBI
FUNDING REQUESTED:	$794,940
PROPOSED DURATION:	November 1, 1997 - October 30, 2000
HEAD OF THE PROGRAMME:	DIRECTOR LABORATORY SCIENCES DIVISION

University of
Maryland
Biotechnology
Institute

OFFICE OF
THE PRESIDENT
4321 HARTWICK ROAD
ROOM 550
COLLEGE PARK, MD 20740
(301) 403-0501
FAX: (301) 454-8123

July 1, 1997

Division of Research Grants
National Institutes of Health
Suite 1040
6701 Rockledge Drive, MSC 7710
Bethesda, Maryland 20892-7710

Re: REVISED SUBMISSION: 1 RO1NR04527-01, PA96-037
"A Simple Water Filtration for Cholera Intervention"

Dear Sir/Madam:

Enclosed is the revised proposal and 5 copies, submitted on behalf of the University of Maryland Biotechnology Institute.

I have addressed all the concerns raised by the reviewers, and I look forward to a favorable decision.

Sincerely yours,

Rita R. Colwell

Rita R. Colwell, Ph.D., D.Sc.
President
University of Maryland Biotechnology Institute

Encl. Revised Proposal (5 copies)

cc: Dr. Anwar Huq
Dr. Estelle Russek-Cohen
[redacted]
Dr. K.M.A. Aziz
Dr. M. Yunus
Mr. William Cooper
Ms. Barbara Cohen-King

RRC/db

Titie: A Simple Water Filtration for Cholera Intervention

Principal Investigator: Dr. Rita Colwell
University of Maryland Biotechnology Institute
4321 Hartwick Road, Suit 500,
College Park, MD 20742, U.S.A.
E-mail: colwell@umbimail.umd.edu

Co PI: Dr. Anwar Huq
University of Maryland, College Park,

Dr. Patrick Vaughan
Community Health Division, ICDDR,B.

Dr. Siraj Islam
Laboratory Sciences Division, ICDDR,B.

Dr. Estelle Russek-Cohen,
University of Maryland, College Park.

Investigators: Dr. K. M.A. Aziz
Community Health Division, ICDDR,B.

Dr. M. Yunus
Community Health Division, ICDDR,B.

Research Location: ICDDR,B, Bangladesh & UMBI.

Funding requested: **$794,940**

Project duration: **November 1, 1997 - October 30, 2000**

proposal\icdfield

Introduction to the Revised Proposal:

The project entitled "A simple filtration for cholera intervention" has been revised in accordance with the comments made by the reviewers. The reviewer's comments were very useful and appropriate which helped us to prepare a stronger proposal. In addition, further consideration was made to the number of study population to obtain useful data for analyses. As a result, the testing will be carried out in a larger population than it was proposed in the original proposal.

Specific response to reviewers comments are as follows:
Critique 1:

(i) No information about projected compliance with consistent filtration practices among those families enrolled in the field. Also, it is not clear who will be carrying out the monitoring in the field and the morbidity potentially related to cholera.

Response: The procedures related to compliance mainly involve simple steps like fastening a sari cloth or a nylon cloth around the neck of the pitcher before the water is filled in the pitcher. According to the prevalent practice in the community, water fetchers use sari cloth or any other similar cloth piece to sieve sand from tube-well water collected for drinking. They also use similar materials to filter surface water only when it contains debris visible by naked eyes. As part of this project, the study population will be motivated and encouraged through verbal communication and pictorial demonstration to use the filtering device. Practical demonstration regarding the outcome of sieving the water will be focused by demonstrating the retained debris in the filtering materials. This study will use visual materials to convince about the benefit of the use of filtration method. In addition, some indices of compliance will be recorded for the two treatment groups. These include whether the filter is used, and whether it is used for drinking water only, for washing vegetables, for utensils or for bathing. A data collection form is presented in the appendix. Only a subset of the participants will be monitored for compliance. Following all these procedures, it is expected that all the households enrolled in the study population would be willing to comply with the use of filtration method. However, it is likely that some of the participants in the study may fail to use the device due to some inevitable reasons and/or events. The proportion of such noncompliance is not likely to exceed 5% of the households. The objective of this portion of the study is to assess practices before and during the study intervention.

The field workers will be trained by the investigators who will implement the project through education and motivation in favor of using the filtration technique. Field workers will also collect data by using questionnaires and participant observations. Senior Health Assistants will supervise the field workers and monitor their activities in the field. Senior Field Research Officer (SFRO) in turn will be responsible for overall supervision and monitoring of the project. Dr. Rita Colwell and Dr. Anwar Huq will visit the study sites at least twice a year to personally observe the progress of the project and to discuss with their counter parts at the ICDDR,B. The study has no plan to do active morbidity surveillance for diarrhoea in the community. Any diarrhoea patient from the

study population admitted at the Matlab hospital will be identified through the Demographic Surveillance System (DSS). Other necessary information will also be collected using the hospital surveillance form shown in the appendix 3. Based on this information, morbidity related to cholera will be assessed.

(ii) No data management section of the application is described and application presents no plans to culture stools or otherwise prove that people ill with diarrhoea are ill because of cholera.

Response: *Data management:* Field workers will use an electronic data entry systems HUSKY HUNTER FS/2 is a portable PC to directly enter data (hardware to be determined) and then it will be subsequently downloaded to a PC. A data management package (FOXPRO) will be used to process data and do an initial screening for errors. Data will be subsequently transferred to the University of Maryland and SAS will be used for statistical analysis. Parts of this system are already in place for another joint US-Bangaldesh collaborative effort. Electronic versions of forms specific for this project will be developed during the pilot phase.

Microbiological analysis: Water and plankton samples from the field and rectal swab specimens from the patients attending hospitals from the study area will be cultured on selective media and subsequent identification will be carried out following standard procedures. The reduction in the number of vibrios following the use of the filtration devices will be measured by DFA-DVC techniques (7). The reduction of plankton will be measured using the Sedgerick-Rafter (S-R) counting cell following the procedures described by Khondkar and Chowdhury (25-A). Each analysis will be conducted with samples before and after filtration. This will be performed with 20 families within each filtration treatment group on four separate occasions. Education will be provided during the first and third such visit.

Critique 2.

(I) Application protocol to be rewritten and there are concerns on the clinical trial study design, the estimated results, the sample size required and how the data will be analyzed.

Response: *Study design:* Three villages will serve as our study population. These villages will be selected from 142 registered villages in the demographic surveillance system (DSS) of ICDDR,B, Matlab, Bangladesh. Villages with a higher incidence rate of cholera based on the previous three years data will be selected. Each village has approximately 4,000 families comprising about 24,000 individuals. Within each village, families will be randomized to one of three treatment groups. Families will either receive nylon cloth, cotton sari cloth and/or nothing, so as to serve as a control. Families with at least one child under the age of five years and a mother between 20 and 44 years of age will be actively recruited. Younger children have a considerably higher incidence rate of cholera (five cases per 1000 individuals per year) than do adults and older children. In the surveillance area around Matlab, the overall rate is approximately three cases per 1000 individuals per year.

There will be two phases to the study. The first six-month pilot phase will be used to refine our methodology and will involve a smaller number of families in each of the three villages. The data collection in both phases will be similar. However, methods in the second phase will be refined in accordance with our findings during the first phase. The second phase will last 2.5 years.

Phase1. The first phase will last six months and will only consist of evaluating the two filtration devices and their acceptability. During this time the field workers will encourage acceptance of the filtration devices in the field setting by education and motivation. The results regarding the reduction of vibrios following the use of the filtration devices will be compared to the results found previously (Huq et al, (20)). During this pilot phase, 750 households in each village will be given interventions or be monitored as part of the control group. In addition to being provided with filters, there will also be educational information given regarding filtering, storage and the use of surface water. A poster containing the magnified pictures of a zooplankton and algae will be shown to emphasize the critical requirement of filtration. Each poster will be laminated so as to ensure durability and provided to each household receiving a filtration device. During periods of increased risk for cholera, i.e., during the presence of large number of plankton in the water in fall and spring, all individuals, whether in an intervention or the control group will be notified and cautioned of risks in using river or pond water. During that period, emphasis will be given to use of filtered water for drinking, for washing utensils and for bathing.

