Attachment I.

Date 11/X/87

ETHICAL REVIEW COMMITTEE

Principal Investigator Dr. K.A. Monsur

Application No. 87-022

Title of Study Incidence of diarrhoea due to Escherichia coli

Trainee Investigator (if any)

Supporting Agency (if Non-ICDDR,B) 28

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 a 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.

Please refer to the enclosed letter to chairman E.R.C.

(PTO)

I 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

7-10-87 . OCT 28 1987

Trainee

INTERNATIONAL CENTRE FOR
DIARRHOEAL DISEASE
RESEARCH, BANGLADESH

Memorandum

TO : The Chairman, ERC

DATE: 1/9/87

FROM : Dr. K.A. Monsur

SUBJECT : Ethical clearance of a protocol entitled, "Incidence of diarrhoea due to Escherichia coli" by: Dr. K.A. Monsur

From the MacConkey plates to be inoculated for routine culture of stool in Surveillance patients (Protocol No. 86-022), about 15 isolated, presumptive E. coli colonies will be picked and preserved in agar Slant for the present study.

This is for your kind information and approval.

Thank you.

87-022
11/X/87

d:kminci.pro

SECTION I - RESEARCH PROTOCOL

1.	TITLE	:	Incidence of diarrhoea due to Escherichia coli.
2.	PRINCIPAL INVESTIGATOR	:	Dr. K.A. Monsur
	CO-INVESTIGATORS	:	Dr. Zia Uddin Ahmed Dr. A.N. Alam Dr. B.A. Kay
3.	STARTING DATE	:	January 1, 1988 or date of starting
4.	COMPLETION DATE	:	2 years after starting
5.	TOTAL DIRECT COST	:	US$ 46,258.00
6.	SCIENTIFIC DIVISION HEAD	:	Dr. Ivan Ciznar

This protocol has been approved by the Laboratory Sciences Division.

Ivan Ciznar

Head, Lab Sciences Division

Date: Oct. 6, 1987

7. ABSTRACT SUMMARY

From the surveillance series of patients attending the ICDDR,B Treatment Centre about 400 cases of diarrhoea will be selected for study per year. Patients in whom a known enteric pathogen can be isolated will be excluded from the study. Fifteen isolated E. coli colonies from each of the remaining patients will be tested for their phage sensitivity pattern. Those which are found to contribute the predominant E. coli flora in the patient, as indicated by the uniformity of their phage susceptibility pattern, will be tested exhaustively for detection of EPEC, ETEC, EIEC and enterohaemorrhagic E. coli. The study will select and make in depth study of the suspect group of E. coli among which the pathogenic ones are expected to be found. The study will be continued for two years and is expected to give the spectrum and seasonal variation of the E. coli infection among the diarrhoeal patients attending ICDDR,B.

8. Reviewers:

a) Ethical Review Committee _____

b) Research Review Committee _____

c) Director _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective:

To identify the spectrum of Escherichia coli infections among the diarrhoeal cases attending ICDDR,B.

2. Background:

Escherichia coli is one of the major causes of diarrhoea in the developing countries. The recognized pathogenic strains presently include E. coli that are classfied as enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC) and enterohaemorrhogic (1,2,3). It is possible that there are other pathogenic E. coli for which we do not know the identification characteristics and they, therefore, remain undiagnosed.

Normally, E. coli in the gut is present as a changing mixture of many different strains (4,5,6,7). But in clinical cases of E. coli diarrhoea the pathogenic strain is usually present as the predominant E. coli population so that if one examined 10 colonies individually most of them would belong to the same pathogenic strain (8). However, in endemic areas pathogenic E. coli can be present in the gut even in asymptomatic healthy individuals. In such cases they are usually present in small numbers and are detected if large numbers of E. coli colonies are examined from the same person (9).

To confirm that an E. coli isolate is not a pathogen, the isolate has to be examined for all the possible pathogenic

characteristics known for E. coli. From the culture plate the different E. coli cannot be distinguished from each other by their colonial appearance. Therefore, a large number of different colonies have to be examined to make sure that a pathogen, if present, will not be missed. It can, therefore, be assumed that many of the E. coli infections remain undiagnosed because such large number of colonies cannot be routinely examined from all cases of diarrhoea for all their possible pathogenic characteristics.

In clinical cases of diarrhoea with no identified etiology the possibility of E. coli being the causative organism should be considered. The most promising approach to detect the pathogenic E. coli will be to examine those E. coli which are present as the predominant E. coli flora in the intestine and which have been obtained from diarrhoeal cases in which no other pathogen has been isolated. Examination of an adequate number of isolates, which fulfill the above criteria for all the pathogenic properties that are known for E. coli, is expected to give the most complete picture of the spectrum and frequency of E. coli infection in the population.

Differentiation between E. coli strains can be made by their complete serological profiles (10) the determination of which is too laborious, expensive and time consuming and beyond the scope of most laboratories. The phage lysotype of an E. coli isolate tested against a suitable selection of phages provides an alternative simple and useful method to determine the identity or otherwise of most E. coli isolates (11). The technique is simple

and a large number of colonies can be examined in a day. If 10 or 15 different E. coli colonies from a normal healthy individual or from a clinical case of diarrhoea not due to E. coli, are examined for their phage lysotypes, the lysotype patterns will usually differ. In the case of diarrhoea due to E. coli, the pathogen will normally constitute the predominant E. coli flora and the phage lysotypes will be similar for nearly all of the colonies. If, therefore, 10 to 15 E. coli colonies from all diarrhoea cases are routinely examined for their phage lysotypes in those cases where the diarrhoea is due to a pathogenic E. coli most of the colonies will belong to a single strain which will be indicated by a similar lysotype pattern.

