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

Memorandum

2 January 2003

To : Dr. Md. Sirajul Islam
Principal Investigator of protocol # 2002-024
Laboratory Sciences Division

From: Professor Barkat-e-Khuda
Acting Chairman, Research Review Committee (RRC)

Sub : Approval of Research Protocol # 2002-024

Thank you for your memo dated 2nd January 2003 with the modified version of your research protocol # 2002-024 entitled "Cholera Risk Management in Mozambique and Bangladesh, Phase I". The modified version of the protocol is hereby approved upon your addressing the issues raised by the RRC in its meeting held on 5th September 2002.

Thank you.

Copy: Associate Director
Laboratory Sciences Division

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
Mail : ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh
Phone : 871751-60, Telex : 675612 ICDD BJ
Fax : 880-2-883116, 886050, 871568, 871686, Cable : Cholera Dhaka

CENTRE
FOR HEALTH AND
POPULATION RESEARCH

Memorandum

Date : 02 January 2003

To : Acting Chairman, RRC

From : Dr. Md. Sirajul Islam M. S. Islam
Principal Investigator of Protocol #2002-024
Laboratory Sciences Division

Subject : Research Protocol #2002-024

Thank you for your memo of December 18, 2002 regarding the Reviewers' comments on the above mentioned Protocol. I have addressed all the comments raised by the Reviewers (copy attached). I have also made necessary modifications according to the Reviewers' comments. I am submitting the revised protocol for your kind approval.

Best regards.

Copy: Associate Director, LSD

Dr. P. Bardhan 2/1/03

Response to Reviewers' Comments

Protocol # 2002-024

1. Activities to be undertaken in the second phase have been elaborated in the revised protocol as suggested by the Reviewers. Page 15 para 3 and 4 of the revised protocol.

2. A provision has been created for Senior Data Management Officer (SDMO) in page 38 of the revised protocol.

3. The proprietorship of the data between the ICDDR,B and the Ministry of Health of Mozambique has been spelt out in the page 29 of the revised protocol as suggested by the Reviewers.

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

RESEARCH PROTOCOL Protocol No. 2002-024	**FOR OFFICE USE ONLY**
	RRC Approval: ☐ ☐Yes / ☐No Date:
	ERC Approval: ☐Yes / ☐No Date:
	AEEC Approval: ☐Yes / ☐No Date:

Project Title: Cholera Risk Management in Mozambique and Bangladesh, Phase I

Theme: ***(Check all that apply)***

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

Key words: ***Vibrio cholerae,*** **Ecology, Epidemiology, Phytoplankton, Zooplankton, Intervention**

Relevance of the protocol: Studies demonstrated that *V. cholerae* are the autochthonous flora of brackish and estuarine environment. It was also found that the aquatic organisms e.g. phytoplankton and zooplankton in the aquatic environment of Bangladesh can act as reservoirs of *V. cholerae*. Studies in Bangladesh have shown that the cholera epidemic seasons in Bangladesh are also coincided with the phytoplankton and zooplankton bloom formation in the aquatic environment. The plankton bloom formation is related with the changes of various physicochemical conditions of water e.g., pH, salinity, conductivity etc. However, it is not known whether similar situation exists in other parts of the world. Therefore a comparative ecological and epidemiological study will be undertaken in Mozambique and Bangladesh. This study will provide the information regarding the comparative ecology of *V. cholerae* and epidemiology of cholera in these two countries. This study will help to find out the intervention strategies for cholera, which will have an ultimate impact in reducing the mortality and morbidity due to cholera in Mozambique. This study will also facilitate the technology transfer from ICDDR,B in Bangladesh to Environmental Hygiene Laboratory at Beira in Mozambique.

Programmes

- ☐ Child Health Programme
- ☐ Nutrition Programme
- ☑ Programme on Infectious Diseases & Vaccine Science
- ☐ Health and Family Planning Systems Programme
- ☐ Population Programme
- ☐ Reproduction Health Programme

Principal Investigator: Dr. Md. Sirajul Islam **Division: LSD** **Phone: 8811751-60, Ext. 2407**

Address: Environmental Microbiology Lab. LSD, ICDDR,B **Email: sislam@icddrb.org**

Co-Principal Investigator(s): **Dr. Andrew E. Collins, University of Northumbria, UK.**
Dr. Marcelino Lucas, Maputo, Mozambique

Co-Investigator(s): **Dr. Robert F. Breiman, ICDDR, B**
Dr. G. Balakrish Nair, ICDDR, B

Student Investigator/Intern: N/A

Collaborating Institute(s): **University of Northumbria, UK.**
Ministry of Health, Mozambique

Population: Inclusion of special groups (*Check all that apply*): **Not Applicable**

Gender
- ☐ Male
- ☐ Females

Age
- ☐ 0 – 5 years
- ☐ 5 – 9 years
- ☐ 10 – 19 years
- ☐ 20 – 64 years
- ☐ 65 +

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

Project / study Site (*Check all the apply*):
- ☐ Dhaka Hospital
- ☐ Matlab Hospital
- ☑ Matlab DSS area
- ☐ Matlab non-DSS area
- ☐ Mirzapur
- ☐ Dhaka Community
- ☐ Chakaria
- ☐ Abhoynagar
- ☐ Mirsarai
- ☐ Patyia
- ☐ Other areas in Bangladesh _____
- ☐ Outside Bangladesh
  name of country: **Mozambique**
- ☐ Multi centre trial
  (Name other countries involved)
  _____

Type of Study (*Check all that apply*):
- ☐ Case Control study
- ☐ Community based trial / intervention
- ☐ Program Project (Umbrella)
- ☐ Secondary Data Analysis
- ☐ Clinical Trial (Hospital/Clinic)
- ☐ Family follow-up study
- ☐ Cross sectional survey
- ☐ Longitudinal Study (cohort or follow-up)
- ☐ Record Review
- ☐ Prophylactic trial
- ☑ Surveillance / monitoring
- ☐ Others

Targeted Population (*Check all that apply*): **Not Applicable**
- ☐ No ethnic selection (Bangladeshi)
- ☐ Bangalee
- ☐ Tribal groups
- ☐ Expatriates
- ☐ Immigrants
- ☐ Refugee

Consent Process (*Check all that apply*): **Not Applicable**
- ☐ Written
- ☐ Oral
- ☐ None
- ☐ Bengali language
- ☐ English language

Proposed Sample size: **Not Applicable**
Sub-group _____ ☐
_____ ☐

Total sample size: _____ ☐
_____ ☐
_____ ☐

Determination of Risk: Does the Research Involve (*Check all that apply*): **Not Applicable**
- ☐ Human exposure to radioactive agents?
- ☐ Fetal tissue or abortus?
- ☐ Investigational new device?
  (specify_____)
- ☐ Existing data available from Co-investigator
- ☐ Human exposure to infectious agents?
- ☐ Investigational new drug
- ☐ Existing data available via public archives/source
- ☐ Pathological or diagnostic clinical specimen only
- ☐ Observation of public behaviour
- ☐ New treatment regime

Yes/No

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

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

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

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

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

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

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

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

Yes/No

☑ ☐ Is the proposal funded?

If yes, sponsor Name: **DFID, UNICEF**

Yes/No

☐ ☐ Is the proposal being submitted for funding ?

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

(2) _____

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

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

Dates of Proposed Period of Support

(Day, Month, Year - DD/MM/YY)

Beginning date : **01/10/2002**

End date : **30/09/2005**

Cost Required for the Budget Period ($)

	1st Year	*2nd Year*	*3rd Year*	*Other years*
a.	**84,716**	**81,876**	**79,286**	

b. ***Direct Cost :*** **1,96,703** ***Total Cost :*** **2,45,878**

Approval of the Project by the Associate Director of the Applicant

The above-mentioned project has been discussed and reviewed at the Division level as well by the external reviewers. The protocol has been revised according to the reviewer's comments and is approved.

DR. G. BALAKRISH NAIR — Name of the Associate Director

Signature

29/08/2002 — Date of Approval

Certification by the Principal Investigator

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

Signature of PI M. S. Islam

Date: 29/08/2002

Name of Contact Person (if applicable)

DR. MD. SIRAJUL ISLAM

Table of Contents

☐ Check here if appendix is included

PROJECT SUMMARY: Describe in concise terms, the hypothesis, objectives, and the relevant background of the project. Describe concisely the experimental design and research methods for achieving the objectives. This description will serve as a succinct and precise and accurate description of the proposed research is required. This summary must be understandable and interpretable when removed from the main application. (TYPE TEXT WITHIN THE SPACE PROVIDED).

Principal Investigator : **Dr. Md. Sirajul Islam**

Project Name : **Cholera Risk Management in Mozambique and Bangladesh, Phase I**

Total Budget : **2,45,878** Beginning Date : **01/10/2002** Ending Date : **30/09/2005**