Phase2. The second phase will be based in part on the results of the first phase. We will expand the use of the treatments, unless initial evidence suggests that one of the filter treatments is not acceptable. Educational efforts may also be redirected in accordance with the results of the first phase. Educational efforts will be continued for those enrolled in the first phase through verbal communication by field workers. An additional 2000 families will be recruited within each village in the second phase.

Data Forms and Questionnaires: The data forms received from different sources including field, hospital and laboratory will be systematically compiled at the Matlab Project Office. These forms will be scrutinized for the completeness and any possible error. If any query arises, those will be referred to the respondents and informants concerned for completion of data collection. Then the data will be computerized and cleaned following established procedures. The task of computerization will begin on completion of baseline data collection and will continue as the data collection progresses through different phases. However, if we use an electronic data system, the above-mentioned procedures will be reduced and minimize manipulation of the data.

Additional funding: Due to increase in the study population and inclusion of electronic data entry system, an additional fund has been requested for supplies and salary for field workers in Bangladesh.

Objectives

The project originally comprised two phases. The first phase, conducted during the past two years (ending July, 1996), was aimed at establishing the feasibility of using sari cloth (unstitched 5-6 yard sections of flat material worn by most women in Bangladeshi villages) to remove *Vibrio cholerae* attached to plankton in surface water used for drinking and designing a device for convenient use by villagers without economic, social or a cultural impediment. The first phase has been successfully completed (20). The second phase is focused on a field trial of the method for the purpose of reducing the number of cholera cases in villages where the method is used, compared to controls. We propose to undertake a three-year study with two parts: 1) evaluation of the acceptability by the community of the filtration; and, 2) efficacy of filtration in reducing the number of cases of cholera.

The specific aim of the first part of this proposal is to evaluate the acceptability of the method by the community under controlled field conditions. The study is based on previous laboratory findings and limited field study data, showing that the method of filtration employing 'sari' cloth as a filter, without modification, can reduce the number of *Vibrio cholerae* in water by removing plankton and other particulates present in natural surface water. Thus, the initial phase of the project will be conducted in the first year. Upon successful completion of the first phase, application and implementation of the method to reduce the number of cholera cases will be evaluated in community-based field studies.

Specific aims:

a. To conduct a community evaluation in Matlab, Bangladesh, to determine the acceptability of filtration in reducing the number of vibrios in surface water used for drinking and willingness of villagers to use the method we have successfully developed and optimized in the laboratory. The percentage of the population employing the device will be monitored as well as the number of index cases appearing in the test population.

b. A field evaluation will be conducted of the filtration device to determine **quantitatively** effectiveness and efficacy in reducing the number of cholera cases in the same community. The bulk of the field evaluation will be carried out in the second and the third years of the project, after acceptance and active practice of filtration is achieved.

Background and Significance

Cholera and other diarrheal diseases remain major killers of children globally, especially in developing countries. According to one estimate, ca. 5.5 million cases of cholera occur annually (23). More than 14,000 deaths, many of them children, were reported in Rwandan refugee camps in 1994. It has long been known that cholera is a waterborne disease, i.e., the infectious agent, *Vibrio cholerae* is transmitted via water (2). This bacterium

is an autochthonous member of the aquatic environment (8,9). Furthermore, surface water has been implicated in the transmission of cholera (11). The association of vibrios, particularly *Vibrio cholerae*, specifically, with phytoplankton (24,25) and zooplankton (18,20,22,32) has een established from extensive studies carried out in our laboratory for the past 30 years. The muciligenous sheath of *Anabaena variabilis* has been found to serve as a site of attachment of *Vibrio cholerae*, with survival for >15 months (24). In addition, *Vibrio cholerae* O1 was observed to be associated with *Volvox*, a common colonial alga (34). The accumulated data show that planktonic copepods play a major role in multiplication, survival, and potential transmission of cholera (10,11,15,16). Larger copepods provide a greater surface area for attachment of the bacteria.

Based on human volunteer trials and depending on the health of a given individual, ca. 10^4 - 10^6 *Vibrio cholerae* O1, if ingested, is likely to produce clinical cholera (6). During the summer phytoplankton blooms occur in Bangladesh followed by zooplankton blooms in September and October. Cholera outbreaks almost always follow the zooplankton bloom (18). The majority of the members of the zooplankton population consist of crustaceans, known to support attachment of vibrios (32). Furthermore, they demonstrate a seasonal distribution, both in size of individuals and in species composition (26,27). Recently, the presence of *Vibrio cholerae* in cargo ship ballast water has been reported, suggesting international dissemination of *Vibrio cholerae* via aquatic organisms carried in ballast water discharged in harbors remote from the original source of the ballast water (29). Our work on ballast water shows that *Vibrio cholerae* is present in, and on copepods in the ballast water of ships arriving in Baltimore, Maryland, from ports of origin else where in the world. Thus, the zooplankton offers a means of global transport of *Vibrio cholerae* (results to be published elsewhere).

Copepods, a dominant group of the zooplankton community in riverine and brackish water, have a characteristic seasonal distribution in size and species (33) and can carry a large number of *Vibrio cholerae*, enough to cause cholera if ca.1-10 copepods are ingested via water (6,10). In addition, copepods feed on phytoplankton, with the result that *Vibrio cholerae* attached to the phytoplankton are ingested by the copepods as demonstrated by Huq et al. (20). Fortunately, copepods are large enough to be removed by using simple domestic filtration units (16). Studies of the association of *Vibrio cholerae* with plankton have provided new leads on the survival and multiplication of these organisms in the natural aquatic environment.

Filtering water at the time of collection and just before drinking, has been successful in removing cyclopes, a planktonic stage of the guinea worm which causes dracunculiasis. The worm is removed using a nylon net and is now recommended as an effective method of preventing dracunculiasis, a life-threatening disease common in Africa (35). Although boiling water prior to drinking will kill cyclopes and guinea worm larvae, as well pathogenic microorganisms, it is a time-consuming procedure and expensive as well. In a country like Bangladesh, where fuel wood is in very short supply, boiling water, effective a practice as it is, has not been done because of the lack of fuel wood. Furthermore, boiling water is not socially acceptable in most rural villages of Africa (35), a situation which also prevails in Bangladesh.

Moreover, during severe flooding, which occurs every year there are some areas of Bangladesh that experience reduction of conditions to those of mere survival, i.e., even the barest necessities become difficult to obtain and building fires to boil water is simply not possible.

In Bangladesh, a majority of the population in the villages still depends on untreated surface water for household consumption (5). Surface water taken from ponds and rivers is preferred as a source of drinking water, for reasons of taste, convenience, or a local belief that "quality" water is "natural," i.e., not chemically treated (5,12). A family and neighborhood study of cholera transmission demonstrated that those who used *V. cholerae* 01 culture positive water sources for cooking, bathing, or washing, but used culture negative water for drinking, had the same rate of infection as those who used *V. cholerae* 01 culture positive for drinking (17). Family-based clustering of cases is not generally observed (M. Yunus and R. Glass, unpublished data). However, there are *ca.* 14-25 "hot spot" villages in the ICDDR,B surveillance area where the number of cases of cholera is higher then average. Once there is a case reported in a family, the secondary case rate is approximately 10% among the remaining family members (16). However 29% of the family members have chance of being colonized with *V. cholerae* 01 (M. Yunus, S. Islam, K.M.A.Aziz, personal communication). Once the index case is reported, it is most likely that further spread in the family takes place via food or other means, such as direct contact which may not be prevented even if the water brought into the house is free of *V. cholerae* 01. Therefore, we propose intervention at the index case level, which will also help prevent releasing large number of bacteria into the environment when sanitary latrines are not available. The importance of safe water for all household purposes, i.e., cooking, bathing, washing and drinking cannot be over-emphasized. In addition, during monsoons, vast areas in Bangladesh are submerged by floods, with the result that the few shallow, hand-operated tube wells available in the villages are under water. Sanitary latrines are flooded, causing serious problems, with hygiene posing a direct threat of contamination by enteric bacteria, including *V. cholerae*. Based on previous studies, during late monsoons, phytoplankton blooms occur, followed by zooplankton blooms, to which vibrios are attached and multiply, amplifying the number of *V. cholerae* in the natural water of the environment (12,18,24). When consumption of surface water cannot be avoided, particularly during flooding or other natural disasters which occur every year in Bangladesh, a simple method that is effective in reducing the number of *V. cholerae* will be very useful.

