

Attachment 1.  
(FACE SHEET)

Date NOVEMBER 11, 1991

ETHICAL REVIEW COMMITTEE, ICDDR,B.

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Principal Investigator Prof. K. Amako & D. Md. Sirajul Islam Trainee Investigator (if any) \_\_\_\_\_

Application No. 91-014 Supporting Agency (if Non-ICDDR,B) \_\_\_\_\_

Title of Study Investigation of erythrocyte five proteins as receptors for V. cholerae of in freshwater and marine planktons of Bangladesh. Project status:  
 New Study  
 Continuation with change  
 No change (do not fill out rest of form)

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

1. Source of Population:
    - (a) Ill subjects Yes No
    - (b) Non-ill subjects  Yes No
    - (c) Minors or persons under guardianship Yes No
  2. Does the study involve:
    - (a) Physical risks to the subjects Yes No
    - (b) Social Risks Yes No
    - (c) Psychological risks to subjects Yes No
    - (d) Discomfort to subjects Yes No
    - (e) Invasion of privacy Yes No
    - (f) Disclosure of information damaging to subject or others Yes No
  3. Does the study involve:
    - (a) Use of records, (hospital, medical, death, birth or other) Yes  No
    - (b) Use of fetal tissue or abortus Yes  No
    - (c) Use of organs or body fluids Yes  No
  4. Are subjects clearly informed about:
    - (a) Nature and purposes of study Yes No
    - (b) Procedures to be followed including alternatives used Yes No
    - (c) Physical risks Yes No
    - (d) Sensitive questions Yes No
    - (e) Benefits to be derived Yes No
    - (f) Right to refuse to participate or to withdraw from study Yes No
    - (g) Confidential handling of data Yes No
    - (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure Yes No
  5. Will signed consent form be required:
    - (a) From subjects Yes No
    - (b) From parent or guardian (if subjects are minors) Yes No
  6. Will precautions be taken to protect anonymity of subjects Yes No
  7. Check documents being submitted herewith to Committee:
    - \_\_\_ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies). Protocol (Required)
    - \_\_\_ Abstract Summary (Required)
    - \_\_\_ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
    - \_\_\_ Informed consent form for subjects
    - \_\_\_ Informed consent form for parent or guardian
    - \_\_\_ Procedure for maintaining confidentiality
    - \_\_\_ Questionnaire or interview schedule \*
- \* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
  2. Examples of the type of specific questions to be asked in the sensitive areas.
  3. An indication as to when the questionnaire will be presented to the Cttee. for review.

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

M. S. Islam  
Principal Investigator

\_\_\_\_\_  
Trainee

91-014  
11/11/91

SECTION I - RESEARCH PROTOCOL

1. Title : Investigation of cryoprotective proteins as receptors for *Vibrio cholerae* 01 in freshwater and marine planktons of Bangladesh.
2. Principal Investigators : Professor Kazunobu Amako  
: Dr. Md. Sirajul Islam  
Co-Investigator : Dr. M. John Albert  
Advisor : Professor R. Bradley Sack.
3. Starting date : December 8, 1991
4. Completion date : December 24, 1991
5. Total Director Cost : US\$445.00
6. Scientific Program : Community Health Division

Abstract Summary:

A study will be carried out to investigate the presence of cryoprotective protein (CPP) in the surfaces of fresh & marine water planktons of Bangladesh which can act as a receptor for *Vibrio cholerae* and can protect vibrios from death at low temperature.

8. i. Ethical Review Committee : \_\_\_\_\_
- ii. Research Review Committee : \_\_\_\_\_
- iii. Director : \_\_\_\_\_

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objectives

To find out the presence of cryoprotective protein (CPP) on the surfaces of Fresh and marine planktons of Bangladesh.

## 2. Background

Cholera is a water and food borne diarrhoea infection. In Japan *Vibrio cholerae* are generally isolated from the imported seafoods e.g. lobsters, prawns, shrimps, etc. (Fukumi, 1979). It is known that *Vibrio cholerae* are sensitive to low temperatures (Miller *et al.*, 1984; Nalin *et al.*, 1979). It has been reported that chitin and amino acids are cryoprotective for *Vibrio cholerae* at refrigerator temperatures (Amako *et al.*, 1987). However, these substances are completely ineffective at temperatures below 0°C. Since most seafoods are imported in a frozen condition, it needs to be determined why *Vibrio cholerae* are isolated so frequently from such frozen foods.