Cholera epidemics occur every year in Mozambique and Bangladesh. The coastal areas of Mozambique that have regularly experienced epidemics of cholera in the past are now endemic. During the 1990s, particularly 1992 and 1998, it had the highest rate of incidence in the world. The Governments of Mozambique and Bangladesh are committed to reducing their diarrhoeal disease burdens and keen that the most appropriate and effective approaches and methods are used in cholera prevention and control. Survival of *Vibrio cholerae* is dependent on key environmental characteristics including levels of alkalinity, salinity, and microbiological flora and fauna that play a crucial role in its ecology and transmission to people who become infected. The comparative study will be able to confirm or negate the wider applicability of different types of variable for assessment and management of cholera in these two regions and beyond. Other than both regions supporting many cases of cholera, Bangladesh and Mozambique have comparable characteristics of cholera endemism. Further similarities include a tropical climate and similar range of vegetation, coastal areas that are annually in flood, high levels of poverty, areas of high population density around urban areas, and limited access of many people to a safe water supply. The main aims and objectives of this protocol are as follows: Transfer of technology from ICDDR,B laboratories in Bangladesh to the Center for Environmental Hygiene and Medical Exams (CHAEM) at Beira of Mozambique, to find out the role of phytoplankton and zooplankton as habitat of *V. cholerae* in the aquatic environment of Mozambique, to see the role of various physicochemical parameters in multiplication of *V. cholerae* O1 in the aquatic environment of Bangladesh and Mozambique. To find out intervention strategies to reduce cholera incidence at Beira, Mozambique. The project is an interdisciplinary and collaborative activity between three institutions that in the first instance focuses on the development of cholera monitoring and prediction in Mozambique. This is based on the consolidation of earlier research findings from a wide variety of sources including those based on Mozambique and Bangladesh through the recognized studies of those coordinating this proposal. The initial phase of the project focuses on testing the hypothesis that environmental influences on cholera ecology are similar in Mozambique to those in Bangladesh. This necessitates transfer of technology from Dhaka to Beira, for microbiological monitoring of cholera epidemic, and comparison with studies in Bangladesh. The collaboration requires capacity building in the Mozambique laboratories to streamline some existing procedures and to introduce new ways of monitoring *V. cholerae* from the environment and from the people who become cholera patients. The specific techniques for the microbiological analysis will include the following: culturing of diarrhoeal pathogens, monitoring of standard water quality indicators, further work on preserved samples, analysis of phytoplankton and zooplankton in well water and neighboring water bodies, and the use of rapid detection technologies such as fluorescent antibody technique. The isolation and enumeration of *V. cholerae* O1 and O139 from various environmental samples by culture, FA, and colony blot hybridization will be related with the physicochemical parameters of water and the seasonality of cholera. The data from Bangladesh will be compared with the data from Mozambique to find out the role of environmental factors in the seasonality and endemicity of cholera in these two countries. This study will provide the information regarding the ecology of *V. cholerae* and epidemiology of cholera in Mozambique. This study will help to find out the intervention strategies for cholera which will have an ultimate impact in reducing the mortality and morbidity due to cholera.

KEY PERSONNEL (List names of all investigators including PI and their respective specialties)

Name	Professional Discipline/ Specialty	Role in the Project
1. Islam, M.S.	Environmental Microbiologist	PI
2. Collins, A. E.	Sustainable Development Specialist	Co-PI
3. Lucas, M.	Environmental Health Specialist	Co- PI
4. Breiman, R.F.	Epidemiologist	Co-I
5. Nair, G.B.	Microbiologist	Co-I

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be tested:

Concisely list in order, in the space provided, the hypothesis to be tested and the Specific Aims of the proposed study. Provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

We postulate that the ecology of *Vibrio cholerae* O1 in Mozambique will be similar to Bangladesh and similar environmental intervention may be applicable to reduce the incidence of cholera in Mozambique.

Specific Aims:

Describe the specific aims of the proposed study. State the specific parameters, biological functions/ rates/ processes that will be assessed by specific methods (TYPE WITHIN LIMITS).

1) Transfer of technology from ICDDR,B laboratories in Bangladesh to the Center for Environmental Hygiene and Medical Exams (CHAEM) at Beira of Mozambique.
2) To find out the role of phytoplankton and zooplankton as habitat of *V. cholerae* in the aquatic environment of Mozambique
3) To see the role of various physicochemical parameters in multiplication of *V. cholerae* O1 in the aquatic environment of Bangladesh and Mozambique.
4) To find out intervention strategies to reduce cholera incidence at Beira, Mozambique.

Background of the Project including Preliminary Observations

Describe the relevant background of the proposed study. Discuss the previous related works on the subject by citing specific references. Describe logically how the present hypothesis is supported by the relevant background observations including any preliminary results that may be available. Critically analyze available knowledge in the field of the proposed study and discuss the questions and gaps in the knowledge that need to be fulfilled to achieve the proposed goals. Provide scientific validity of the hypothesis on the basis of background information. If there is no sufficient information on the subject, indicate the need to develop new knowledge. Also include the **significance and rationale** of the proposed work by specifically discussing how these accomplishments will bring benefit to human health in relation to biomedical, social, and environmental perspectives. **(DO NOT EXCEED 5 PAGES, USE CONTINUATION SHEETS).**

Incidence of cholera persists in Mozambique and Bangladesh. Whilst there have been advances in reducing mortality rates, there continues to be high levels of severe morbidity. Furthermore, coastal areas of Mozambique that have regularly experienced epidemics of cholera in the past are now endemic. Meanwhile, regions previously free of cholera are suffering from this disease for the first time, such as the northeast region of South Africa, which neighbours Mozambique's southern boundary. Cholera continues to plague many parts of the world but also has largely been concentrated in Africa which constitutes more than 80% of the total cases world wide (Naidoo and Patric, 2002). The incidence rate of cholera in Africa in 1993 has been shown in Figure 1 (WHO, 1994a).

The recent pandemic is thought to have been responsible for hundreds of thousands of cases and tens of thousands of deaths in Africa alone (CDD, 1994). Among the African countries, Mozambique, Malawi and South Africa are the most affected countries. Figure 2 shows the latest cholera cases and deaths in various countries of Africa from January 2002 to June 2002.

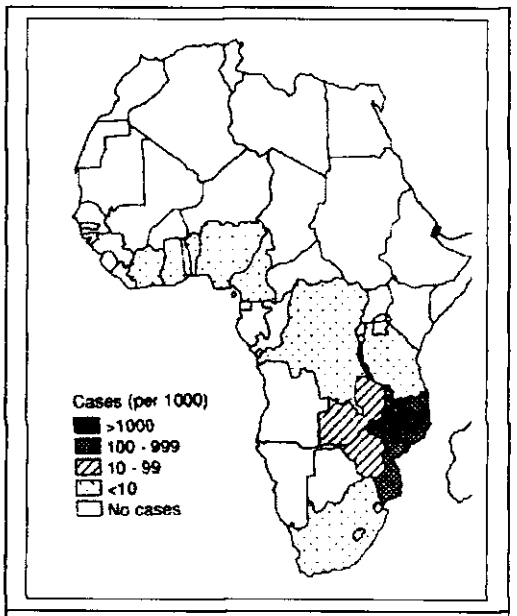


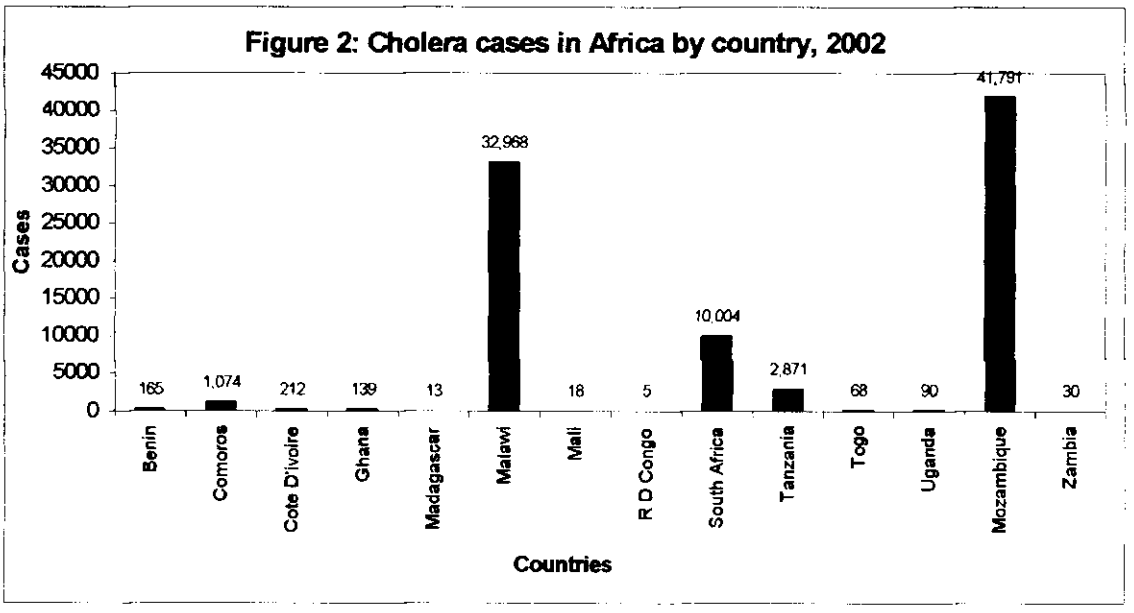
Figure 1: Incidence of cholera cases in Africa in 1993

Mozambique has suffered particularly badly from this disease. In 1992, it had the highest notified incidence of cholera in Africa with 20,802 cases and 726 deaths (WHO, 1993, 1994b). Again, this year from January to June, the highest number of cholera cases have been reported from Mozambique (Figure 2). In Mozambique (Figure 3), the cholera epidemic started in 1981 and continued upto 1984. Then again the epidemic started in 1989 and continued upto 1994. During 1995 and 1996, no cholera case was reported from Mozambique. However, from 1997, cholera became endemic in Mozambique (Personal communication with Dr. Kahozi, WHO, Mozambique).

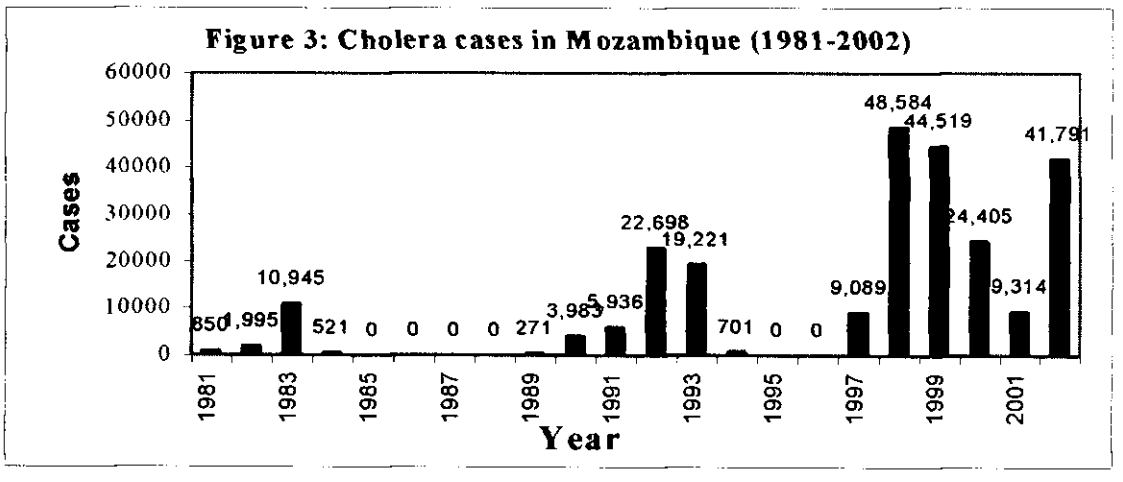
In Mozambique, Beira is the most affected city. It had the highest rate of incidence of cholera in Mozambique from 1991 to 1994 (Collins, 1998). Beira is situated in the sedimentary basin and composed of sands at the surface and layers of clay. Flooding occurs every year in the low-lying area during rainy seasons. The average temperature of the hottest month (January) was reported as 28°C and average of the coolest month (July) as 21°C; the average rainfall per anum was recorded as 1551 mm (Servico Provincial de Estatistica, 1993).