It is a common practice in villages in Bangladesh to use cloth, frequently a piece of an old sari, to filter homemade drinks, usually containing sugar/molasses and served, with rose water or lemon juice for flavoring, to guests. Thus, there is no ethical, cultural, or a social barrier to using sari cloth to filter drinking water. A primary objective of this project is to develop a method of filtration that will be readily acceptable to villagers in their homes and without reservation.

Considering all our findings, we have hypothesized that our simple and inexpensive filtration method to sieve outplankton to which *V. cholerae* is attached will curb or at least reduce cholerae epdiemics. The latter presumes *V. cholerae* per volume of drinking water, whether from ponds, rivers or other natural water supplies, will be reduced below a potentially infectious dose. Results of experiments (see Tables 1 and 2) recently published (20), demonstrated that simple filtration, in fact, can be a useful practice to

institute, since it is affordable by even the poorest of the poor, to reduce the incidence of cholera, especially during extreme weather conditions, e.g., floods and monsoons.

A concept of filtration acceptable to villagers is the basis of this project. We have been able to obtain data showing that simple filtration using Bangladeshi household material can reduce the number of cells of *Vibrio cholerae* 01 (up to 2 logs) in water. We are suggesting a method for preventing or reducing the number of cases of cholera, since the disease is dose-dependent, a community-based study targeted toward underserved rural populations and aimed at cholera intervention involving direct community participation. In addition, mothers of households will be responsible for implementation, thereby, ensuring ultimate success.

Preliminary Studies

During the past 18 months, extensive laboratory-based experiments have been conducted with results demonstrating that *V. cholerae* attached to copepods can be filtered out of water using sari material of a type that is readily available in nearly every household in villages of Bangladesh. Different kinds of sari material were used, in addition to nylon net of maximum pore size of 200 μm (the same nylon net as used to control dracunculiasis in Africa (35)). The emphasis in this study was on sari material because it was our aim to develop a method of filtration that bore no additional cost to villagers for household water filtration. It is not intended to eliminate cholera by this method but to reduce the number of cholera cases to a minimum.

Results of experiments showed that either four layers of sari cloth of the commonest type used by Bangladeshi villagers, or one layer of nylon net, retained 99% of *V. cholerae* cells attached to zooplankton (20,21). In separate experiments we demonstrated that equal volume of phytoplankton and zooplankton, when inoculated with a known number of *V. cholerae* cells, showed a two-log difference in attachment (Table 1). From these results, it is concluded that smaller sized plankton passing through the filters contains less than 1% of the total cells, a significant reduction in *V. cholerae* counts. Experiments were also done using a variety of copepods collected throughout different seasons, with incubation at different temperatures, and employing four kinds of sari material. Field experiments done in Bangladesh showed that naturally contaminated copepods, i.e., in the "wild," are effectively removed by filtration, with the filtrate remaining DFA negative for both *V. cholerae* 01 and 0139 (Table 2).

The sari material used to filter contaminated water, i.e., to separate out suspended particles, including copepods, can be washed and air dried each time for repeated use. Complete drying of the filtering material is desirable, with four hours, or more than 24 hours, required for drying, depending on humidity (20), i.e., monsoons vs. non-monsoon season. From results obtained in the preliminary studies, four hours of sun exposure or 24 hours of air drying in a shaded environment should be employed, since *V. cholerae* was culture negative in experiments where the filter material was soaked with up to 10^6 cells per ml and then washed and dried. The concentration of cells in the experiments was high, compared to the number of vibrios occurring in the natural environment, to ensure effectiveness of the procedure. In addition, by using very large numbers of vibrios, it was possible to determine whether washing and sunlight drying would reduce counts sufficiently for re-use of the sari cloth. The decontamination procedure was more effective if the sari cloth was thoroughly rinsed with

water before drying. However, during the monsoon in Bangladesh, when the humidity is *ca.* 100%, fully complete drying is not usually achieved. Considering such situations, thorough washing of each filter after every use is recommended, using the same river or pond water to remove concentrated plankton from the filter, followed by rinsing with filtered water and drying, if possible. Drying provides an advantage, but is not critical (manuscript in preparation). If bleach is available, the filter should be soaked for 45 minutes in bleach solution, followed by spreading out the unfolded sari cloth to dry. This achieved reduction of the *Vibrio* counts to non-detectable by culture.

There is a general belief in Bangladesh among villagers that filtering water can prevent diarrheal disease. However, a scientific study, providing evidence that such action is effective, has not been done that provides results sufficiently convincing to reinforce use of filtration. The method proposed in this project may not be entirely novel, but the data will be valuable in proving scientifically that the method is effective. There is no hazard or risk associated with application of the proposed method, either to participants or to workers carrying out the study.

Research Design and Methods

Two kinds of filtration devices will be field tested. One of the devices comprises a nylon net of known mesh size, the same as that used to control dracunculiasis in Africa, under the auspices of the World Health Organization (35). The second device consists of four layers of sari cloth, shown to be effective in laboratory experiments (20). Four folds of sari material from an old sari or a piece of nylon material is placed on the neck of the water collecting pot, locally called "kalash" in Bangladesh, in a way so that water may not enter the container without passing through the sari cloth filter.

Emphasis will be on instructions for cleaning the filter after each use. There are several possible ways, some of which are now being tested in our laboratory. As discussed above, each filter (approximately 1 sq. yd. of sari material) will be washed, after every use, using river or pond water to remove concentrated plankton from the filter, followed by rinsing with previously filtered water and subsequent drying when possible. Because of high humidity, rapid drying may not be possible, in which case the villagers will be instructed to use several different pieces of sari material, allowing enough time for drying between application. To filter water, no more than 1 sq. ft. of an old sari is required. Thus, a simple sari can yield as many as 50 pieces of filtering cloth. Field Health Assistants will pay regular visits to families every month to reinforce the filtration practice, as per previously set instructions. The field Health Assistants will also replace broken or damaged filtering devices, as necessary. Data forms and questionnaires (Appendix I) will be filled out on a regular basis in the field. The information collected via the questionnaire will be compared with information obtained by observation at the water collection sites and by means of in-depth interviewing of the users, employing a sub-sample of the population covered by the questionnaire. This method will allow for verification of information provided by villagers, a useful procedure for providing reliable data for analysis.

Prior to initiating the survey, study families, especially the mother and other female members of the household, responsible for collecting water will be trained to use the filter device properly and the significance of its application. Matlab has the largest continuously operating population surveillance system in the world, established in 1963 (1). Several major studies have been conducted successfully in that population during the past 30 years, possible because the outstanding cooperation and goodwill between the Center and the people of Matlab. Dr. K.M.A. Aziz, Senior Social Scientist and Dr. M. Yunus, an epidemiologist, have worked at the Center since its founding and are investigators on this project. According to Dr. Aziz and Dr. Yunus, from their long experience working in the Matlab community, they are confident that the proposed filtration device will be acceptable in households, as well as in other groups in the community (letter enclosed). A questionnaire has been developed for this study (Appendix I) and has been designed to determine how many families are motivated to filter their water at the collection site. The data will be correlated with index cases of cholera as they are reported at the Matlab treatment center for the study population.

Bathing is often done at the river's edge. In this study, during increased periods of risk for cholera, i.e., when large numbers of plankton is present in the water during late fall and late spring, the villagers will be alerted to the risk of cholera in using river or pond water. During the period of plankton blooms, therefore, instructions will be given to use, filtered water for drinking, washing utensils, and bathing.

Microbiological analysis: Ten water and plankton samples will be collected per day, once each week, at 10 different sites randomly selected from the study areas to monitor plankton populations. Also, ten water samples will be collected from household stored water. Both water and plankton samples will be used to enumerate *V. cholerae* 01 and 0139, using DFA-DVC (7). **In addition the rectal swab specimens taken from the patients attending hospitals from the study area will be cultured on selective media and subsequent identification will be carried out following standard procedures.** Aliquots of plankton and water samples will be fixed with 2-3% formaldehyde for plankton species identification and further analysis at the University of Maryland.

The reduction in the number of vibrios following the use of the filtration devices will be measured by DFA-DVC techniques (7). The reduction of plankton will be measured using the Sedgerick-Rafter (S-R) counting cell following the procedures described earlier (25-A). Each analysis will be conducted with samples before and after filtration. This will be performed with 20 families within each filtration treatment group on four separate occassions. Education will be provided during the first and third such visit.