Substances cryoprotective for *Vibrio cholerae* were detected from prawn shells. It has been found that *Vibrio cholerae* can survive longer in association with zooplankton and phytoplankton than the surrounding water (Huq *et al.* 1983; Islam *et al.*, 1989; 1990). It is not known whether these planktons have cryoprotective protein (CPP) on their surfaces which provide advantages for longer survival of *Vibrio cholerae*. Therefore, studies will be carried out to investigate the presence of CPP on surfaces of planktons collected from both marine and freshwater environments of Bangladesh.

## 3. Rationale:

The presence of CPP on the surfaces of planktons will provide a clue about the longer survival of *Vibrio cholerae* in association with the aquatic organisms at lower temperatures.

B. SPECIFIC AIMS:

To search for cryoprotective proteins (CPP) as receptors for *Vibrio cholerae* on the surfaces of planktons collected from river and sea water of Bangladesh.

C. METHODS AND PROCEDURES

Field sites: Freshwater planktons will be collected from Buriganga river in Dhaka and sea water in Teknaf, Bangladesh.

Planktons will be sampled by putting a plankton net (mesh size 0.77  $\mu$ m) through the water. Plankton will be collected in a 4 oz wide-neck bottles. The plankton samples will be fixed at 3% formaldehyde. Then the plankton will be treated with anti CPP serum and then with anti IgG fluorescence labeled antibody and will be examined finally under fluorescent microscope to detect the presence of CPP in the surfaces of the planktons and the results will be recorded in photomicrographs. A total of 100 planktons samples (50 marine + 50 freshwater planktons) will be examined.

D. COLLABORATION ARRANGEMENT:

The principal investigator will come to Dhaka for about two weeks to perform the study and Dr. Md. Sirajul Islam, Dr. M. John Albert and Prof. Bradley Sack will be involved in this study. Technical help may be requested and the cost will be reimbursed. The findings of this study will be published with Co-authorship from ICDDR,B. This project will reimburse ICDDR,B all the expenses in the laboratory including technician's time. Professor K. Amako will bring anti-CPP serum, fluorescence labeled antibody and glass slides with him.

## Literature Cited

- Amaku, K., S. Shimodori, T. Imoto, S. Miake, and A. Umeda. 1987. Effects of chitin and its soluble derivatives on survival of *Vibrio cholerae* 01 at low temperature. *Appl. Environ. Microbiol.* 53:603-605.
- Fukumi, H. 1979. Epidemiological aspects on cholera outbreak in Japan originating from wedding dinner parties in Ikenohata Bunka Center, Tokyo, in 1978. In Proceedings of the 15th U.S.-Japan Cooperative Medical Science Program Symposium on Cholera. National Institutes of Health publication no. 80-2003. U.S. Department of Health, Education and Welfare, Bethesda, Md. p.107-120.
- Huq, A., P.A. West, E.B. Small, M.I. Huq, and R.R. Colwell. 1984. Influence of water temperature, salinity, and  $p^H$  on survival and growth of toxigenic *Vibrio cholerae* serovar 01 associated with live copepods in laboratory microcosms. *Appl. Environ. Microbiol.* 48:420-424.
- Islam, M.S., Drasar, B.S. and Bradley, D.J. (1989). Attachment of toxigenic *Vibrio cholerae* 01 to various freshwater plants and survival with a filamentous green alga, *Rhizoclonium fontanum*. *J. Trop. Med. & Hyg.*, 92: 396-401.
- Islam, M.S., Drasar, B.S. and Bradley, D.J. (1990). Long term persistence of toxigenic *Vibrio cholerae* 01 in the mucilaginous sheath of a blue green alga, *Anabaena variabilis*. *J. Trop. Med. & Hyg.* 93: 133-139.
- Miller, C.J., B.S. Drasar, and R.G. Feachem. 1984. Response of toxigenic *Vibrio cholerae* 01 to physiochemical stress in aquatic environments. *J. Hyg.* 93:475-495.
- Singleton, F.L., R.W. Attwell, M.S. Jangi, and R.R. Colwell. 1982. Effects of temperature and salinity on *Vibrio cholerae* growth. *Appl. Environ. Microbiol.* 44:1047-1058.

Budget

Salary

Technician, ICDDR,B	100%	\$	95.00
(for two weeks)			

Supplies

Plankton nets, PBS, Normal saline, Tissue paper, etc.		\$	100.00
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Travel

Dhaka-Teknaf-Dhaka(2 persons)		\$	150.00
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Miscellaneous

Transport, Telephone, Fax, etc.		\$	100.00
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Total			----- US\$ 445.00
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