

ETHICAL REVIEW COMMITTEE, ICDDR, B.

28

Principal Investigator Prof. S.C. Sanyal Trainee Investigator (if any) _____
 Application No. 81-045 Supporting Agency (if Non-ICDDR,B) _____
 Title of Study Studies on the clinical Project status:
manifestations and toxicities by Aeromonas New Study
hydrophila strains isolated from cases of () Continuation with change
diarrhoea and their serological responses. () 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 *NA*
 - (c) Physical risks Yes No *NA*
 - (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 *NA*
 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:
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.

Principal Investigator

Trainee

81-0415

SECTION I - RESEARCH PROTOCOL

Recd 27-10-81

1. Title : Studies on the clinical manifestations and toxicities by Aeromonas hydrophila strains isolated from cases of diarrhoea and their serological responses.
2. Principal Investigator : Prof. Suhas C. Sanyal
Co-Investigators : Dr. P.K. Bardhan
Dr. M.I. Huq
Dr. B. Stoll
Dr. A.S.M.H. Rahman
Dr. Thelma Leifert
3. Starting Date : November 1, 1981
4. Completion Date : October 31, 1982
5. Total Direct Cost : US \$ 32,785.00; (out of which US \$ 21,735.00 is required for personnel services only)

6. Scientific Program Head :

This protocol has been approved by the Disease Transmission
Working Group

Signature of the Scientific Program Head :

Date :

[Signature]
23/10/81

7. Abstract Summary :

Strains of Aeromonas hydrophila are increasingly being isolated from cases of diarrhoea almost all over the world. It has been reported that these organisms may be responsible for nearly 5 percent of the diarrhoea cases in a community. A large number of A. hydrophila strains are also isolated at ICDDR,B from diarrhoeal patients. They have been reported from various animals and the environment as well. Most of these organisms isolated from various sources were found to be enterotoxic and cytotoxic. The enterotoxin was found to be a heat and pH labile protein that stimulates the production of cAMP

in the intestinal epithelial cells of experimental animals. There is supportive evidence that the cytotoxins may be haemolysins. However, very little is known about the clinical features of the cases although the available literature indicates that they may cause mild to severe choleraic diarrhoea in man. Except their isolation in pure form on primary culture and demonstration of enterotoxin in-vitro, very little is known about its role in the aetiology of diarrhoea. Knowledge regarding the epidemiology of the organism is also meagre. The proposed study aims at determining the clinical features of the disease produced by them and delineate some of the epidemiologic parameters which will be of great help in formulating proper intervention and prevention measures. The study also envisages to see if the strains isolated from acute diarrhoeal cases in Bangladesh are all enterotoxic and/or cytotoxic, and if so, to compare them with isolates from healthy individuals and if possible, animal and environmental isolates of this country to determine their relative toxicities. Through serological studies with acute convalescent sera against homologous and heterologous strains an attempt will be made to establish their exact role in the aetiology of diarrhoea. The study may also explore the possibility of any correlation between biochemical characteristics and enterotoxicity and detection of tox gene by DNA which may enable us to avoid cumbersome, time consuming and expensive animal experimentation

8. Reviews :
- a. Research Involving Human Subjects : _____
 - b. Research Review Committee ; _____
 - c. Director ; _____
 - d. BMRC ; _____
 - e. Controller/Administrator ; _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective :

- a. To study the clinical features of the cases of diarrhoea wherefrom strains of A. hydrophila are isolated as the only suspected aetiological agent.
- b. To examine the enterotoxigenicity of A. hydrophila strains isolated from cases of diarrhoea, healthy individuals, water sources and sweage in Bangladesh.
- c. To study the cytotoxicity of the strains isolated from different sources as noted in (b).
- d. To study the serological responses like neutralisation of enterotoxic and cytotoxic activities of the A. hydrophila strains isolated from cases of diarrhoea caused by these organisms with antisera collected from the convalescent patients.
- e. To examine the possibility of any correlation between the biochemical characteristics and enterotoxigenicity.