Morbidity data: **For the environmental data, the location and date of the collection will be recorded in addition to the water and plankton values previously described. Attempt will be made to match up families to the water source nearest their residence. However, it will not be feasible to monitor each and every water source. Approximately 10 sources within each group will be monitored. Both water and plankton samples will be collected and analyzed.**

Compliance data: The procedures related to compliance mainly involve simple steps like fastening a sari cloth or a nylon cloth around the neck of the pitcher before the water is filled in the pitcher. According to the prevalent practice in the community, water fetcher uses sari cloth or any other similar cloth piece to sieve sand from tube-well water collected for drinking. They also use similar materials to filter surface water only when it contains debris visible by naked eyes. As part of this project, the study population will be motivated and encouraged through verbal communication and pictorial demonstration to use the filtering device. Practical demonstration regarding the outcome of sieving the water will be focused by demonstrating the retained debris in the filtering materials. This study will use visual materials to convince about the benefit of the use of filtration method. In addition, some indices of compliance will be recorded for the two treatment groups. These include whether the filter is used, and whether it is used for drinking water only, for washing vegetables, for utensils or for bathing. A data collection form is presented in the appendix. Only a subset of the participants will be monitored for compliance. Following all these procedures, it is expected that all the households enrolled in the study population would be willing to comply with the use of filtration method. However, it is likely that some of the participants in the study may fail to use the device due to some inevitable reasons and or events. The proportion of such non-compliance is not likely to exceed 5% of the households. The objective of this portion of the study is to assess practices before and during the study intervention.

The field workers will be trained by the investigators who will implement the project through education and motivation in favor of using the filtration technique. Field workers will also collect data by using questionnaires and participant observations. Senior Health Assistants will supervise the field workers and monitor their activities in the field. Senior Field Research Officer (SFRO) in turn will be responsible for overall supervision and monitoring of the project. Dr. Rita Colwell and Dr. Anwar Huq will visit the study sites at least twice a year to personally observe the progress of the project and to discuss with their counter parts at the ICDDR,B. The study has no plan to do active morbidity surveillance for diarrhoea in the community. Any diarrhoea patient from the study population admitted at the Matlab hospital will be identified through the Demographic Surveillance System (DSS). Other necessary information will also be collected using the hospital surveillance form shown in the appendix 3. Based on these information, morbidity related to cholera will be assessed.

Study design: Three villages will serve as our study population. These villages will be selected from 142 registered villages in the demographic surveillance system (DSS) of ICDDR,B, Matlab, Bangladesh. Villages with a higher incidence rate of cholera based on the previous three years data will be selected. Each village has approximately 4,000 families comprising about 24,000 individuals. Within each village, families will be randomized to one of three treatment groups. Families will either receive nylon cloth, cotton sari cloth and or nothing, so as to serve as a control. Families with at least one child under the age of 5 years and a mother between 20 and 44 years of age will be actively recruited. Younger children have a considerably higher incidence rate of cholera (5 cases per 1000 individuals per year) than do adults and older children. In the

surveillance area around Matlab, the overall rate is approximately 3 cases per 1000 individuals per year.

There will be two phases to the study. The first 6 month pilot phase will be used to refine our methodology and will involve a smaller number of families in each of the three villages. The data collection in both phases will be similar. However, methods in the second phase will be refined in accordance with our findings during the first phase. The second phase will last 2.5 years.

Phase1. The first phase will last 6 months and will only consist of evaluating the two filtration devices and their acceptability. During this time the field workers will encourage acceptance of the filtration devices in the field setting by education and motivation. The results regarding the reduction of vibrios following the use of the filtration devices will be compared to the results found previously (Huq et al, 1996). During this pilot phase, 750 households in each village will be given interventions or be monitored as part of the control group. In addition to being provided with filters, there will also be educational information given regarding filtering, storage and the use of surface water. A poster containing the magnified pictures of zooplankton and algae will be shown to emphasize the critical requirement of filtration. Each poster will be laminated so as to ensure durability and provided to each household receiving a filtration device. During periods of increased risk for cholera, i.e. during the presence of large number of plankton in the water during fall and spring, all individuals whether in an intervention or the control group will be notified and cautioned of risks in using river or pond water. During that period, emphasis will be given to use of filtered water for drinking, for washing utensils and for bathing.

Phase2. The second phase will be based in part on the results of the first phase. We will expand the use of the treatments, unless initial evidence suggests that one of the filter treatments is not acceptable. Educational efforts may also be redirected in accordance with the results of the first phase. Educational efforts will be continued for those enrolled in the first phase through verbal communcation by field workers. An additional 2000 families will be recruited within each village in the second phase.

Data Forms and Questionnaires: The data forms received from different sources including field, hospital and laboratory will be systematically compiled at the Matlab Project Office. These forms will be scrutinized for the completeness and any possible error. If any query arises, those will be referred to the respondents and informants concerned for completion of data collection. Then the data will be computerized and cleaned following established procedures. The task of computerization will begin on completion of baseline data collection and will continue as the data collection progresses through different phases. However, if we use data acquisition system that directly enters data electronically, the above mentioned procedures will be reduced and minimize manipulation of the data.

Facilities Available: The facilities in ICDDR,B consists of a well-equipped environmental microbiology laboratory available to this project and headed by Dr. Sirajul Islam, co-PI of this project. Dr. Patrick Vaughan, an experienced epidemiologist is also a Co-PI of this project. In addition, an experienced epidemiologist/community health scientist and a sociologist, Drs. M. Yunus and K. M. Ashraful Aziz, respectively and Dr. Estelle Russek-Cohen, a biostatistician at the University of Maryland, are Co-Investigators of this project. Dr. R.R. Colwell, PI of the project was recently appointed to the Board of Trustees of ICDDR,B, and will be able to provide full information to ICDDR,B related to this project, as well as closely supervise the project with Dr. Anwar Huq during twice yearly visits to Bangladesh.

Table 1. Total number of *V. cholerae* 01 associated with plankton

	Total number of *V. cholerae* O1 (cfu/g wet weight)	
Plankton Size (µm diameter)	LB	TCBS
>64	$(1.34 \pm 0.25) \times 10^9$	$(9.19 \pm 0.70) \times 10^7$
>20 & < 64	$(8.97 \pm 0.09) \times 10^6$	$(5.99 \pm 0.85) \times 10^5$
>20 & <200	$(1.86 \pm 0.10) \times 10^9$	$(1.58 \pm 0.95) \times 10^8$

Data showing colony forming units (cfu) of *V. cholerae* 01 attached to plankton are per gram wet weight. The total surface area per gram of smaller size plankton is significantly greater than that of larger sized plankton. However, the lower concentration on of *V. cholerae* 01 on smaller size plankton showed that the sari filter was more effective in filtering out the vibrios since they were attached to the layer plankton, namely the zooplankton.

Table 2. Detection of *V. cholerae* by direct fluorescent antibody (DFA)

Pond 6*	Plankton Collected at Sunrise	Plankton Collected at Sunset
Plankton	Positive	Positive
Filter Water	Negative	Negative

*Site 6 pond in Matlab. The community surrounding this pond had a high incidence of cholera in 1993, and is one the 70 villages covered by the demographic surveillance system (DSS) of ICDDR,B.

Evaluation/survey

A survey will be carried out on the practice of filtration, i.e., use of, or use, either of the filters, and index cases. The degree of diarrheal illness among the study population will be recorded in both the control group (using normal methods of water collection and use without

filtration) and the group practicing filtration. The results obtained via the questionnaires will be subjected to analysis. For statistical analysis, see below.

Statistical Analysis

The number of cholera cases per family over the entire study period will be analyzed using a Poisson regression model (30). Covariates used in the model will include treatment, village, number of months in which a child under age 5 is in the household and number of person-months on the study. Environmental data recorded just prior to each cholera case will also be considered. The model may be modified to consider alternative discrete response distributions or the presence of extra-poisson variation (3). Mixed-model Analysis of variance models will be used to analyze the reduction in bacterial counts (log transformed) as a result of filtration and the analysis of patterns in bacterial levels over time (36).