Strains of *Vibrio cholerae* that are historically associated with cholera epidemics in Bangladesh and around the Bay of Bengal later become established in Africa. Mozambique has frequently been amongst those countries that are the most affected. During the 1990s, particularly 1992 and 1998, it had the highest rate of incidence in the world. There is additional concern that the new O139 strain of *V. cholerae*, which was identified in 1993, may at some point spread to Africa and have a further widespread impact on the health of the continent. In a study in Bagladesh by Islam *et al.* (1993a) showed that *V. cholerae* O139 may be even hardier with a survival advantage over *V. cholerae* O1. They noted that whereas *V. cholerae* O1 was

normally isolated from <1% of water samples during epidemics, 12% of water samples in their study of 92 water samples from ponds, lakes, rivers, and canals in rural Matlab and urban Dhaka yielded *V. cholerae* O139. Therefore, environmental surveillance of *V. cholerae* O139 along with *V. cholerae* O1 will also be carried out in both Mozambique and Bangladesh. The Governments of Mozambique and Bangladesh are committed to reducing their diarrhoeal disease burdens and keen that the most appropriate and effective approaches and methods are used in cholera prevention and control.



Cholera research during the last twenty years has progressed understanding of the disease but the prevalence of cholera cases continues to be very high. The accumulated findings of this work indicates that incidence of the disease is now potentially much more predictable and preventable than hitherto realized. However, relatively little has been done to apply this knowledge to those regions that continue to suffer the highest burden of the disease. Furthermore, no predictive model has yet been developed that can be offered to local government environment and health departments as a tool for prevention. One key to progress in understanding cholera risks is that *V. cholerae* was more recently found to be resident in environmental reservoirs where there are the right physical and chemical conditions, and that it is associated with other micro-organisms that can be easily removed from water.



Indication of some of the chemical environmental influences was offered as an explanation for differing patterns of incidence of cholera in coastal cities during work carried out on environment, health and population displacement in Mozambique (Collins, 1993a, 1993b, 1996a, 1996b). More microbiologically detailed work carried out by ICDDR,B through a number of extensive studies in Bangladesh explains how environmental reservoirs of *V. cholerae* are influential on incidence of cholera (Islam *et al.*, 1993a, 1994a,

1994b, 1994c, 1994d, 1997). More specifically, this has confirmed that *V. cholerae* is resident in the aquatic environment of Bangladesh. Its survival is dependent on key environmental characteristics including levels of alkalinity, salinity, and microbiological flora and fauna that play a crucial role in its ecology and transmission to people who become infected.

An underlying rationale of this project proposal is that comparison of environmental influences on incidence of cholera between Bangladesh and Mozambique alongside identification of most at risk groups of people and locations will help test findings from both countries in establishing the appropriate components of a predictive model. The model will support an early warning system and will be adaptable to both the South Asia and Southern Africa. The comparative study will be able to confirm or negate the wider applicability of different types of variable for assessment and management of cholera in these two regions and beyond. Other than both regions supporting many cases of cholera, Bangladesh and Mozambique have comparable characteristics of cholera endemism. Further similarities include a tropical climate and similar range of vegetation, coastal areas that are annually in flood, high levels of poverty, areas of high population density around urban areas, and limited access of many people to a safe water supply (Adesina, 1987; Merson, 1980; Miller *et al.*, 1982; Learmonth 1988; WHO Features 1990).

Cholera is endemic in certain parts of Bangladesh. Cholera epidemics in endemic areas occur twice every year and maintain a regular seasonal pattern (Glass *et al.*, 1982; Islam *et al.*, 1993a). However it was not known until recently, how the endemicity and seasonality of cholera are maintained. One interesting observation is that during epidemic seasons, the causative agent, *V. cholerae* O1 have been isolated from the patients as well as from the aquatic environment but when the epidemic is over, *V. cholerae* O1 can not be isolated in culturable form from the aquatic environment (Khan *et al.*, 1984; Islam *et al.*, 1988). Therefore the question is where do the *V. cholerae* O1 hide or survive during in between epidemics ? What are the reservoirs of *V. cholerae* O1 in the aquatic environment during inter epidemic periods ? This bacterium was first discovered by a German Scientist, Dr. Robert Koch in 1884. After about 100 years later Professor Rita R. Colwell of Maryland University, USA hypothesized that *V. cholerae* O1 is an authocthonous flora of the brakish and estuarine environment (Colwell *et al.*, 1977, 1990). It has been found that *V. cholerae* O1 secrets an enzyme chitinase (Nalin, 1976) and can make an association with the chitinous fauna e.g., zooplankton mainly copepods (Huq *et al.*, 1983, 1984). The attachment of *V. cholerae* with copepod is shown in Fig. 4.

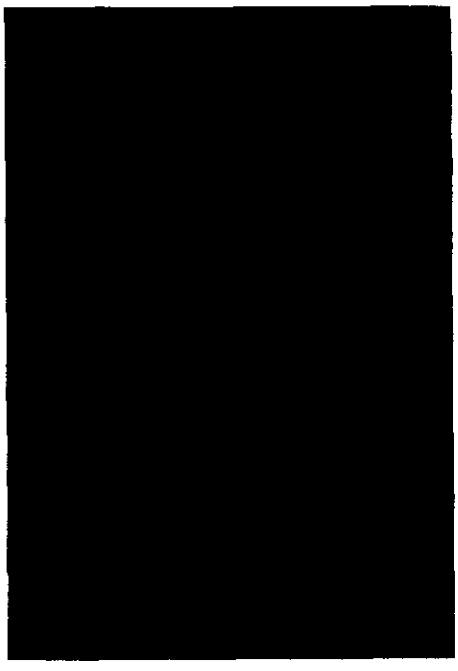


Fig. 4. Attachment of *V. cholerae* O1 in caudal setae of copepod (*Mesocyclops* sp.) is shown. Arrow shows *V. cholerae* O1. Original magnification X 400.

Similarly it was also found that *V. cholerae* secrets an enzyme mucinase (Schneider *et al.*, 1982) and the role of the mucinase is to degrade the mucin from the environment. The mucin is present in the plant cell wall therefore it was thought that *V. cholerae* can also make an association with the aquatic plant (Islam and Aziz, 1981). Studies have shown that various aquatic plants e.g. water hyacinth, marine algae, duck weeds, green algae can act as temporary reservoirs of *V. cholerae* O1 in the aquatic environment (Spira *et al.*, 1981; Islam *et al.*, 1988, 1989, 1990b). However, finally it was found by both laboratory and field based studies that a blue green alga, *Anabaena* spp. can act as a long term reservoir of *V. cholerae* O1 in the aquatic environment (Islam *et al.*, 1990a, 1994a). Fig. 5 shows *V. cholerae* O1 inside the mucilaginious sheath of *Anabaena variabilis*.

The months of cholera seasons in Mozambique has a correlation with rainfall ($p < 0.001$) (Fig. 6) which is different from the cholera seasons in Bangladesh (Fig. 7). In Bangladesh, cholera epidemics have two peaks, the highest peak during post monsoon and a second small peak during pre-monsoon. This difference in seasonality of cholera epidemics needs to be investigated from ecological point of view. It is not known during rainy season what changes take place in various parameters of water (eg. pH, salinity, conductivity etc.) which may be favorable for multiplication of bacteria in the aquatic environment in Mozambique.

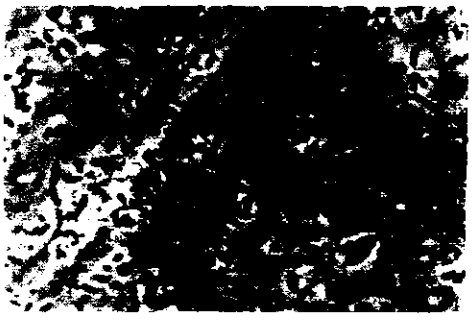


Fig. 5. Phase-contrast microscopy showing *V. cholerae* O1 inside the mucilaginous sheath of a blue green alga, *Anabaena variabilis*. Arrows show a filament of *Anabaena variabilis*. Original magnification X 630.

There is no information about the occurrence of phytoplankton and zooplankton in the aquatic environment in Mozambique during epidemic seasons. Therefore the present study aims at investigating the possible environmental factors responsible for different epidemiological pattern of cholera in Mozambique and Bangladesh.

In epidemiological and ecological study in Bangladesh for the last 5 years, it has been observed that phytoplankton and zooplankton can act as microhabitat for *V. cholerae* O1 in the aquatic environment of Bangladesh (Table. 1). We do not know whether similar situation exists in Mozambique.