2. Background : Strains of A. hydrophila have been isolated from cases of diarrhoea, healthy individuals, animals, water sources and sewage and have been implicated as causative agent of diarrhoea in children in various countries (von Graevenitz

and Mensch, 1968; Zajc-Satler et al, 1972; Chatterjee and Neogy 1972; Bhat et al, 1974; Sanyal et al, 1972, 1975 a,b, 1977; Wadström et al, 1976; Cumberbatch et al, 1979; Trust and Chipman 1979, Bhatia et al, 1980; Daily et al, 1981]. These organisms have been found to produce an enterotoxin and cytotoxins irrespective of their sources of isolations (Annapurna and Sanyal, 1975; 1977; Wadström et al, 1976; Dubey and Sanyal, 1978, 1979). The enterotoxin has been purified to electrophoretic homogeneity. (Dubey et al, 1980) and cytotoxins were found to be haemolysins which could be separated from the enterotoxin (Ljungh et al, 1978; Dubey et al, 1981 b). It has been observed in experimental models that A. hydrophila enterotoxin may act through mediation of cAMP (Sanyal et al, 1978; Dubey et al, 1981a). Attempts have been made to correlate biochemical characters with enterotoxicity (Ljungh et al, 1977; Burke et al, 1981), but the studies had certain lacunae and the results are equivocal.

However, no work has yet been done to prove the diarrhoeagenic capability of A. hydrophila strains except their isolation in pure form on primary culture from culture from cases of diarrhoea and demonstration of enterotoxin production by them in vitro. Although they have been reported to be isolated from mild to severe cases of diarrhoea (von Graevenitz and Mensch, 1968; Chatterjee and Neogy 1972; Sanyal et al, 1975; Cumberbatch et al, 1979; Trust et al, 1979), very little is known about the clinical features. Except

one report wherein the incidence rate was shown to be 5 per cent of all the cases of diarrhoea in a community (Sanyal et al, 1977) and its isolation from different sources as noted earlier, no data is available regarding its epidemiological aspects.

3. Rationale : A. hydrophila has been implicated as a causative agent of diarrhoea in many countries. A large number of strains are isolated at ICDDR,B from diarrhoeal patients where no other enteropathogen is detected. However, very little is known about the clinical features of the disease caused by this organism and no systematic study has yet been done regarding the enterotoxicity and cytotoxicity of the strains isolated at ICDDR,B. Further, except its isolation in pure form on primary culture of diarrhoeal stools and demonstration of enterotoxin and cytotoxin in the isolates, nothing is known about its diarrhoeagenic capability. The proposed study will allow us to delineate the clinical features of diarrhoea caused by this organism which will be of immense value in formulating appropriate intervention measures. The study will also provide informations regarding enterotoxicity and cytotoxicity of the organisms isolated from various sources. These data are essential for proper understanding of the pathogenesis of the disease and planning of intervention and prevention measures. This study also envisages to establish conclusively through serological studies, the role played by this organism in the aetiology of diarrhoea. Moreover, studies of biochemical characters vis-a-vis enterotoxicity

may lead to the exploration of some correlation between enterotoxin production and certain biochemical characters which will be of great value in the laboratory for easy detection of enterotoxigenic strains. Thus the proposed study will allow us to determine the overall significance of frequent isolations of A. hydrophila strains from diarrhoeal patients in Bangladesh.

B. SPECIFIC AIMS

1. To determine the clinical features of cases of diarrhoea caused by A. hydrophila.
2. To determine the enterotoxicity of A. hydrophila strains isolated from diarrhoeal patients and other sources in Bangladesh in experimental models as well as by P32 DNA probe technique.
3. To determine the cytotoxicities of A. hydrophila strains isolated from different sources in Bangladesh.
4. To see anti-enterotoxin response by neutralisation test in ileal loops or skin PF with convalescent patients' sera.
5. To see anti-cytotoxin response by neutralisation test in CHO or Y1 cell culture with convalescent patients' sera.
6. To examine cross reactivity on neutralisation of A. hydrophila enterotoxin by antibodies against cholera and E. coli enterotoxins.
7. To find out simple biochemical tests to detect toxigenic strains.