In addition, severity of illness will be examined for those with a reported case of cholera-like symptoms. This will include comparing number of hospitalizations required, and days in which symptoms of diarrhea, fever and/or vomiting are recorded. The latter will necessarily include illnesses not directly attributable to cholera. However, the filtration method may be successful in reducing a number of pathogens, since the filtration may well reduce fecal contaminants in surface water. Environmental data will be incorporated into this phase of the analysis since one can look at cholera levels in the water and plankton just prior to disease onset. For data recorded on individual patients, the analysis will account for correlation of observations within individuals and within families (14).

Sample Size Calculations

The overall rate of cholera in Matlab is 3/1000 individuals/year. The final phase of the study will be a 2.5 year study. Approximately 7.5 individuals per 1000 will have cholera at that time. Few individuals will have more than one episode of cholera during that time period because of acquired immunity subsequent to the first episode. The expected number of cases per family over the two year period is approximately .045 since the typical family consists of two adults and 4 children. The rate may actually be higher since villages with a higher than average rate of cholera incidence will be selected. The goal is to reduce cholera by 1/3 or to 2/1000/year with either of the two filtration treatments. Thus under the null hypothesis and assuming a poisson distribution, all three treatments will have a mean of .045 cases per family and a variance of .045. Under the alternative, the control will have the same mean and variance as under the null and the two intervention treatments will have a mean and variance of .030. Assuming that the mean number of cases per family is approximately normally distributed (i.e. using a central limit theorem), a weighted analysis of variance, a type I error rate of 5% and a 80% detection rate, we needed 2620 families per treatment. By allowing for migration and mortality, we assumed 2750 families per treatment with 750 to be recruited during the first phase. Such a high participation in the study is not unreasonable since ICDDR,B guarantees medical care to households in the surveillance area. It is believed that both

cholera morbidity and mortality is lower in the surveillance area than in many other areas of Bangladesh. This has led to a high degree of cooperation between villagers and ICDDR,B.

Data management: Field workers will use electronic data entry systems. HUSKY HUNTER FS/2 is a portable PC to directly enter data (hardware to be determined) and then it will be subsequently downloaded to a PC. A data management package (FOXPRO) will be used to process data and do an initial screening for errors. Data will be subsequently transferred to the University of Maryland and SAS will be used for statistical analysis. Parts of this system are already in place for another joint US-Bangaldesh collaborative effort. Electronic versions of the forms specific for this project will need to be developed during the pilot phase.

Time Schedule

The field survey study will be conducted during first 6 months, beginning ~~November~~ July 1998, 1997 through ~~April~~ June 30, 2001, after which the evaluation of efficacy will be conducted over a period of two and a half years, covering two major and two minor cholera seasons in Bangladesh. It is important to note that any given year is never identical to the previous, with respect to time of onset of an epidemic, since the occurrence of cholera outbreaks and the arrival of monsoon flooding differs from year to year.

Budget Justification

In this revised proposal, sample size has been recalculated to obtain data to provide statistically significant results. Additional funds for added field workers and electronic data systems have been requested.

Results of pilot study: The results of the pilot study are provided in appendices 1-4.

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APPENDIX I
QUESTIONNAIRE FOR FILTRATION PROJECT

Data Collection Form (Subject to Revision)

Date of Interview _____

Interviewer ID _____

Village Code: ___ ___ ___

Family Code: ___ ___ ___

1. Where is container cleaned? (Check all that apply)
   At home _____ At source _____ Others (specify)

2. Type of water used to clean container (Check all that apply)
   Tube-well water _____
   Filtered surface water _____
   Unfiltered surface water _____
   Others (specify)

3. Type of container
   Copper _____ Brass _____ Aluminum _____ Clay _____
   Other (specify)

4. How is container cleaned?
   By water only _____
   Water and hand, hand washed before _____
   Water and hand, hand not washed before _____
   Other (specify)

5. Was pitcher covered with a lid? Yes _____ No _____

6. Is a filter used at all? If No then skip remainder of form. Yes _____ No _____

7. Fixation of filter on pitcher mouth
   At home _____ At collection site _____ Not at all _____

8. Was filtering material intact while collecting water? Yes _____ No _____

9. When was filter removed?
   At home _____ At collection site _____ Other _____

10. After removing filter, how was filter cleaned?
    Tube-well water _____
    Filtered water _____
    Unfiltered water _____

11. Where was filter cleaned?
    At home _____ At collection site _____ Other _____

12. Was filter dried after washing? Yes _____ No _____

13. Purposes of use of filtered water (check all that apply):
    Hand washing ☐
    Utensils ☐
    Ritual purposes ☐
    Washing vegetables ☐
    Bathing ☐
    Drinking ☐
    Other ☐

II Principal Investigator/Program Director *(Last, first, middle)*: Colwell, Rita R.

CHECKLIST

TYPE OF APPLICATION *(Check all that apply.)*

☐ NEW application. *(This application is being submitted to the PHS for the first time.)*

☒ REVISION of application number: 1 RO1 NR04527-01
(This application replaces a prior unfunded version of a new, competing continuation, or supplemental application.)

INVENTIONS AND PATENTS *(Competing continuation appl.only)*

☐ COMPETING CONTINUATION of grant number: _____ ☐ No ☐ Previously reported

☐ Yes. If "Yes," ☐ Not previously reported

☐ SUPPLEMENT to grant number: _____

☐ CHANGE of principal investigator/program director.
Name of former principal investigator/program director._____

☒ FOREIGN application or significant foreign component.

1. ASSURANCE/CERTIFICATIONS

The following assurances/certifications are made and verified by the signature of the Official Signing for Applicant Organization on the Face Page of the application. Descriptions of individual assurances/certifications begin on page 27 of Section III. If unable to certify compliance where applicable, provide an explanation and place it after this page.

•Human Subjects; •Vertebrate Animals; •Debarment and Suspension; •Drug-Free Workplace *(applicable to new [Type 1]I or revised [Type 1] applications only)*; •Lobbying; •Delinquent Federal Debt.; •Research Misconduct; •Civil Rights (Form HHS 441 or HHS690); •Handicapped Individuals (Form HHS 641 or HHS 690); •Sex Discrimination (Form HHS 639-A or HHS 690); •Age Discrimination (Form HHS 680 or HHS 690); •Financial Conflict of Interest.

2. PROGRAM *(See instructions, page 20.)* *NOT APPLICABLE*

All applications must indicate whether program income is anticipated during the period(s) for which grant support is requested. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)

3. INDIRECT COSTS

Indicate the applicant organization's most recent indirect cost rate established with the appropriate DHHS Regional Office, or, in the case of forprofit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office. If the applicant organization is in the process of initially developing or renegotiating a rate, or has established a rate with another Federal agency, it should, immediately upon notification that an award will be made, develop a tentative indirect cost rate proposal. This is to be based on its most recently completed fiscal year in accordance with the principles set forth in the pertinent *DHHS Guide for Establishing Indirect Cost Rates*, and submitted to the appropriate DHHS Regional Office or PHS Agency Cost Advisory Office. Indirect costs will *not* be paid on foreign grants, construction grants, grants to Federal organizations, grants to individuals, and conference grants. Follow any additional instructions provided for Research Career Awards, Institutional National Research Service Awards, and specialized grant applications.

☒ DHHS Agreement dated: 03/10/93 ☐ No Indirect Costs Requested.

☒ DHHS Agreement being negotiated with Philadelphia/E. McKune Regional Office.

☐ No DHHS Agreement, but rate established with _____ Date _____

CALCULATION* *(The entire grant application, including the Checklist, will be reproduced and provided to peer reviews as confidential information. Supplying the following information on indirect costs is optional for forprofit organizations.)*

a. Initial budget period: Amount of base $ 253,796 x Rate applied 48 % = Indirect costs (1) $ 290,106

b. Entire proposed project period: Amount of base $ 707,622 x Rate applied 48 % = Indirect costs (2) $ 794,940

(1) Add to total direct costs from form page 4 and enter new total on Face Page, Item 7b.
(2) Add to total direct costs from form page 5 and enter new total on Face Page, Item 8b.

*Check appropriate box(es):

☐ Salary and wages base ☒ Modified total direct cost base ☐ Other base *(Explain)*

☐ Off-site, other special rate, or more than one rate involved *(Explain)*

Explanation *(Attach separate sheet, if necessary)*

4. SMOKE-FREE WORKPLACE

Does your organization currently provide a smoke-free workplace and/or promote the nonuse of tobacco products or have plans to do so?