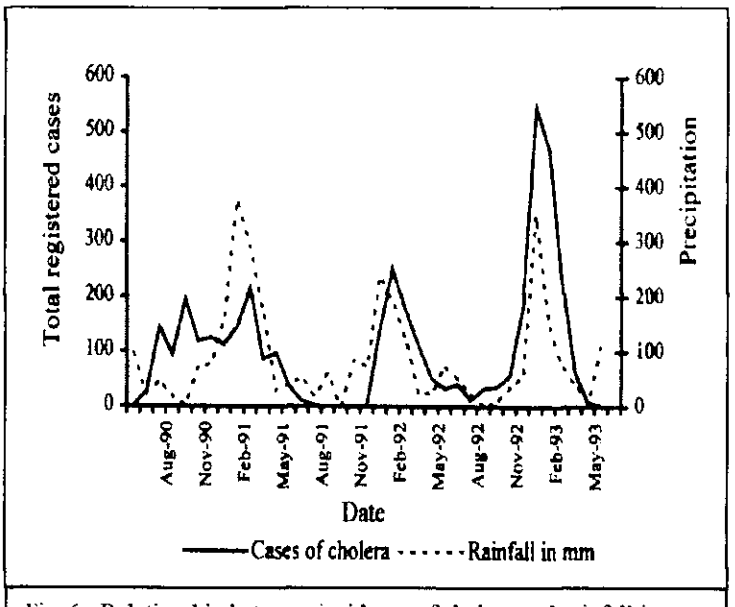


Fig. 6. Relationship between incidence of cholera and rainfall in Mozambique

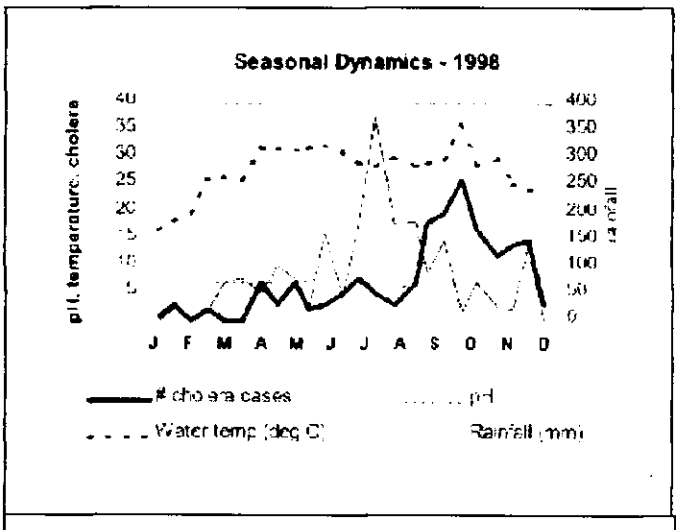


Fig. 7. Seasonal dynamics of pH and water temperature in a fresh water lake in Bangladesh, compared rainfall and the number of cholera cases in the surrounding area. Note that the peak in cholera cases follows the peak in rainfall and coincides with a temperature peak.

Table 1: Isolation of culturable *V. cholerae* O1 from the various components of aquatic environments of Bangladesh during 1999 to 2002

Components	Total number of samples	Total number of isolates
Water	1923	21
Zooplankton	1923	12
Phytoplankton	1923	12
Water Hyacinth	758	2

It has been observed that the seasonality of cholera in Bangladesh is also associated with the seasonality of plankton bloom formation in the aquatic environment of Bangladesh (Islam *et al.*, 1994b). The multiplication of the phytoplankton and zooplankton in the aquatic environment is also related with the changes of various physicochemical parameters of water (Islam *et al.*, 1994b). As phytoplankton is the food for zooplankton, the phytoplankton bloom is followed by the zooplankton bloom. It is not known whether similar changes of the physicochemical parameters of water in the cholera endemic areas of Mozambique also take place during cholera seasons, Therefore in the present study a comparison of all these factors will be considered.

Studies have also shown that the toxigenic *V. cholerae* during association with the aquatic plants do not loose their pathogenic properties (Islam *et al.*, 1990a, 1990b, 1993b) but after certain times become nonculturable (Colwell and Huq, 1994; Colwell *et al.*, 1996; Islam *et al.*, 1990a, 1994b; Huq *et al.*, 1990). These viable and nonculturable *V. cholerae* have been detected both in association with the blue green algae and zooplankton from the aquatic environment in Bangladesh (Islam *et al.*, 1994b; Huq *et al.*, 1990). Therefore attempts will be made to see whether *V. cholerae* also remains in association with the plankton in the aquatic environment of Mozambique in viable but nonculturable (VBNC) form.

Besides ecological factors, other factor like human passage of *V. cholerae* enhances subsequent water-borne spread of the cholera bacterium by effectively lowering the infectious dose in secondary individuals (Merrell *et al.*, 2002). There is also evidence that biological differences are considered as risk factors in contracting cholera also include lowered level of gastric acid, not breastfeeding and presence of O blood group (Glass *et al.*, 1985, 1991, 1992; Van Loon 1993).

In Beira, the diarrhoeal patients are treated in the Catholic hospital. The diagnosis of cholera patients is occasionally done by direct plating of stool and rectal swab samples on to TCBS agar and subsequent confirmation is done by antisera. No attempts have ever been made to detect *V. cholerae* O1 from clinical samples by using dark field microscope. This quick method will also be introduced in the clinical pathology laboratory of the Catholic Hospital of Beira, Mozambique.

The Centre for Environmental Hygiene and Medical Exams (CHAEM) laboratory at Beira monitors the faecal pollution of water from various sources during cholera seasons using MPN technique. Membrane filtration technique will be introduced for better assessment of the faecal pollution of water that will be collected from various water sources. The isolation of culturable *V. cholerae* O1 from various environmental samples will be carried out following enrichment technique. The detection of VBNC *V. cholerae* O1 from the

environmental samples will also be carried out using direct fluorescent antibody (DFA) technique. All these techniques are routinely followed in the Environmental Microbiology Laboratory of ICDDR, B. These simple technologies will be transferred to the Environmental Hygiene laboratory (CHAEM) of Beira from ICDDR, B. Purchase of a fluorescent microscope and other equipment are in progress.

Fig 8. Village woman filtering pond water by using 8-fold old saree.

A study in Matlab is in progress to find out the efficacy of 8 fold saree material as a filtering device of surface water. The hypothesis is that as the *V. cholerae* O1 remains attached with the plankton, if the water is passed through the saree material, it will sieve out the plankton and as the plankton contain *V. cholerae*, it will ultimately sieve out the *V. cholerae* . If this filtered water is used for various household purposes, it will help to reduce the incidence of cholera. The efficacy of saree material as filtering device has been tested in the laboratory and found to be very effective (Huq *et al.*, 1996).

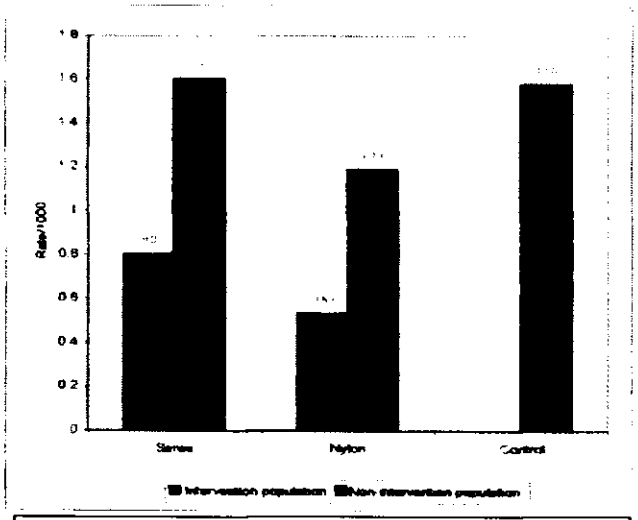


Fig. 9: Cholera attack rate/1000 population at Matlab with and without intervention

This study is going on in 60,000 (Sixty thousand) population at Matlab. According to Huq *et al.*, 2001, it has been revealed from the preliminary results of the study that by using 8 folds saree material as (Fig. 8) filtering device, there is a 50% reduction of cholera incidence in the saree study group in comparison to the control group (Fig. 9). In Mozambique if the similar situation prevail like matlab, similar environmental intervention by using capulanas (two pieces of cloths used like saree by the African women) may be applied to reduce the morbidity and mortality due to cholera.

When a particular water source like pond or river will be found to contain *V. cholerae* in association with blue green algae and copepod, that water source will be considered as a potential reservoir of *V. cholerae.* The surrounding communities of the water source who use the water for various household purposes will be considered as risk group. It is likely that during cholera seasons, the bio-physicochemical parameters of that particular water source will be favourable for multiplication of *V. cholerae*, as a result, the water source will be heavily contaminated by *V. cholerae*. When this contaminated water will be used by the surrounding population, they will most likely contract the disease. If similar phenomenon is found every year, then on the basis of the bio-physico-chemical conditions of the aquatic environment vaccine will be used to that targeted population at risk.

The data obtained from this study will provide information of cholera ecology and epidemiology in Mozambique which will help to consider the targeted use of vaccine as a preventive measure of cholera epidemic. However, it must be emphasized that the research and development represented in this proposal intends to provide the information that will help to address the question of where and when such intervention should take place, final evaluations for such policy decisions only being made through the local policy makers.

Research Design and Methods

Describe in detail the methods and procedures that will be used to accomplish the objectives and specific aims of the project. Discuss the alternative methods that are available and justify the use of the method proposed in the study. Justify the scientific validity of the methodological approach (biomedical, social, or environmental) as an investigation tool to achieve the specific aims. Discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Point out safety procedures to be observed for protection of individuals during any situations or materials that may be injurious to human health. The methodology section should be sufficiently descriptive to allow the reviewers to make valid and unambiguous assessment of the project. **(DO NOT EXCEED TEN PAGES, USE CONTINUATION SHEETS).**

Methods

The project is an interdisciplinary and collaborative activity between three institutions that in the first instance focuses on the development of cholera monitoring and prediction in Mozambique. This is based on the consolidation of earlier research findings from a wide variety of sources including those based on Mozambique and Bangladesh through the recognized studies of those coordinating this proposal. The initial phase of the project focuses on testing the hypothesis that environmental influences on cholera ecology are similar in Mozambique to those in Bangladesh. This necessitates transfer of technology from Dhaka to Beira, for microbiological monitoring of cholera epidemic, and comparison with studies in Bangladesh. The collaboration requires capacity building in the Mozambique laboratories to streamline some existing procedures and to introduce new ways of monitoring *V. cholerae* from the environment and from the people who become cholera patients. The specific techniques for the microbiological analysis will include the following: culturing of diarrhoeagenic pathogens, monitoring of standard water quality indicators as well as physicochemical parameters of the water samples, further work on preserved samples, analysis of phytoplankton and zooplankton in selected water bodies, and the use of specific detection technologies such as fluorescent antibody technique and colony blot hybridization technique. In Bangladesh, we will carry out environmental surveillance of one pond and river in Matlab and the clinical data will be obtained from the hospital records of Matlab. This environmental and clinical surveillance will be carried out to have a comparative data of cholera endemic areas of Bangladesh and Mozambique.