C. METHODS

1. Epidemiology and Clinical Features :

Dacca hospital surveillance patients, a 4% systematic sampling of all patients coming to ICDDR,B will be studied. To enrich the sample we will also study the first five patients attended to the inpatient treatment centre each day from 9:00 a.m. to 5:00 p.m. Only those patients excreting A. hydrophila as the sole enteropathogen will be included in the study. Twenty A. hydrophila diarrhoea cases from each of the following age groups will be studied: 0-5 years, 5-9 years, 9-14 years and 14 +. Five age and sex matched control subjects will be selected from non- diarrhoeal patients attending the ICDDR,B Staff Clinic each day. The standard surveillance study questionnaire will be administered to all patients. Each inpatients will be examined by one of the investigators at the time of admission and will be followed closely. The average incidence of A. hydrophila diarrhoea is expected to be 5 percent. Therefore, a total of 1,600 patients need be screened for the study.

2. Determination of enterotoxigenicity :

Twenty-five strains of A. hydrophila isolated and preserved in the Microbiology Laboratory of ICDDR,B from each group of diarrhoea cases, healthy individuals and water, sewage and animals will be included in this study. ^{if available} In addition all the

freshly isolated strains will also be tested. The following tests will be done :

i. Heat-labile enterotoxin

- a. Adult rabbit ileal loop test
- b. Skin permeability assay

Although A. hydrophila enterotoxin was found to be toxic for the following cell cultures (Sanyal et al, 1978), the tests will be repeated with isolates from Bangladesh.

- c. Chinese hamster ovarian cell assay
- d. Mouse adrenal (Y-1) cell assay

ii. Heat-stable enterotoxin

- a. Time course of fluid accumulation in ileal loops of adult albino rabbits with a representative group of strains.
- b. Suckling mice assay

iii. P32 DNA probe technique

It is understood that the P32 DNA probe technique of Mosley et al (1980) will be available this year in this laboratory. It will, therefore, give a wonderful opportunity to test A. hydrophila strains for unequivocal proof of the presence in the genome of the genetic information coding for the proteins of heat-labile and heat-stable enterotoxins. This may confirm that A. hydrophila possesses the genetic information to produce these toxins.

3. Tests for cytotoxicity :

- a. Chinese hamster ovarian cell culture
- b. Mouse adrenal cell culture
- c. HeLa cell culture
- d. Chick embryo system
- e. Other tests, if possible, like fibroblast cells etc.

4. Tests for serological responses :

Blood specimens will be collected during acute and convalescent periods from all patients having documented A. hydrophila infection.

Hospitalised patients will have an acute sera drawn in hospital.

As soon as a surveillance patient is found to be positive, we

will send a fieldworker to collect an acute sera at home. Follow-up

visits will be done on all patients for convalescent sera. Blood

will be drawn by capillary tube method, as used at ICDDR,B from

young children and by syringe in 2 ml quantities from older

children and adults. The sera will be diluted 1 in 10 in normal

saline, distributed in 0.5 ml aliquots wherever possible in

small vials and preserved frozen at -20°C or if possible at -70°C

until the following tests are done :

- a. Neutralisation of enterotoxic activity in adult rabbit ileal loops.
- b. Neutralisation of skin permeability factor activity in rabbits.
- c. Neutralisation of cytotoxic activity in CHO, Y-1, HeLa cell cultures.
- d. As haemolysins have been provisionally identified as cytotoxins, neutralisation of haemolytic activities using sheep erythrocytes will be performed.
- e. Agglutination test.

5. Cross reactivity between A. hydrophila, cholera and E. coli enterotoxins :

Although A. hydrophila enterotoxin was found to be neutralised by cholera and E. coli antitoxins in ileal loop and skin permeability tests (Sanyal et al, unpublished data), cross reactivity on neutralization by antibodies against cholera and E. coli enterotoxin will be tested with isolates of Bangladesh in rabbit gut and skin.

6. Biochemical characterisation to correlate toxin production will be done with toxigenic strains on the basis of utilisation of various carbohydrates, different compounds as the sole sources of carbon, haemolysin production etc.