☒ Yes ☐ No *(The response to this question has no impact on the review or funding of this application.)*

Competing Continuation Applications
PERSONNEL REPORT

All Personnel for the Current Budget Period

Name	Degree(s)	SSN	Role on Project *(e.g. PI, Res. Assoc.)*	Date of Birth *(MM/DD/YY)*	Annual % Effort
Rita R. Colwell	Ph.D., D.Sc.	014-26-7178	PI	11/23/34	1%
Anwaru Huq	Ph.D.	213-94-7811	Co-PI	03/16/51	10%
Sirajul Islam	Ph.D.	N/A	Co-PI	01/01/52	8.3%
Estelle Russek-Cohen	Ph.D.	084-42-2698	Co-PI	07/23/51	10%
K.M.Ashraful Aziz	Ph.D.	N/A	Co-Investigator	–	10%
Mohammed Yunus	M.D.	N/A	Co-Investigator	01/05/45	10%
Imdadul Huq	–	N/A	Field Officer	–	50%
To be named	–	N/A	Sr. Health Assist.	–	200%
To be named	–	N/A	Res. Officer	–	200%
To be named	–	N/A	Field Workers	–	2000%

BUDGET FOR ENTIRE PROPOSED PERIOD OF SUPPORT
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED 2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits Applicant organization only*		$ 34,346	$ 35,720	$ 37,149		
CONSULTANT COSTS		—	—	—		
EQUIPMENT		—	—	—		
SUPPLIES		$ 6,900	$ 6,900	$ 6,900		
TRAVEL		$ 9,400	$ 9,800	$ 9,800		
PATIENT CARE COSTS	INPATIENT	—	—	—		
	OUTPATIENT	—	—	—		
ALTERATIONS AND RENOVATIONS		—	—	—		
OTHER EXPENSES		—	—	—		
SUBTOTAL DIRECT COSTS		$ 50,646	$ 52,420	$ 53,849		
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	$ 155,076	$ 131,431	$ 133,880		
	INDIRECT	$ 48,074	$ 40,744	$ 41,503		
TOTAL DIRECT COSTS		$ 253,796	$ 224,595	$ 229,232		
TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PERIOD OF SUPPORT *(item 8a, Face Page)* →						$ 707,622

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed

Personnel:

Dr. Rita R. Colwell, Principal Investigator and Project Director, will be overall charge of the project, however, her salary will not be charged to this project. Dr. Colwell will visit Dhaka at least two times per year and will not charge this project for her travel as the cost will be paid by ICDDR,B to attend board of trustees meeting. She will have the opportunity for extensive interaction with the investigators involved in this project.

Dr. Anwarul Huq, Co-Principal Investigator and Co-Project Director will assume the responsibility along with Dr. Colwell for the planning, coordination and execution of all phases of the project. In addition, his responsibility will include oversight of the testing and confirmation of the detection of *V. cholerae* in plankton and water transported to the University of Maryland after fixation. Dr. Huq will be helped out by the graduate who will be working on this project and use data for her/ his thesis.

Supplies:

Conservative funding has been requested for chemical, reagents for DFA tests and other consumable needs in the laboratory. In addition, funding for a **electronic data entry system** has been requested to be used by the field **workers.**

Travel:

Dr. Huq will visit Bangladesh at least once in a year to an overview the progress and to ensure the procedures are followed as stated out in the grant proposal. In the first year, it will be necessary to have a meeting for all the investigators to lay out the details of the trial. Two round trip tickets to attend national meeting for scientific presentation have been sought.

ICDDR,B BUDGET

Project Title: A SimpleWater Filtration for Cholera Intervention
Project Dates: July 1, 1997 through June 30, 2000

Personnel	Role on Project	Type of Appt	% Eff.	Inst. Base Salary	Salary Requested	Year 1 Total
Dr. M.S. Islam	Co-PI	12	8.3%	$ 93,000	$ 7,719	$ 7,719
Dr. K.M.A. Aziz	Co-PI	12	10.0%	$ 31,687	$ 3,169	$ 3,169
Dr. M. Yunus	Co-PI	12	10.0%	$ 27,108	$ 2,711	$ 2,711
Mr. Imdadul Huq	SFRO	12	50.0%	$ 9,818	$ 4,909	$ 4,909
Seniqr Health Assistant (2)	SHA	12	200.0%	$ 6,384	$ 12,768	$ 12,768
Field Workers (20)	FA	12	2000.0%	$ 900	$ 18,000	$ 18,000
Research Officer (2)	RO	12	200.0%	$ 4,800	$ 9,600	$ 9,600
Subtotal Personnel:						$ 58,876
Equipment						
Electronic Data Entry System (10) $2600						$ 26,000
Subtotal Equipment:						$ 26,000
Supplies						
Chemicals and Media						$ 15,500
Petri dishes and consumable supplies						$ 4,000
Filtration Devices and Nylon material						$ 25,000
Subtotal Supplies:						$ 44,500
Travel						
R/T ticket plus per diem between MD and Dhaka						$ 3,200
Other Expenses						
Transport for the field, Boat time for family visits						$ 10,000
Communication/Rent/Utilities						$ 1,000
Publication Cost						$ 1,000
Posters						$ 5,000
Repair and Maintenance						$ 500
Office Supplies						$ 1,000
Xerox/Mimeography						$ 4,000
Subtotal Other Expenses						$ 22,500
Total:						$ 155,076
Overhead: (31%)						$ 48,074
TOTAL:						$ 203,150

APPENDIX-1

Results of pilot study:

A pilot study was carried out ot find out the efficiency and acceptability of the proposed filtering devices. The results show that by using 4 fold sari material 61% and 77% of phyto and zooplankton can be reduced (Table-1). However, we are not getting 100% reduction of total phytoplankton because some of the very small unicellular phytoplankton e.g., *Trachelomonas* sp., *Chlorella* sp. etc. are passing through even in 8 folds of both poplin and markin cloths but these phytoplankton are not our concern as *V. cholerae* do not attach to them.

The table-2 shows that *V. cholerae* O1 can be reduced to 60, 80 and 60% using 4 folds of sari, poplin and markin cloths respectively.

We interviewed 28 families and found that 93% of the family are willing to adapt the filtering devices for cleaning the surface water. We also found that 82% of the families are already using pieces of different cloths as filtering devices for cleaning surface water. However, 33% of the families use old sari as filtering material and 67% use pieces of other cloths e.g. napkin, mosquito net, cover of the quilt etc. as filtering material. Regarding folding of the filtering material 79% and 21% families use one fold and two folds respectively (Table-3).

APPENDIX-2

Table 1: Results of plankton reduction using different type of filter materials

Date & time of collection	Pond Name	Type of Filter used	Total phyto before filtration	Total phyto after filtration	% reduction of total phytoplankto	Total zoo before filtration	Total zoo after filtration	% reduction of total zoo
9.3.98 12:30 pm	Mosque pond	4-fold sari	1.14×10^7	1.13×10^7	<1.0	Not done	Not done	Not done
9.3.98 1:30 pm	Kaladi pond	4-fold sari	1.33×10^6	5.10×10^5	61.50	4.90×10^5	1.10×10^5	77.55
9.3.98 6:30 pm	Kaladi pond	8-fold sari	1.00×10^6	2.65×10^5	73.55	1.22×10^6	2.88×10^5	76.22
10.3.98 1:00 pm	Mubarakdi pond	8-fold sari	8.08×10^6	1.90×10^6	76.48	8.0×10^4	Nil	100
10.3.98 3:30 pm	Kaladi pond	4-fold chinese poplin	1.70×10^6	1.40×10^5	91.76	1.0×10^5	Nil	100
10.3.98 3:30 pm	Kaladi pond	4-fold Bangladeshi poplin	1.70×10^6	8.00×10^5	53.0	1.0×10^5	3.70×10^4	63
11.3.98 12:30 pm	V-10, F-160 pond	4-fold sari	5.80×10^5	4.58×10^5	21.03	2.00×10^5	Nil	100
11.3.98 1:00 pm	Chairman bari pond	4-fold sari	2.72×10^7	1.83×10^7	32.2	1.00×10^6	9.0×10^4	10
11.3.98 2:00 pm	V-10, F-149 pond	4-fold sari	3.08×10^6	2.65×10^6	14.00	4.00×10^5	Nil	100
11.3.98 4:30 pm	Mubarakdi pond	4-fold sari	6.26×10^6	2.71×10^6	56.00	1.20×10^6	1.0×10^5	91,6