The sampling at Beira, Mozambique for the analysis of cholera ecology will include the sea, river, well water, and other surface water bodies. Basic water chemistry tests at sites will include conductivity, pH, temperature, salinity, dissolved oxygen and total dissolved solids. In the laboratory we will analyze free carbondioxide, chlorides and hardness. Tests for *V. cholerae* will include culture by enrichment technique to (qualitative) assess the presence of vibrios in water, phytoplankton and zooplankton. Faecal coliforms in water will be counted by the membrane filtration technique; fluorescent microscopy will indicate the non-culturable vibrios, alive or dead. Colony blots will provide the detailed quantitative assessment of vibrios in the sample. All of these tests will be carried out every 15-day interval throughout the study. The CHAEM laboratory at Beira has been selected as the main base for developing capacity and for carrying out some of the microbiological analysis for this research. Procedures will be set up as part of the future monitoring system. In course of time, the more routine laboratory procedures will also eventually be enhanced at Quelimane and Maputo. Equipment for chemical and microbiological testing used in this research will remain with the centre at Beira after completion of the project.

Phase II: Like Bangladesh, if it is found in the first phase of the study that phytoplankton and zooplankton are also acting as reservoirs of cholera in Mozambique, then environmental cholera intervention strategy will be introduced using capulanas as a filtering device in the second phase. The intervention study will be carried out in two phases. The first phase study will be in-depth laboratory-based investigation to find out the effectiveness of capulanas to remove phytoplankton and zooplankton associated *V. cholerae* O1 and O139. In the second phase, acceptability and effectiveness of capulanas will be investigated in the community to reduce cholera incidence.

After successful completion of the first phase, which includes the identification of risk factors of cholera and its environmental intervention, attempts will be made in the second phase to focus on the identification of the risk factors for other infectious diseases in Mozambique particularly for Shigellosis.

Environmental monitoring

At 15-day intervals, various environmental samples will be collected including water, phytoplankton and zooplankton for three years. A three-year study will provide the data needed to obtain statistically valid results and conclusions. One-time sampling will be done for consecutive ten days and weekly for one month to find out whether there is any day-to-day or weekly variation in pathogen load. As we plan to collect sample every 15 days interval, 26 samples from each kind from each site will be collected in a year. Apart from being logistically feasible, this sample size will permit us to demonstrate two-log reduction in the count of microbes in each site. Two water systems (one lentic and one lotic) at Matlab will be selected for sampling. A total of 468 (26 X 2 X 3 =156 each of phytoplankton, zooplankton and water) samples will be collected. All the samples will be transported to Dhaka and will be processed with in 24 hours of collection. Culturable and VBNC *V. cholerae* O1 and O139 will be isolated, counted and detected following conventional culture, colony blot and fluorescent antibody techniques. The procedures to be followed are described below:

Sample collection and Processing

Phytoplankton

One litre water from aquatic environment will be collected in a 1 litre capacity narrow mouth Nalgene plastic bottle with 3 ml Lugol's iodine solution for the qualitative and quantitative analysis of phytoplankton. Bottles will be kept in a dark undisturbed place at room temperature for 48 to 72 hours for sedimentation of phytoplankton. Concentrated phytoplankton will carefully be collected from the bottom of the bottles in l00ml vials. If the abundance is low, the phytoplankton will be concentrated by passing through plankton net of mesh size 20μm and then will be kept in a 4-oz bottle. The concentrated phytoplankton samples will be preserved by adding 4% formalin. Then the samples will kept for identification and enumeration.

The concentrated preserved samples will be shaken gently for proper mixing from which 1 ml sub-sample will be drawn by pipette and transferred into a Sedgewick-Rafter (S-R) counting cell following the standard procedures (APHA, 1998). The samples will then be placed under a compound binocular microscope at a magnification of l0x, 40x or 100x and then observed for identification and enumeration. The phytoplankton will be identified following the procedures described by various authors (Smith, 1950; Ward & Whipple, 1959; Desikachary, 1959; Needham and Needham, 1961; Islam and Khatun, 1966, Islam and Nahar,1967; Islam *et al.*, 1992; Prescott, 1984; Khondker and Parveen, 1992; APHA, 1998).

After identification of phytoplankton in each sample, they will then be enumerated to study their abundance. A number of strips (sub-cells) of S-R cell will be randomly examined for each sample. The total numbers of phytoplankton will be estimated by the formula given below.

$$N = \frac{C \times 1000mm^3}{L \times D \times W \times S} \times F$$

Here,
N = Total number of phytoplankton per litre of original water.
C = Average number of phytoplankton observed.
L = Length of a strip (sub-cell) of S-R cell = 1 mm.
D = Depth of strip of S-R cell = 1 mm.
W = Width of strip of S-R cell = 1 mm.
S = Number of counted strips of the S-R cell.
F = Volume of sample concentrated from 1 litre original sample.

Thus the formula can be summarized as follows:

$$N = \frac{C \times 1000mm^3}{S} \times F$$

Zooplankton

One hundred liters or an appropriate volume of water (depending on concentration) will be filtered through 60μm mesh size plankton net (Millipore, Ges.m.b.H, Hietzinger Hauptstrasse 145, A-1130 Wien, Austria, Cat# NY6000010) and the concentrated samples will be collected in 4oz glass bottles. The samples will be brought to the laboratory within 24 hours after collection in an insulated icebox using cool packs to maintain the temperature at 4°C. The collected zooplankton samples will further be concentrated by the plankton net so that the ultimate volume of the concentrated sample become 50 ml. The concentrated zooplankton samples from different sites will be kept in separate scintillation glass vials and will be preserved by adding 4% formalin. Then the samples will be kept for identification and enumeration.

The preserved samples will be shaken gently for proper mixing from which Iml sub- samples will be drawn by pipette and transferred into a Sedgewick-Rafter (S-R) counting cell following standard procedure (APHA, 1998). The samples will then be placed under a compound binocular microscope at a magnification of l0X and then observed for identification and enumeration. The magnification of the compound microscope are sometimes adjusted to 4X, 40X or 100X according to the need for better observation and identification. The zooplanktons are identified by using the taxonomic keys, identifying characteristics and specific figures following Ward & Whipple (1959), APHA (1998), Needham & Needham (1961), Bhouyain & Asmat (1992) and other relevant literatures.

After identification of zooplankton in each sample, they will then be enumerated for their abundance. At least a total of 100 strips (sub-cells) of S-R cell will randomly examined for each sample and the zooplankton lying within that area will be counted for each species, genera, group and phylum wise. The total numbers of zooplankton will be estimated following the formula described above for phytoplankton.

Water

One liter water sample will be collected from each sampling site in a one liter capacity Nalgene (Nalgene company, USA) plastic bottle. This water will be collected after passing through a 20-μm mesh size Millipore plankton net to make water free from zooplankton and phytoplankton. This water will be used for both microbiological and chemical analyses.

Culture of *V. cholerae* from zooplankton, phytoplankton and water

Zooplankton and phytoplankton samples will be collected using 64 μm and 20 μm mesh sizes plankton net respectively. Then the plankton will be homogenized using Sted Fast homogenizer (Fisher Scientific Co.). A portion of the homogenized materials will be inoculated into alkaline bile peptone enrichment media and incubated overnight and then plated onto thiosulphate citrate bile salt sucrose (TCBS) agar and tellurite taurocholate gelatin agar (TTGA). The inoculated plates will be incubated at 37°C for 18 to 24 hours. Vibrio-like organisms (from colony appearance) will be picked up and streaked on gelatin agar medium (Monsur, 1961) and incubated at 37°C to determine the production of gelatinase and will be inoculated into Kliglers Iron Agar (KIA) and Motility Indole Urea (MIU) agar. Presumptive vibrio isolates will be checked for lysine and ornithine decarboxylase, arginine dihydrolase and for fermentation of mannitol, sucrose, mannose and arabinose and growth in 0.3, 8 and 10% NaCl. Serology will be done if indicated. Isolation and

identification of *V. cholerae* O1 and O139 will be carried out following the procedures described by Islam *et al.* (1990a, 1990b, 1994b).

Three hundred milliliter of water sample will be filtered through 0.20 µm Millipore filter paper and the filter paper will be enriched in alkaline bile peptone in 120 ml glass bottle and will be incubated overnight at 37°C. Then the rest of the procedures will be followed as described above.

Direct Fluorescent Microscopy (DFA)

All the samples will be tested for both *V. cholerae* O1 and O139 by fluorescence microscope following the procedures described by Hasan *et al.* (1994, 1995). The procedures are briefly described below.

A thin smear of 5-µl of resuspended sample will be prepared on a well. Controls will be run simultaneously. 5-µl of absolute ethanol will be added in control or sample well to fix the smear, again will be air dried. 10-µl of reconstituted DFA reagent will be added to each well. The slides will be placed in a covered, moist

chamber, and will be incubated at 37°C for 30 ± 5 minutes protecting from light. Then the slides will be rinsed thoroughly with PBS protecting from light. Excess moisture will be absorbed using a blotting paper. A drop of fluorescent mounting medium will be added on the slide and covered with a 22 x50mm, No. 1 cover slip. For best results, the slides will be read immediately at a magnification of 1000 X with oil immersion. The slides will, however, be kept cool, in the dark, or will be kept humid to prevent drying.

Colony blot hybridization technique

Preparation of colony blots and hybridization will be carried out according to published procedures (Wright *et al.* 1992 and Maniates *et al.* 1982). Environmental water samples will be plated directly or upon appropriate 10 fold serial dilutions on non selective LB agar plates and incubated overnight at 37°C. Colonies will be transferred to a Whatman # 541 filter paper from the culture plates. The filters will be transferred with colony side up on to # 3 Whatman filters that are pre-soaked with denaturation solution (4 ml) for 10 minutes. The filter paper will be saturated with neutralizing solution (4 ml) for 3 min then the filter will be air dried and soaked in pre hybridization buffer at 42°C for 1 hour. Then hybridization will be carried out with DIG lebelled ctx probe at 42°C for over night. Then the washing, blocking and signal detection will be carried out following the procedures provided by the manufacturers (Boehringer Mannheim, Japan).