D. SIGNIFICANCE :

A. hydrophila are being increasingly isolated from case of diarrhoea in different parts of the world. A large number of isolations of these organisms are being made in Bangladesh as well. As very little is known about the clinical features of diarrhoea caused by this organism, this study will allow us to document all these features which are essential for proper diagnosis and prompt management. This study will also enable us to know whether all the strains isolated from diarrhoeal patients and their environment are enterotoxic and cytotoxic or not. Knowledge regarding these factors are very important to understand the disease and its epidemiology in their proper perspective in

Bangladesh. These will also help in formulating intervention and prevention measures. As A. hydrophila is yet to be established in the aetiology of diarrhoea in spite of their frequent isolations and toxin production, the proposed serological studies will clarify its role in causation of the disease. Delineation of simple biochemical characters that could be correlated with enterotoxin production will make the detection of enterotoxigenic A. hydrophila very simple and easy and can be performed in all ordinary diagnostic laboratories where the cumbersome ileal loop and PF tests are not possible to perform.

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SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

NAME	POSITION	% OR NO. OF DAY	ANNUAL SALARY	BUDGET REQUIREMENT	
				TAKA	DOLLAR
Prof. Suhas C. Sanyal	Prin. Investi.	30%			13,500
✓ Dr. M.I. Huq	Chief, Microbiol.	5%			1,800
✓ Dr. B.J. Stoll	Asst. Scientist	5%			1,000
✓ Dr. P.K. Bardhan	Sr. Med. Officer	10%	60,000	6,000	
Dr. Thelma Leifert	Staff Cli. Phy.	5%			
Dr. A.S.M.H. Rahman	Veterinarian	10%	66,360	6,336	
Mr. Khorshed Alam	Sr. Res. Officer	50%	38,196	19,098	
To be named	Res. Officer*	50%	25,272	12,636	
To be named	Lab. Technician*	50%	18,036	9,018	
✓ Dr. Arun Ch. Das	Asst. Animal Care Taker	10%			2,720
Md. Ishaque	Lab. Attendant	50%	18,096	9,048	
To be named	Field Asst.	50%	19,200	9,600	
To be named	(Secretary)	50%	25,000	12,500	

2. SUPPLIES AND MATERIALS:

ANIMALS	AMOUNT REQUIRED	UNIT COST	PROJECT REQUIREMENT	
			TAKA	DOLLAR
Adult albino rabbits	250.00	120.00	30,000.00	
Suckling mice	220.00		2,500.00	
<u>Tissue Cultures</u>				
CHO)	200			
Y1)	tests each	14.50	2,900.00	
He La	200 tests	20.00	4,000.00	
Haemeolysis	100 tests		2,000.00	
<u>Aeromonas Culture</u>	3200	6.00	19,200.00	
<u>P32 DNA Probe</u>				1,500.00
Eggs	100 tests		1,000.00	

3. EQUIPMENT:

Glass Equipments 1,000.00
Space in Deep Freeze

4. PATIENT HOSPITALIZATION:

15,000.00

5. OUTPATIENT CARE:

Nil

* At present no Research Officer and Lab. Technician available since all are engaged with research activities. These can be recruited against the budget of this protocol.

	<u>AMOUNT REQUIRED</u>	<u>COST UNIT</u>	<u>PROJECT REQUIREMENT</u> <u>TAKA</u>	<u>DOLLAR</u>
6. <u>ICDDR, B TRANSPORT:</u>		4.00 / Mile 1,000 Mile	4,000.00	
7. <u>TRAVEL AND TRANSPORTATION OF PERSONS:</u>				3,000.00
8. <u>TRANSPORTATION OF THINGS:</u>				200.00
9. <u>RENT, COMMUNICATION AND UTILITIES:</u>	Nil			
10. <u>PRINTING AND REPRODUCTION:</u>			5,000.00	
11. <u>OTHER CONTRACTUAL SERVICES:</u>	Nil			
12. <u>CONSTRUCTION, RENOVATION ALTERATION ETC.:</u>	Nil			