APPENDIX- 3

Table 2: DFA results of water before and after filtration using various filtration cloths

Sl. no.	Name of the pond	Type of cloth	Number of fold	Total count of V. cholerae O1/ml	% of reduction
01	Mubarakdi	Before filtration	Before filtration	2.04×10^5	--
02	,,	Sari	4-fold	8.16×10^4	60
03	,,	Sari	5-fold	6.12×10^4	70
04	,,	Sari	6-fold	6.12×10^4	70
05	,,	Sari	8-fold	6.12×10^4	70
06	,,	Poplin	4-fold	4.08×10^4	80
07	,,	Poplin	5-fold	4.08×10^4	80
08	,,	Poplin	6-fold	2.04×10^4	90
09	,,	Poplin	8-fold	$<4.5 \times 10^3$	100
10	,,	Markin	4-fold	8.16×10^4	60
11	,,	Markin	5-fold	6.12×10^4	70
12	,,	Markin	6-fold	6.12×10^4	70
13	,,	Markin	8-fold	4.08×10^4	80

APPENDIX-4

Table 3: Response of families regarding some questions used in baseline survey

1.	Families are interested in using filtration device	93%
2.	Families already using filtration device	82%
3.	Use of one fold filter material	79%
4.	Families are using old sari as filter materials	33%
5.	Use of pitcher for collecting water	93%
6.	Social problem in using old sari as filter material	64%

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Rita R. Colwell	Professor of Microbiology and President, University of Maryland Biotechnology Institute

EDUCATIONAL/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
Purdue University	B.S.	1956	Bacteriology
Purdue University	M.S.	1958	Genetics
University of Washington	Ph.D.	1961	Microbiology
Harriott-Watt University	D.Sc. (Hon)	1987	
Hood College, Frederick, MD	D.Sc. (Hon)	1991	
Purdue University	D.Sc. (Hon)	1993	

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.**

RECENT AWARDS

International Institute of Biotechnology Gold Medal, 1990; Purkinje Medal for Achievement in the Biological Sciences, Czechoslovakian Academy of Sciences, 1991; National Scholar, Phi Kappa Phi, 1992.

EXPERIENCES

1957-1958	Research Assistant, University of Washington
1959-1960	Predoctoral Associate, University of Washington
1961-1964	Assistant Research Professor University of Washington
1961-1963	Guest Scientist, Division of Applied Biology, National Research Council of Canada, Ottawa
1963-1964	Visiting Assistant Professor of Biology, Georgetown University
1964-1966	Assistant Professor of Biology, Georgetown University
1966-1972	Associate Professor of Biology, with tenure, Georgetown University
1977-1983	Director, University of Maryland Sea Grant College
1980-1981	Acting Director, Center for Environmental and Estuarine Studies
1983-1987	Vice President for Academic Affairs, University of Maryland
1987-1991	Director, Maryland Biotechnology Institute, University of Maryland
1987-1991	Director, Center of Marine Biotechnology, University of Maryland
1991-	President, University of Maryland Biotechnology Institute, University of Maryland System

RESEARCH

Marine and estuarine microbial ecology; microbial systematics; marine microbiology (ecology, physiology, genetics, and fine structure of marine and estuarine bacteria); temperature and high pressure effects on marine bacteria; microbial degradation; applications of computers in biology and medicine; release of genetically engineered microorganisms.

Dr. Rita R. Colwell

PROFESSIONAL ACTIVITIES

Phi Beta Kappa; National Commercial Company Research Award; Fellow - Washington Academy of Sciences, American Association for the Advancement of Science, Sigma Delta Epsilon, American Academy of Microbiology, Canadian College of Microbiologist; Board of Governors - Society for Industrial Microbiology, 1976-79; Board of Managers - Washington Academy of Sciences, 1976-79; Board of Trustees - American Type Culture Collection, Board of Trustees, 1970-85; Chairman - American Type Culture Collection, Board of Trustees, Chairman, 1980-81; International Union of Biological Sciences, U.S. National Committee, 1980-1984; International Union of Microbiological Societies (IUMS), and Secretary-Treasurer, Vice Chairman of Microbiology; Governing Board - American Institute of Biological Sciences, 1987-92; Marine Technology Society, 1980-86; Editorial Board - *Microbial Ecology*, 1972-1990; *Applied and Environmental Microbiology*, 1969-81; *Estuaries*; Associate Editor - *Canadian Journal of Microbiology*, 1972-75; Assistant Editor - *Canadian Journal of Microbiology*, 1975-80; North American Editor - *World Journal of Microbiology and Biotechnology*, 1990-; Chairman and member of various committees - American Society for Microbiology Steering Committee for IUMS Congress, 1979-82, ASM Foundation Lecturer, 1981-1982 and 1985-1986; American Society for Microbiology, President 1984-85; ASM Fisher Award, 1985 and ASM Alice Evans Award, 1985; National Science Foundation, 1970-present; National Academy of Sciences, Member Committee preparing Report on "Field Testing Genetically Modified Organisms", 1989; Alpha Chi Sigma Professional Service Award; Society for Experimental Biology and Medicine, Distinguished Scientist and Lecturer Award, 1979; Presidential appointment to National Sciences Board 1984-1990; Advisory Committee - Food and Drug Administration ("Blue Ribbon Committee"), 1990-91; Chairman, Biotechnology Scientific, Biotechnology Policy Board, 1990-1994, President, AAAS, 1995-1996; Member, Board of Trustees, International Centre for Diarrhoeal Disease, Research, Bangladesh.

PUBLICATIONS

Author or editor of 12 books; produced award-winning film, *Invisible Seas*, a 28-minute, color, sound film on marine microbiology.

Author or co-author of 462 papers and articles, 69 chapters, 67 reports and 387 abstracts or presentations.

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Anwarul Huq	Research Associate Professor

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Karachi	B.Sc. (Hon)	1973	Zoology
University of Karachi	M.Sc.	1973	Marine Zoology
University of Maryland	Ph.D.	1984	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.**

EXPERIENCES

1975-76	Research Assistant, Johns Hopkins University, ICMRT
1976-80	Research Office, International Center for Diarrhoeal Disease Research, Bangladesh, ICDDR,B
1980-84	Faculty Research Assistant, University of Maryland, College Park, Maryland
1984-87	Assistant Scientist and Manager, Laboratory Services Department, ICDDR,B
1987-92	Associate Scientist, ICDDR,B
1987-92	Head, Research Microbiology Branch, ICDDR,B
1989-1995	Research Assistant Professor, Dept. of Microbiology, University of Maryland, College Park, MD
1995-	Research Associate Professor, Dept. of Microbiology, University of Maryland, College Park, MD

PUBLICATIONS

Author or Co-Author of 52 papers and articles, (published) 17 chapters 8 (pending) published and 63 abstracts or presentations made.

Selected Publications 1990-96:

Tamplin, M.L., P.R. Brayton, **A. Huq**, D.A. Sack and R.R. Colwell, 1990. Attachment of *V. cholerae* serogroup O1 to zooplankton and phytoplankton of Bangladesh waters. Appl. Environ. Microbiol. 56:1977-80.

Huq, A., R.R. Colwell, R. Rahman, A. Ali, M.A.R. Chowdhury, Salin Parveen, D.A. Sack and E. Russek-Cohen, 1990. Occurrence of *V. cholerae* in the aquatic environment measured by fluorescent antibody and culture method. Appl. Environ. Microbiol. 56:2370-73.

Parveen, S. and **A. Huq**, 1990. Enterotoxicity of *Aeromonas* spp. isolated from environmental sources. Bangladesh J. Microbiol. 7:89-93.

Alam, M., S.I. Khan and **A. Huq**, 1991. Drug Resistance of Clinical and Environmental isolates of V. *cholerae* O1. Bangladesh J. Microbiol. 8:55-57.

Dr. Anwarul Huq

Huq, A., A. Akhtar, D.A. Sack and M.A.R. Chowdhury, 1991. Optimal growth temperature for the isolation of *Plesiomonas shigelloides* using PDA, IBB and MSS agar. Can. J. Microbiol. 37:800-802.

Alam, M., S.I. Khan and **A. Huq**, 1991. Prevalence of fecal coliform in isolated ponds in a village-setting. Bangladesh J. Microbiol. 8:103-107.