Physicochemical parameters of water

Physicochemical measurement will be made at the time of collection at each sampling site. This will include water temperature, pH (Orion Portable pH Meter, Cat. No. 210 A, Orion Research, USA), salinity, total dissolved solids and conductivity (HACH Conductivity Meter, Cat. No. 51800-18, Sension™ 5, HACH Company, USA) and dissolved oxygen content (HACH Dissolved Oxygen Meter, Cat. No. 51850-18, Sension™ 6, HACH Company, USA). All these readings will be taken in the field using potable meters. The free CO_2, hardness and chloride will be measured in the laboratory following standard procedures (APHA, 1998). The number of hours of sunshine, total rainfall and the ambient temperature will be obtained from the Metrological Department of Bangladesh in Dhaka.

Clinical surveillance

Data on the incidence of cholera in Matlab, Bangladesh will be collected from the Matlab Hospital records of ICDDR,B.

Activities in Mozambique:

Like Bangladesh, environmental samples will be collected from both lotic (open water, e.g., river, canal, sea etc.) and lentic (closed water, e.g., pond, lake, wells etc.) environment of Beira, Mozambique. Phytoplankton, zooplankton and water samples will be collected every two weeks interval. The samples will be transported to the CHAEM laboratory following standard procedures and processed following the same procedures as described in Bangladesh part. The isolation and identification of *V. cholerae* O1 and O139 will be carried out following enrichment technique as described in material and methods section in Bangladesh part. The VBNC *V. cholerae* O1 and O139 will also be detected using direct fluorescent antibody technique. All these methodologies will be set up in CHAEM laboratory by ICDDR,B as a part of technology transfer. Samples will be processed and preserved for detection of *V. cholerae* O1 and O139 by colony blot hybridization from the environmental samples. Preparation of colony blots will also be carried out in CHAEM and the dried blots will be sent to ICDDR,B for hybridization using DIG labelled probe.

The stool sample from the patients having watery diarrhoea from Infectious Diseases Hospital in Beira will be tested by dark field microscope as well as by using culture technique at CHAEM. The information about the patient will be taken from the record of Infectious Diseases Hospital.

The phytoplankton, zooplankton and chemical analysis will be carried out at CHAEM in Beira following the methods described in Bangladesh part.

Facilities Available

Describe the availability of physical facilities at the place where the study will be carried out. For clinical and laboratory-based studies, indicate the provision of hospital and other types of patient's care facilities and adequate laboratory support. Point out the laboratory facilities and major equipment that will be required for the study. For field studies, describe the field area including its size, population, and means of communications. **(TYPE WITHIN THE PROVIDED SPACE).**

The sampling sites will be selected at Matlab, a field study area of ICDDR, B located 70 km southeast of Dhaka in a low lying deltaic region. All the cholera patients from the sampling sites will be reported to the hospital for treatment. We will be able to know the time of cholera epidemic from the hospital records.

All the environmental samples will be processed in the Environmental Microbiology Laboratory in Dhaka. All the techniques required for testing the environmental samples are already in place in this laboratory with adequate manpower and expertise. All the equipments are also available in this laboratory.

Data Analysis

Describe plans for data analysis. Indicate whether data will be analyzed by the investigators themselves or by other professionals. Specify what statistical software packages will be used and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to monitor further progress of the study. (TYPE WITHIN THE PROVIDED SPACE).

The isolation and enumeration of *V. cholerae* O1 and O139 from various environmental samples by culture, FA, and colony blot hybridization will be related with the physicochemical parameters of water and the seasonality of cholera. The data from Bangladesh will be compared with the data from Mozambique to find out the role of environmental factors in the seasonality and endemicity of cholera in these two countries.

Logistic regression analysis will be done to do the qualitative analysis (i.e., the presence and absence of *V. cholerae* O1 and O139 using culture technique). For quantitative analysis, Poisson regression analysis will be used.

The biweekly data from both the lotic and lentic environment will be analyzed by first computing the percentage of samples positive by FA, conventional culture and colony blot hybridization by site combination using McNemar test (Sokal and Rohlf, 1989).

A two-way analysis of variance will be performed with site and month as the effects in the model and model assumptions will be determined by examining residuals for normality following the procedures described by Sokal and Rohlf (1989).

The correlation of association between phytoplankton and zooplankton with *V. cholerae* will be analyzed using Pearson's correlation method.

Ethical Assurance for Protection of Human Rights

Describe in the space provided the justifications for conducting this research in human subjects. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how subject's rights are protected and if there is any benefit or risk to each subject of the study.

No human subject is involved in this environmental study.

Use of Animals

Describe in the space provided the type and species of animal that will be used in the study. Justify with reasons the use of particular animal species in the experiment and the compliance of the animal ethical guidelines for conducting the proposed procedures.

No animal experiments will be carried out in this study.

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however exercise judgment in assessing the "standard" length.

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Dissemination and Use of Findings

Describe explicitly the plans for disseminating the accomplished results. Describe what type of publication is anticipated: working papers, internal (institutional) publication, international publications, international conferences and agencies, workshops etc. Mention if the project is linked to the Government of Bangladesh through a training programme.

Outputs

Core research findings

Research findings will contribute to control of the local and global cholera burden through;

- A comparison of cholera ecology in Bangladesh and Mozambique and identification of environmental variables for prediction of cholera epidemic. First results will be released by end of project year one.
- Identification of the indicators suitable for assessment of cholera epidemic and early warning. Definitive results by end of project year three.

Capacity building:

Capacity to prevent and control cholera will be improved through;

- The transfer of appropriate technology between Bangladesh and Mozambique for the detection of cholera in endemic areas.
- Training of permanent staff in the detection of cholera in the environment and in the patients using appropriate techniques and equipments.

Publications:

Publishable output from this project will include;

- A manual for the collection, assessment, review, monitoring and evaluation of epidemics to be completed within no longer than four months from the end of project year three.
- Comparative cholera research papers in international journals will be published. A minimum of one leading paper published in an international journal of repute for each project year.
- Full series of project reports. Report to donors within one month of the end of each project year.

Collaborative Arrangements

Describe briefly if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization. (DO NOT EXCEED ONE PAGE)

In addition to electronic networking between project personnel, there will be additional mechanisms to share data and coordinate research efforts. Data which will be generated in ICDDR,B will be the property of ICDDR,B and the data which will be generated in Mozambique will be the property of Ministry of Health, Mozambique. The data from both ends will be combined for the publication in which the names of all the investigators will be included.

Of particular note, scientists from ICDDR,B will visit twice every year to Mozambique. Similarly Scientists from Mozambique will also visit ICDDR,B. Mr. Marcelino will be formally trained in the technologies used in ICDDR,B Environmental Microbiology Laboratory. He will use the data for his Ph.D. thesis. He will be enrolled as a doctoral student in the University of Northumbria, under the direct supervision of Dr. Andrew Collins. Dr. Andrew Collins will make annual trips to the ICDDR,B to coordinate the studies. An annual meeting of all the investigators will be organized in Mozambique. This meeting will be held in Maputo and will be coincided with the annual trip of Dr. S. Islam to Mozambique.

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

1 Name : Dr. Md. Sirajul Islam

2 Present position : Environmental Microbiologist

3 Educational background (last degree and diploma & training relevant to the present research proposal) : Ph.D. in Environmental Diseases Transmission from the London School of Hygiene and Tropical Medicine, UK

List of ongoing research protocols
(start and end dates; and percentage of time)

4.1. As Principal Investigator

Protocol Number	Starting date	End date	Percentage of time
2001-018	September, 2001	August, 2004	25%

4.2. As Co-Principal Investigator

Protocol Number	Starting date	End date	Percentage of time

4.3. As Co-Investigator

Protocol Number	Starting date	Ending date	Percentage of time
2002-017	August, 2002	May, 2003	5

5 Publications

Types of publications	Numbers
a) Original scientific papers in peer-review journals	67
b) Peer reviewed articles and book chapters	5
c) Papers in conference proceedings	18
d) Letters, editorials, annotations, and abstracts in peer-reviewed journals	61
e) Working papers	0
f) Monographs	0

6 Five recent publications including publications relevant to the present research protocol

1. **Islam MS**, Siddika A, Khan MNH, Golder MM, Sadique MA, Kabir ANMH, Huq A,and Colwell RR. (2001). Microbiological analysis of tube-well water in a rural area of Bangladesh. Applied and Environmental Microbiology. Vol.67 (7), pp.3328-3330.

2. **Islam MS,** Hossain MA, Khan SI, Khan MNH, Sack RB, Albert MJ, Huq AA, and Colwell RR. (2001). Survival of Shigella dysentery type 1 on fomites. Journal of Health Population and Nutrition. 19: 264-72

3. Zo Young-Gun, Rivera ING, **Islam MS**, Siddique AK, Yunus M, Sack RB, Huq A, Colwell RR. (2002). Genomic Profiles of Clinical and Environmental Isolates of *Vibrio cholerae* O1 in Cholera-Endemic Areas of Bangladesh. The Proceedings of the National Academy of Sciences USA (PNAS); (In Press)

4. **Islam MS**, Golder M, Morshed GM, Khan MNH, Islam MR and Sack RB. (2002). Involvement of the *hap* gene (mucinase) in the survival of *Vibrio cholerae* O1 in association with the blue-green alga, *Anabaena* sp. Canadian Journal of Microbiology; (In press)

5. **Islam MS**, Begum A, Khan SI, Sadique MA, Khan MNH, Albert MJ, Yunus M Huq A, and Colwell RR. (2000). Microbiology of pond ecosystems in rural Bangladesh: Its public health implications. International Journal of Environmental Studies. 58: 33-47.

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

1 Name : Dr Andrew Collins

2 Present position : Leader, Disaster Management and Sustainable Development Programme, Northumbria University, Project Leader, Infectious Disease Risk Management in Mozambique and Bangladesh, Senior Lecturer in Sustainable Development:

3 Educational background :

1996 PhD (London) Environment, Health and Population Displacement in Mozambique: the case of cholera and bacillary dysentery.