In the present study, bacteriological examination of the stool of clinical cases of diarrhoea will be carried out. Colonies of E. coli from those cases of diarrhoea in which no other pathogen had been identified and where the E. coli isolates would give predominantly a single lysotype will be examined in depth for their possible pathogenic characteristics. Examination of an adequate number of the abaove type of isolates is expected to give the most complete picture of the spectrum of E. coli infections in a population. The study is proposed to be carried out over a period of 2 years. Therefore, it is also expected to give the pattern of seasonal variation in E. coli infections.

3. Rationale:

In view of the difficulties in separating pathogenic E. coli from the non-pathogenic ones, many E. coli infections remain

undiagnosed. Using phage lysotype as an identifying marker the study will serve to screen the suspect E. coli population. Study of this suspect population of E. coli for pathogenic markers is expected to give the best picture of the spectrum of E. coli infecion in clinical cases of diarrhoea.

The study which will be carried out over a 2 year period is also expected to give a picture of the seasonal variations in E. coli infection.

B. SPECIFIC AIMS

1. To investigate the diarrhoeal cases attending ICDDR,B for screening the suspect E. coli population among which the pathogenic ones are expected to be found.

2. to examine the above suspect population in depth for pathogenic characteristics of E. coli.

Selection of E. coli isolates:

About 400 cases of diarrhoea reporting to the Treatment Centre of ICDDR,B will be selected per year from among the surveillance series. Main clinical features, naked eye and microscopic characters of the stool will be noted. From the MacConkey plates to be inoculated for the stool culture about 15 isolated, presumptive E. coli colonies from each patient will be randomly picked and preserved in agar slant. Those cases in whom Salmonella, Shigella, V. cholerae or other enteric pathogen are detected will be excluded from the study. The picked up E. coli colonies from those cases in which no known pathogen can be

isolated will be tested for their phage lysotype patterns as described by Monsur et al. (11). Lysotypes giving a predominantly (≥60%) single pattern in a case will be examined in depth for the presence of pathogenic markers for E. coli as mentioned below. The number of isolates which will require detailed examination is expected to be of the order of 200 per year.

Test for pathogenic characteristics:

i) Serotyping of the isolates using commercially available antisera for presence the of EPECs.

ii) Test for LT.

iii) Test for ST.

iv) Sereny test and plasmid profile analysis for EIEC.

Where all the tests (i) to (iv) are negative the isolate will be tested on HeLa cells for the production of verotoxin.

For specific isolates additional tests, e.g. accumulation of fluid in rabbit ileal loop, will be attempted when felt necessary.

D. SIGNIFICANCE

Investigation of diarrhoeal cases for detection of all types of pathogenic E. coli is laborious, expensive and time consuming. Obviously our knowledge of the nature and extent of all types of E. coli infections among diarrhoeal cases in Bangladesh has remained incomplete. The present study will give us a much more complete picture of the spectrum of E. coli infection among diarrhoeal cases attending ICDDR,B.

BUDGET

Expense Category	Description	Amount	
		Year 1 Jan 1 - Dec 31 1988	Year 2 Jan 1 - Dec 31 1989 (15% inflation added)
3100	Local Salaries	9504	9648
3300	Local consultant	3960	4019
3700	Supplies and Mat.	6000	5075
4000	Other costs	500	510
4800	Interdepartmental	4000	3045
		23964	22294

Total Two years' direct cost: 46,258.00

Brief Budget Breakdown of Year 1

Local Personnel (All new recruitment)

Job	No.	Level	Amount/month	Time involvement
Consultant	1	-	333	100%
Molecular Biologist	1	NO-C	300	50%
Sr. Res. Officer	1	GS-6	320	100%
Sr. Res. Officer	1	GS-6	32	10%
Sr. Technician	1	GS-4	140	100%
Lab. Attendant	1	GS-2	120	100%

Supplies and Materials:

Media	4000
Sera	2000
Glassware	1000
Non-stock	4000

Interdepartmental and other costs 7000

REFERENCES

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2. Levine MM, Kaper JB, Black RE et al. New knowledge on pathogenesis of bacterial enteric infections as applied to vaccine development. Microbiol Rev. 1983; 47: 510-50.

3. Edelman R, Levine MM. Acute diarrhoeal infections in infants. II. Bacterial and viral causes. Hosp Pract 1980; 15: 97-104.

4. Cruickshank R, Duguid JP, Marimon BP, Swain RHA. Medical Microbiology, 12th ed., Edinburgh: Livingstone, 1973. 328.

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8. Sack DA, Kamisky DC, Sack RB et al. Enterotoxigenic Escherichia coli diarrhoea of travellers: a prospective study of America Peace Corps Volunteers. Johns Hopkins Medical Journal 1977; 141: 63-70.

9. Merson MH, Sack RB, Kibriya AKMG et al. Use of colony pools for diagnosis of enterotoxigenic Escherichia coli diarrhoea. J Clin Microbiol 1979; 9: 493-497.

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11. Monsur KA, et al. Use of bacteriophae as a marker for identification of freshly isolated individual Escherichia coli strains. J Diarrhoeal Dis Res 1985 Sep; 3(3): 131-137.