Huq, A., M. Alam, S. Parveen and R.R. Colwell, 1992. Occurrence of vibrio-static compound resistant *V. cholerae* O1 in clinical and environmental samples in Bangladesh. J. Clin. Microbiol. 30:219-2321.

Parveen, S., **A. Huq**, J.A.K. Hasan and K.M.S. Aziz, 1992. Prevalence of hemolysin producing *Aeromonas* in the aquatic environment of Bangladesh. Microbios. 70:67-70.

Colwell, R.R., J.A.K. Hasan, **A. Huq**, L. Loomis, Mark L. Tamplin, R.J. Siebeling, M. Torres, S. Galvez, S. Islam and D. Bernstein, 1992. Development and evaluation of a rapid, simple and sensitive monoclonal antibody-based co agglutination test for direct detection of *V. cholerae* O1. FEMS Microbiol. Letters. 97:215-220.

Huq, A., S. Parveen, F. Qadri and R.R. Colwell, 1993. Comparison of *Vibrio cholerae* serotype O1 strains isolated from patients and the aquatic environment. J. Trop. Med. Hyg. 96:86-92.

Huq, A., J.A.K. Hasan, G. Losonsky and R.R. Colwell, 1994. Occurrence of toxigenic *V. cholerae* O1 and *V. cholerae* non O1 in professional divers and dive sites in the United States, Ukraine, and Russia. FEMS Microbiol. Letters. 120:137-142.

Hasan, J.A.K., **A. Huq**, M.L. Tamplin, R. Siebeling and R.R. Colwell. 1994. A novel kit for rapid detection of *V. cholerae* O1. J. Clin. Microbiol. 32:249-252.

Huq, A., J.A.K. Hasan. L. Loomis and R.R. Colwell. 1994. Occurrence of non-culturable bacteria in the marine environment: rapid detection using inexpensive kit. Indian J. Marine Sc. Pp. 17-24.

Sack, D.A., A.T.M. Shamsul Haque, **A. Huq**, and M. Etheridge, 1994. Hypothesis: Natural infection with *P. Shigelloides* protects developing country residents against *S. sonnei* infection. The Lancet. 343:1413-15.

Hasan, J.A.K., D. Bernstein, **A. Huq**, L. Loomis, M. Tamplin, and R.R. Colwell, 1994. Cholera DFA: An improved direct fluorescent antibody staining kit for rapid detection and enumeration of *V. cholerae* O1. FEMS Microbiol. Letters. 120:143-148.

Hasan, J.A.K., M.A.R. Chowdhury, M. Shahabuddin, **A. Huq**, L. Loomis, and R.R. Colwell, 1994. Cholera toxin gene polymerase chain reaction for detection of non-culturable *Vibrio cholerae* O1. World J. Microbiol. Biotechnol. 10:568-571.

Colwell, R.R., **A. Huq**, M.A.R. Chowdhury, P. Brayton, and B. Xu, 1995. Serogroup conversion in *Vibrio cholerae*. Can. J. Microbiol. 41:946-950.

Huq, A., and R.R. Colwell, 1995. A Microbiological Paradox: viable but non-culturable bacteria with special reference to *Vibrio cholerae*. J. Food Technol. 58:1-7.

Colwell, R.R., P. Brayton, **A. Huq**, B. Tall, P. Harrington and M. Levine. 1996. Diarrhea in humans caused by viable non-culturable *V. cholerae*. World J. Microbiol. & Biotech. 12:28-31.

Huq, A., B. Xu, M.A.R. Chowdhury, M. Montilla and R.R. Colwell, 1996. A Simple Filtration method to remove plankton associated *V. cholerae* O1 in raw water supplies in developing countries. Appl. Environ. Microbiol. 62:2508-2512.

Huq, A., and R.R. Colwell, 1996. Vibrios in the Environment: Tracking of *Vibrio cholerae*. J. Ecosystem Health. 2:198-214.

BIOGRAPHICAL SKETCH

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

AME	POSITION TITLE
Sirajul Islam	Scientist and Head Environmental Microbiology Laboratory

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
aulatpur Govt. B.L. College, Khulna	B.Sc. (Hon)	1973	Biology
epartment of Botany, University of Dhaka, Bangladesh	M.Sc.	1976	Ecology, Limnology & Microbiology
epartment of Tropical Hygiene, London, chool of Hygiene and Tropical Medicine, UK	Ph.D.	1987	Environmental Disease Transmission

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

XPERIENCES

77-77	Research Scholar, Asiatic Society of Bangladesh
77-77	Research Scholar, National Council of Science and Technology, Government of Bangladesh
78-79	Research Officer, Microbiology Branch, Matlab, ICDDR,B
79-83	Senior Research Officer, Training Branch, ICDDR,B
83-87	Ph.D. Scholar, London University, United Kingdom
88-92	Assistant Scientist, Laboratory Sciences Division, ICDDR,B
92-94	Associate Scientist, Laboratory Sciences Division, ICDDR,B
94-	Scientist, Laboratory Sciences Division, ICDDR,B

UBLICATIONS

lected Publications 1994-96:

lam, M.S., Hasan, M.K., Miah, M.A., Huq, A., Bardhan, P.K., Sack, R.B., and Albert, M.J., (1994). pecificity of cholera screen™ test during an epidemic of cholera-like disease due to *Vibrio cholerae* O139 nonym Bengal. Transactions of the Royal Society of Tropical Medicine and Hygiene. 88:424-425.

lam, M.S., and Bateman, O.M. (1994). The spread and control of cholera in Bangladesh. Waterlines. 12:20-23.

Islam, M.S., Hasan, M.K., Miah, M.A., Yunus, M., Zaman, K., and Albert, M.J. (1994). Isolation of *Vibrio cholerae* O139 synonym Bengal from the aquatic environment of Bangladesh: Implications for disease transmission. Applied and Environmental Microbiology 60:1684-1686.

Dr. M.S. Islam

Islam, M.S., Miah, M.A., Hasan, M.K., Sack, R.B., and Albert, M.J. (1994). Detection of non-culturable *Vibrio cholerae* O1 associated with a cyanobacterium from an aquatic environment in Bangladesh. Transactions of the Royal Society of Tropical Medicine and Hygiene. 88:298-299.

Islam, M.S., Drasar, B.S. and Sack, R.B. (1994). The aquatic flora and fauna as reservoirs of *Vibrio cholerae*: A review. Journal of Diarrhoeal Diseases Research. 12:87-96.

Islam, M.S., Alam, M.J., Khan, S.I. and Huq, A. (1994). Faecal pollution of freshwater environments in Bangladesh. International Journal of Environmental studies. 46:161-165.

Islam, M.S., Drasar, B.S., and Sack, R.B. (1994). Probable role of blue-green algae in maintaining endemicity and seasonality of cholera in Bangladesh: A hypothesis. Journal of Diarrhoeal Diseases Research. 12:245-256.

Islam, M.S., Alam, M.J., and Khan, S.I. (1995). Occurrence and distribution of culturable Vibrio *cholerae* O1 in aquatic environments of Bangladesh. International Journal of Environmental Studies. 46:217-223.

Islam, M.S., Siddique, A.K.M., Salam, A., Akram, K., Zaman, K., Fronczak, N. and Laston, S. (1995). Microbiological investigation of the diarrhoea epidemics among Rwandan refugees in Goa, Zaire. Transactions of the Royal Society of Tropical Medicine and Hygiene. 89:506.

Islam, M.S., Alam, M.J., Miah, M.A., Felsenstein, A. and Sack, R.B. (1995). Detection of non-culturable *Vibrio cholerae* O139, by PCR and fluorescent antibody methods in laboratory microcosms. World Journal of Microbiology and Biotechnology. 11:597-598.

Islam, M.S., Rezwan, F.B., and Khan, S.I., (1996). Response of Shigella flexneri in artificial aquatic environment: effects of different physicochemical stress factors. Journal of Diarrhoeal Disease. 14:000-000 (In Press).

Islam, M.S., Alam, M.J., Begum, A., Rahim, Z., Felsenstein, A. and Albert, M.J. (1996). Occurrence of culturable *Vibrio cholerae* O139 Bengal with ctx gene in various components of aquatic environment in Bangladesh. Transactions of the Royal Society of Tropical Medicine and Hygiene, 90:128.