1992 BSc (Hons) First Class Geography.

4. List of ongoing research protocols
(start and end dates; and percentage of time)

4.1. As Principal Investigator

Protocol Number	Starting date	End date	Percentage of time
Development of GIS capacity in surveillance to improve malaria prevention and control in Mozambique	October 1998	October 1999	None from end of September 2002
Participatory Monitoring and Evaluation of food and livelihood security in Zambezia	June 1998	September 2002	None from end of September 2002
Hazard and sustainable development awareness via radio broadcast in Zambezia	May 2001	September 2002	None from end of September 2002

4.2. As Co-Principal Investigator

Protocol Number	Starting date	End date	Percentage of time
Infectious disease risk management in Mozambique and Bangladesh	October 2002	October 2005	0.25%

4.3. As Co-Investigator

Protocol Number	Starting date	Ending date	Percentage of time

5 Publications

Types of publications	Numbers
a) Original scientific papers in peer-review journals	5
b) Peer reviewed articles and book chapters	1
c) Papers in conference proceedings	6
c) Letters, editorials, annotations, and abstracts in peer-reviewed journals	1
d) Working papers	12
b) Monographs	1

6 Five recent publications including publications relevant to the present research protocol

1) **COLLINS, A.E**. (2002) 'Health ecology and environmental management in Mozambique' – paper commissioned for special edition of *Health and Place*, Elsevier Science.
2) **COLLINS, A.E**. (2000) Land degradation, health ecology and development. *Land Degradation and Development*. 12, pp.
3) **COLLINS, A.E** (1998) *Environment, Health and Population Displacement: Development and Change in Mozambique's Diarrhoeal Disease Ecology*, Making of Modern Africa Series, Ashgate, Aldershot. (332 page book with 57 figures and 36 tables).
4) **COLLINS, A.E** (1996) The geography of cholera. In Drasar, B.S and Forrest, B.D (eds.) *Cholera and the ecology of Vibrio cholerae*. Chapman and Hall, London 255-94.
5) **COLLINS, A.E** (2002 forthcoming accepted) Cholera in coastal populations. *Social Science and Medicine*, Elsevier Science.

Other less recent paper:

COLLINS, A.E. (1993) Environmental influences on the distribution of incidence of cholera: a case study in Quelimane, Mozambique. *Disasters* 17:4 321-40.

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

1. Name: **Robert F. Breiman, M.D.**

2. Present position: Associate Director, ICDDR,B and Head, Health Systems and Infectious Diseases Division (HSID)

3. Educational background :
(last degree and diploma & training relevant to the present research proposal)

Institution and Location	Degree	Year	Field of Study
University of Arizona	*B.A.*	*1975*	*Political Science*
University of Arizona	*M.D.*	*1979*	*Medicine*
UCLA Affiliated Hospitals	**Residency**	**1979-1982**	**Internal Medicine**
UCLA Affiliated Hospitals	**Chief Resident**	**1982-1983**	**Internal Medicine**
UCLA	**Fellowship**	**1984-1987**	**Infectious Diseases**
CDC	**Fellowship**	**1987-1989**	**EIS Program**

4. List of ongoing research protocols
(start and end dates; and percentage of time)

4.1 As Principal Investigator

Protocol Number	Starting date	End date	Percentage of time
2000-023	10/00	12/02	15%
2002-012	9/02	5/03	20%

4.2. As Co-Principal Investigator

Protocol Number	Starting date	End date	Percentage of time
2001-021	1/02	9/04	5%
2001-029	1/01	6/03	5%

4.3. As Co-Investigator

Protocol Number	Starting date	Ending date	Percentage of time
2001-14	5/02	10/02	10%
2002-10	4/02	7/02	2%
2002-28	10/01	9/03	10%

4.4 Publications

Types of publications	Numbers
a) Original scientific papers in peer-review journals	90
b) Peer reviewed articles and book chapters	9
c) Papers in conference proceedings	>50
c) Letters, editorials, annotations, and abstracts in peer-reviewed journals	10
Working papers	
Monographs/books	6

5. Five recent publications including publications relevant to the present research protocol

1) Feikin DR, Elie CM, Goetz MB, Lennox JL, Carlone GM, Romero- Steiner S, Holder PF, O'Brien WA, Whitney CG, Butler JC, **Breiman RF**. Randomized trial of the quantitative and functional antibody responses to a 7-valent pneumococcal conjugate vaccine and/or 23-valent polysaccharide vaccine among HIV-infected adults. *Vaccine*. 2001;20:545-53.

2) **Breiman RF**, Keller DW, Phelan MA, Sniadack DH, Stephens DS, Rimland D, Farley MM, Schuchat A, Reingold AL. Evaluation of effectiveness of the 23-valent pneumococcal capsular polysaccharide vaccine for HIV-infected patients. *Arch Intern Med*. 2000;160:2633-8.

3) Lieu TA, Ray GT, Black SB, Butler JC, Klein JO, **Breiman RF**, Miller MA, Shinefield HR. Projected cost-effectiveness of pneumococcal conjugate vaccination of healthy infants and young children. *JAMA* 2000;283(11):1460-8.

4) Metlay JP, Hofmann J, Cetron MS, Fine MJ;,Farley MM, Whitney C, **Breiman RF**. Impact of penicillin susceptibility on medical outcomes for adult patients with bacteremic pneumococcal pneumonia. *Clin Infect Dis* 2000;30:520-528.

5) Nuorti JP, Butler JC, Farley MM, Harrison LH, McGeer A, Kolczak MS, **Breiman RF**. Cigarette smoking and invasive pneumococcal disease. *N Engl J Med* 2000;342:681-689.

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

1 Name : **G. BALAKRISH NAIR**

2 Present position : Associate Director

3 Educational background :

(last degree and diploma & training relevant to the present research proposal)

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Loyola College, Madras University, India	B.Sc.	1973-75	Zoology, Chemistry and Botany.
Centre of Advanced Study in Marine Biology, Annamalai University, India	M.Sc.	1975-77	**Marine Biology.**
Centre of Advanced Study in Marine Biology, Annamalai University, India	Ph.D.	1978-82	Marine Microbiology

4. List of ongoing research protocols
(start and end dates; and percentage of time)

4.1. As Principal Investigator

Protocol Number	Starting date	End date	Percentage of time
2000-20	01/08/2000	31/07/2002	10%
Training protocol (Nepal AMR Project	01/12/2000	Continued	10%

4.2. As Co-Principal Investigator

Protocol Number	Starting date	End date	Percentage of time
2002-002	01/01/2002	31/12/2004	5%

4.3. As Co-Investigator

Protocol Number	Starting date	Ending date	Percentage of time
2000-019	01/08/2002	31/08/2005	5%

4.4. Publications

Types of publications	Numbers
a) Original scientific papers in peer-review journals	240
b) Peer reviewed articles and book chapters	47
c) Papers in conference proceedings	>100
c) Letters, editorials, annotations, and abstracts in peer-reviewed journals	
d) Working papers	
d) Monographs	

5. Five recent publications including publications relevant to the present research protocol

1) Sarkar, A., R. K. Nandy, **G.B. Nair** and A.C. Ghose. 2002. Vibrio pathogenicity island and cholera toxin genetic element-associated virulence genes and their expression in Non O1 and Non O139 strains of *Vibrio cholerae*. **Infect. Immun.** 70. 4735-4742

2) Faruque, S.M., Asadulghani, M. Kamruzzaman, R.K. Nandi, A.N. Ghosh, **G.B. Nair**, J.J. Mekalanos and D.A. Sack. 2002. RS1 element of *Vibrio cholerae* can propagate horizontally as a filamentous pahge exploiting the morphogenesis genes of CTXϕ. **Infect. Immun.** 70: 163-170.

3) Khan, A., S. Yamasaki, T. Ramamurthy, A. Pal, S. Datta, N. Roy Chowdhury, S.C. Das, A. Sikdar, T. Tsukamoto, S.K.Bhattacharya, Y. Takeda and **G. B. Nair**. 2002. Prevalence and genetic profiling of virulence determinants of non-O157 Shiga toxin-producing *Escherichia coli* isolated from cattle, beef and from human cases in Calcutta, India. **Emerg. Infect. Dis.** 8: 54-62.

4) Bhuiyan, N.A., M. Ansaruzzaman, M. Kamruzzaman, Khorshed Alam, N.R. Chowdhury, M. Nishibuchi, S. M. Faruque, D. A. Sack, Y. Takeda and **G. B. Nair**. 2002. Prevalence of the pandemic genotype of *Vibrio parahaemolyticus* in Dhaka, Bangladesh, and significance of its distribution across different serotypes. **J. Clin. Microbiol.** 40: 284-286.

5) Chakraborty, S., P. Garg, T. Ramamurthy, J.K. Gautam, C. Kumar, S. Maiti, S. Yamasaki, T. Shimada, Y. Takeda, A. Ghosh and **G.B. Nair**. 2001. Comparison of antibiogram, virulence genes, ribotypes and DNA fingerprints of *Vibrio cholerae* of matching serogroups isolated from hospitalized diarrhoea cases and from the environment during 1997-1998 in Calcutta, India. **J. Med. Microbiol**. 50: 879-888.