B. BUDGET SUMMARY

	<u>Taka</u>	<u>US\$</u>
1. Personnel	86,956.00	16,300.00
2. Supplies	61,600.00	1,500.00
3. Equipment		1,000.00
4. Hospitalization	15,000.00	
5. Outpatients	None	
6. ICDDR,B Transport	4,000.00	
7. Travel-Persons		3,000.00
8. Transportation Things		200.00
9. Rent/Communication	None	
10. Printing/Reproduction	5,000.00	
11. Contractual Services	None	
12. Constructions	None	
	<u>172,556.00</u>	<u>22,000.00</u>

Grand Total: US\$ 32,785.00
Plus 30% : US\$ 8,700.00

ABSTRACT SUMMARY

Although Aeromonas hydrophila has been implicated in the aetiology of diarrhoea in many countries on the basis of its isolation in pure culture, demonstration of enterotoxin production and its mode of action, very little is known about the clinical profile of the design and the exact role played by this organism in causation of diarrhoea. This study will help in delineating the clinical features, establishment of A. hydrophila in the aetiology of diarrhoea in Bangladesh and demonstration of toxicities of the strains isolated from acute diarrhoeal cases to be compared with those from healthy individuals, animals and the environment of this country.

1. Subject population : All age groups
2. Risks : No potential risk to the subject involved.
3. Protection against risk : Sterile methodology will be used.
4. Confidentiality : Not applicable
5. Privacy : Not applicable
6. Interview : Not applicable
7. Benefit : This study will help in establishing A. hydrophila in the aetiology of diarrhoea. It will help in proper diagnosis and formulations of proper intervention and prevention measures.
8. Use of Record : Hospital records will be used.

Patient number	Date	Name	Father's name and address	Duration of illness	Types of diarrhoea ¹	Abd.pain	Fever	Vomiting	Dehydration ²	Recommended ³ fluids
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Discharge Date & Time	weight	height	Duration of illness after stating treatment	Volume of fluid administered		Losses(volume) Diarrhoea	Vomit	Other complication	Any laboratory test & results	Diarrhoea in family ⁴ during last 1 week
				oral	I.V.					

Home visit and its outcome

Comments :

¹. Watery/liquid/loose/bloody/mucoid

². mild/mod/mod.severe/severe/none

³. oral/I.V.

⁴. yes/no/who

81-045

ইরোমোনাস রোগীর সন্মতি পত্র

হাসপাতালে আপনান্ন/আপনান্ন শিশুর পায়খানা পরীক্ষা করে ইরোমোনাস নামক একপ্রকার জীবাণু পাওয়া গিয়াছে। এই জীবাণু পায়খানার সাথে অবলম্বন করে। এই জীবাণুই আপনান্ন/আপনান্ন শিশুর রোগের কারণ কিনা তা দেখার জন্য যদি আপনি রাজী হন তবে আপনান্ন/আপনান্ন শিশুর আংগুল হতে পরীক্ষার জন্য সামান্য রক্ত নেওয়া হবে। আপনান্ন/আপনান্ন শিশুর দেহে এই রোগের কোন প্রতিশ্রম্বিয়া হয়েছে কিনা তা দেখার জন্য আপনান্ন সন্মতক্রমে দু'সপ্তাহের মধ্যে আবারও সমপরিমান রক্ত নিতে হবে। আপনি যে কোন সময় আপনান্ন/আপনান্ন শিশুর নাম প্রত্যাহার করতে পারেন।

আপনান্ন/আপনান্ন শিশুর সম্পর্কিত তথ্যাবলী গোপন রাখা হবে। আশা করি আপনান্ন সহযোগীতা বাংলাদেশে ইরোমোনাস রোগের চিকিৎসা ও দমনে সহায়তা করবে।

আমি স্বেচ্ছায় সব কিছু জানার পর এই গবেষণায় অংশগ্রহণ করতে রাজী আছি।

রোগী/অভিভাবকের

স্বাক্ষর/টিপ সহি-----

তারিখ-----