Detailed Summary Budget for New Proposal

Project Title: **Cholera Risk Management in Mozambique and Bangladesh, Phase 1**

Name of PI: **Dr. Md. Sirajul Islam**

Protocol Number: **2002- 024** Name of Division: **Laboratory Sciences Division**

Funding Source: **Northumbria University and UNICEF** Amount Funded (direct): **2,03,043**

Total: **2,45,878** Overhead (%): **42835 (25% Northumbria University fund)**

Starting Date: **10.01.2002** Closing Date: **09.31.2005**

Strategic Plan Priority Code(s):

Sl. No	Account Description	Salary Support			US $ Amount Requested		
	Personnel	Position	Effort %	Salary	1st Yr	2nd Yr	3rd Yr
1	Dr. Md. Sirajul Islam	Environmental Microbiologist	25%	8905	26715	28051	29453
2	Research Officer (3)		300%	353	12708	13343	14011
3	Sr. Data Management Officer		10%	459	551	579	607
	Sub Total				39974	41973	44071
3	Local Travel in Mozambique (UNICEF fund)				2500	2700	2500
4	Local Travel in ICDDR,B (~~DFID fund~~) (Northumbria University)				2500	2500	2500
5	International Travel				8000	8000	8000
	Sub Total				13000	13700	13000
	Supplies and Materials (Description of Items)						
5	Media, Petridishes, Glasswares, Disposables etc.				8391	6120	4048
6	Chemicals, reagents, antisera etc.				6908	4948	3110
	Sub Totals				15299	11068	7158

	Other Contractual Services			
	Repair and Maintenance			
	Rent, Communications, Utilities	**500**	**300**	**200**
	Training Workshop, Seminars			
	Printing and Publication			
	Staff Development			
	Sub Total	**500**	**300**	**200**

	Interdepartmental Services	**1st Yr**	**2nd Yr**	**3rd Yr**
	Computer Charges			
	Pathological Tests	**400**	**400**	**400**
	Microbiological tests	**500**	**500**	**500**
	Biochemistry Tests			
	X-Rays			
	Patients Study			
	Research Animals			
	Biochemistry and Nutrition			
	Transport	**200**	**200**	**200**
	Xerox, Mimeographs etc.			
	Sub Total	**1100**	**1100**	**1100**
	Other Operating Costs			
	Capital Expenditure			
TOTAL DIRECT COST		**69873**	**67641**	**65529**
Overhead (25%) on Northumbria University fund only		**14843**	**14235**	**13757**
Total		**84716**	**81876**	**79286**

Total	**= 2,45,878**

US $ 2,14,178 only (Funded by Northumbria University) will be administered by ICDDR,B

1/1/2003

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

Budget for Mozambique Part

	Amount in US$		
	YEAR1	YEAR2	YEAR3
Staff costs:			
Payment of local field workers	3,600	3,600	3,600
Travel:			
From Mozambique to Bangladesh and UK@1550 per return trip	3,100 (x2)	1,550	3,100
Visas @ approx 100 each	200	100	200
Accomodation and food whilst in the field @100 per day	11700 (117 days)	8,300	6,700
Local Travel: i.e., Maputo-Beira-Maputo	6,000	6,000	6,000
Consumables:			
Media, petri dishes, Glassware, disposables etc.	8,850	8,850	8,850
Chemicals, reagents, antisera	6,150	6,150	6,150
Additional cost included for food contamination tests.	3,500	3,500	0
Equipment: (DO meter, conductivity meter, pH meter, fluorescent microscope, dark field microscope, plankton nets, etc.)	25,000	5,000	3,000
GIS equipment: 2 computers, software, GPS's	7,500	0	0
GIS training:	2,000	0	0
Communications: Maputo-Beira-Dhaka-Newcastle	500	500	500
Total	**78,100**	**43,550**	**38,100**
Grand Total			**=1,59,750**

Budget Justifications

Please provide one page statement justifying the budgeted amount for each major item. Justify use of manpower, major equipment, and laboratory services.

The 25 % salary of the local PI and 3 Research Officers have been budgeted in this project for 3 years. The expenses for local travel to Matlab, has been budgeted. The expenses for doing various microbiological analyses have been included as reagents and chemical costs. Two international travels from ICDDR,B to Mozambique every year has been budgeted in this protocol.

Other Support

Describe sources, amount, duration, and grant number of all other research funding currently granted to PI or under consideration. (DO NOT EXCEED ONE PAGE FOR EACH INVESTIGATOR)

ISLAM, M. S.

ACTIVE

NSF

Genomic assessment of phenotypic plasticity in an aquatic bacterium: Water quality vs. microbial habitats	1/10/2001 – 30/09/2004 US$: 2,50,872	25%

The major goals of this grants are to examine the long-term progression of responses by unattached and attached *V. cholerae* after transition in nutrient condition, to examine genome-wide expression in a response to environmental factors by attached and unattached *V. cholerae* and to look at in situ seasonality effects on *V. cholerae* in fresh water ponds.

APPENDIX

International Centre for Diarrhoeal Disease Research, Bangladesh
Voluntary Consent Form

Title of the Research Project:

Principal Investigator:

Before recruiting into the study, the study subject must be informed about the objectives, procedures, and potential benefits and risks involved in the study. Details of all procedures must be provided including their risks, utility, duration, frequencies, and severity. All questions of the subject must be answered to his/ her satisfaction, indicating that the participation is purely voluntary. For children, consents must be obtained from their parents or legal guardians. The subject must indicate his/ her acceptance of participation by signing or thumb printing on this form.

Not applicable.

Signature of Investigator/ or agents
Date:

Signature of Subject/ Guardian
Date:

Check List

After completing the protocol, please check that the following selected items have been included.

1. **Face Sheet Included** ✓
2. **Approval of the Division Director on Face Sheet** ✓
3. **Certification and Signature of PI on Face Sheet, #9 and #10** ✓
4. **Table on Contents** ✓
5. **Project Summary** ✓
6. **Literature Cited** ✓
7. **Biography of Investigators** ✓
8. **Ethical Assurance** N/A
9. **Consent Forms** N/A
10. **Detailed Budget** ✓

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

Memorandum

10 September 2002

To : Dr. Md. Sirajul Islam
Principal Investigator of protocol # 2002-024
Laboratory Sciences Division

From: David A. Sack, MD
Chairman, Research Review Committee (RRC)

Sub : Protocol # 2002-024

Thank you for your protocol # 2002-024 entitled "Cholera Risk Management in Mozambique and Bangladesh, Phase I", which the RRC considered in its meeting held on 5th September 2002. After review and discussion, the Committee made the following observations on the protocol:

a) Specific Aim: It was not clearly stated how specific aim # 4 will be addressed in this phase of the protocol.

b) Background: The epidemiology of cholera in Africa, particularly the cholera epidemics, could have been described in a bit more detail to make it easier for the readers to compare and contrast with those of in Bangladesh (Naidoo a et al. Cholera: a continuous epidemic in Africa. J R Soc health, 2002 Jun, 122:89-94).

c) The factors enhancing transmission of *V. Cholerae* during epidemics are still not well defined, and the investigators have rightfully pointed out the importance of environmental and ecological factors. However, other factors may also be involved, and they could have been mentioned, to make a balanced view (Merrell DS et al. Host-induced epidemics spread of the cholera bacterium. Nature 2002 Jun; 417:642-5).

d) Design and Methodology: The protocol is stated to comprise Phase I. It would have been useful if the subsequent phases were also outlined – this would put the project in clearer perspective and relevance.

e) Detailed plan for analysis of data should be provided.

f) The budget does not include any provision for capital expenditure. Will there be a separate budget for the equipments to be procured for CHAEM? The investigators should clarify this. Further, the total budget with breakdown for Mozambique part and ICDDR,B part, should be provided.

g) Several references in the in the Reference List are not cited, there are discrepancies the year of publication of two references.

h) Comments of the external reviewers and the investigators' response thereto should be provided.

i) The protocol should clearly state which activities would be undertaken in Bangladesh and which in Mozambique. The investigators should also consider inclusion of O139 in the Mozambique part of the protocol.

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

You may, however, start the 'Technology Transfer' component of the protocol before the approval of the protocol.

Thank you.

Copy: Associate Director
Laboratory Sciences Division

Responses to Reviewers' Comments

Protocol # 2002-024

a) The specific aim # 4 has been addressed in page 14, para 3, 4 and 5 of the revised protocol.

b) The epidemiology of cholera in Africa, particularly the cholera epidemics in Mozambique has been described in more details in the background section (page 9 and 10) of the revised protocol.

c) The other factors enhancing transmission of *Vibrio cholerae* during epidemics have been mentioned in the background section (page 13, para 3) of the revised protocol.

d) A brief outline of Phase II has been included in the 'Research Design and Method' section of the revised protocol (page 15, para 3).

e) Detailed plan of analysis of data has been provided in the 'Data Analysis' section (page 21) of the revised protocol.

f) For the work in Bangladesh, no capital expenditure is required as all the instruments needed for this study is available in the Environmental Microbiology Laboratory of ICDDR,B. There will be separate budget for the equipments to be procured for CHAEM. The budget for Mozambique and ICDDR,B part has been provided in page 40 of the revised protocol.

g) References have been corrected in the revised protocol.

h) Comments of external reviewers' have been enclosed.

i) The overall activities in Bangladesh and Mozambique have been described in page 15, para 2 and page 16 to page 19. The specific activities in Mozambique have been described in the revised protocol (page 19). The monitoring of *V. cholerae* O139 in both Bangladesh and Mozambique has been included in the revised protocol.

[redacted]

[redacted]

Dec 4th, 2002

[redacted]
Head of Laboratory Science Division
ICDDR-B
Dhaka, Bangladesh

Dear Dr. G. B. Nair,

Thank you for giving me an honour to review a research proposal entitled "Cholera Risk Management in Mozambique and Bangladesh, Phase I". I would like to apologize for a delay in giving my opinion.

The proposal was very well written. The hypothesis to be tested is appropriate. The background and review of literature are adequate. Scientific merit of the proposal is sound. The researchers are conscientious. The methodology and time frame and budget requested are appropriate.

I give full moral support to the research.

Yours sincerely,

Wanpen Chaicumpa D.V.M., Ph.D.
Professor of Microbiology and Immunology
Faculty of Allied Health Sciences, Thammasat University Rangsit Center

CONFIDENTIAL

FAX MESSAGE FOR DR. G. BALAKRISH NAIR

TOTAL PAGES = 3 (INCLUDING THIS PAGE)

Title:

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

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

CONCLUSIONS

I support the application:

a) without qualification ☑

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

I do ~~not~~ support the application ☐

Name of Referee: DR. S. K. BHATTACHARYA

Signature: S Bhattacharya Date: 4-12-2002

Position: Director

Institution: NICED, Kolkata, India

Detailed Comments

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

(Use additional pages if necessary)

Title: Cholera Risk Management in Mozambique and Bangladesh, Phase I

PI: Dr. Md. Sirajul Islam

Reviewer:

The project is well designed and is expected to give useful information on cholera management. The investigators are well known experts in the field. I strongly support the project proposal.

G Bhattacharya

4-12-2002