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ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 28.7.83

ncipal Investigator Dr. T. Butler

lication No. 83-033(P)

le of Study Detection of Shiga-like xin in E. coli isolates from arrheal patients in Bangladesh

Trainee Investigator (if any) _____

Supporting Agency (if Non-ICDDR,B) _____

Project status:
(x) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

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

Source of Population:
(a) Ill subjects (Yes) No
(b) Non-ill subjects Yes No NA
(c) Minors or persons under guardianship Yes No NA

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)

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

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 perticipate 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) Already on other protocol
(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.

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

T Butler 27.7.83
Principal Investigator

Trainee

SECTION I- RESEARCH PROTOCOL

(LIMITED STUDY)

1. Title: Detection of Shiga-like toxin in E. coli isolates from diarrheal patients in Bangladesh

2. Principal Investigator: Dr. T. Butler

   Co-Investigator: Dr. Alison O'Brien, Dr. M.I. Huq Dr. A.R. Samadi and Dr. Nigar Shahid

3. Starting Date: August 1983

4. Completion Date: October 1983

5. Total Direct Cost: US$ 985

6. Scientific Program Head: Dr. A.R. Samadi

This protocol has been approved by the Disease Transmission Working Group.

Signature of the Program Head : A Samadi

Date : 25/7/1983

7. Abstract Summary:

One hundred strains of E. coli isolated from diarrheal patients in Bangladesh will be tested for LT, ST, and Sereny test. The same strains will be sent to Dr. O'Brien in USA for testing for Shiga-like toxin. Correlations among these virulence determinants will be sought.

8. Reviews:

   a. Research Involving Human Subjects: _____

   b. Research Review Committee: _____

   c. Director: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objectives:

   a. Establishment of detection of shiga-like toxin in isolates of E. coli in ICDDR,B following achievement of objective No.b to justify the transfer of technology.

   b. To attempt to detect Shiga-like toxin in isolates of E. coli.

   c. To correlate the presence of Shiga-like toxin in E. coli with other virulence determinants LT, ST, and Sereny test.

2. Background:

Two mechanisms have been described by which Escherichia coli can cause diarrheal disease in humans and animals (1). Some strains colonize the small bowel and cause fluid secretion by elaboration of heat-labile (LT) and/or heat-stable (ST) enterotoxins. In contrast to these noninvasive, enterotoxic organisms, certain strains of E. coli penetrate and multiply within colonic epithelial cells and are able to produce keratoconjunctivitis in guinea pigs (the Sereny test). Infection with these enteroinvasive strains may cause symptoms that mimic those seen in patients with shigellosis. That E. coli must be capable of mediating gastroenteritis by other means is indicated by the finding that some strains associated with diarrheals disease produce no detectable LT or ST and are Sereny test-negative. O'Brien et al (2) have detected Shige-like toxin in certain strains of E. coli associated with diarrhea in the USA. Because some of these strains did not have other virulence factors of LT, ST, or Sereny positivity, this cytotoxin could play a role in diarrhea pathogenesis. The E. coli Shiga-like toxin showed biological activity of enterotoxity in the rabbit ileal loop, lethality for mice, and inhibition of protein synthesis in He La cells.

3. Rationale:

Some patients with diarrhea still do not have identifiable causes and are infected with E. coli not possessing known virulence characters. Thus, it is relevant to look for new virulence determinants in E. coli strains from diarrheal patients.

B. SPECIFIC AIMS:

1. To isolate and test 100 strains of E. coli for LT, ST, and Sereny test.
2. To examine these same strains for the presence of the newly described Shiga-like toxin.
3. To make correlations among these putative virulence determinants.

C. METHODS OF PROCEDURE:

1. Strains of E. coli will be selected from patients in the Diarrheal Surveillance Study from whom no other pathogen was isolated. The isolates will be fresh stocks.
2. LT will be detected by CHO cell assay at ICDDR,B.
3. ST will be detected by infant mouse assay at ICDDR,B.
4. Sereny tests will be carried out by Guinea pig conjunctivitis test at ICDDR,B.
5. The strains will be sent to Dr. O·Brien in Bethesda, Maryland, USA shiga-like toxin testing. She will quentitate the level of toxin production by measuring cytotoxicity for He La Cells. The toxin will be neutralized by antiserum against shiga toxin (2).

D. SIGNIFICANCE

Some patients with diarrhea are infected with *E. coli* strains that do not possess known virulence determinants. Finding shiga-like toxin in these strains may implicate another pathogenic mechanism for *E. coli* diarrhea.

E. FACILITIES REQUIRED:

1. ICDDR,B Microbiology lab and Animal House facilites.

F. COLLABORATIVE ARRANGEMENTS

Dr. O'Brien in USA will carry out shiga-like toxin testing.

REFERENCES

1. DuPont, H.L., Formal, S.B., Hornick, R.B., Snyder, M.J., Libonati, J.P., Sheahan, D.G., LaBrec, E.H., Kalas, J.P. Pathogenesis of Escherichia coli diarrhea. N. Engl. J. Med. 285:1-9, 1971

2. O'Brien, A.O., LaVeck, G.D., Thompson, M.R., and Formal, S.B. Production of Shigella dysenteriae Type 1-like cytotoxin by Escherichi coli. J. Infect. Dis. 146:763, 1982

ABSTRACT SUMMARY

1. Population will be bacterial E. coli isoaltes from patients with diarrhea. The reason is to test the E. coli for virulence determinants.

2. There are no risks to patients

3. NA

4. Patients' identities will be kept confidential. Bacterial strains will carry a laboratory number.

5. NA

6. No interveiws.

7. There are no risks. Benefits are learning about a new mechanism of diarrhea.

8. NA

SECTION III - DETAILED BUDGET

1. **Personnel Services:**

	Position	% Effort	Ann.Salary Taka	Ann.Salary Dollar	Actual Cost Tk.
Dr. T. Butler	Pr.Investigator		-	-	
Dr. O'Brien	Co.Investigator		-	No cost	
Dr. M.I. Huq	"		-	"	
Dr. A.R. Samadi	"		-	-	
Dr. Nigar Shahid	"		-	-	
Technician (Microbiology)		20%	50,000		10,000
Technician (Animal)		20%	50,000		10,000

2. **Supplies and Materials:**

Sereny test 100 x Tk.36.00 (50 G.P.)		3,600	3,600

3. **Equipment** - None

4. **Patient hospitalization** - None

5. **Transport** - None

6. **Rent, Communications, and Utilities** - None

7. **Other contractual services** - None

Total : Tk. 23,600

Actual Cost in US$ 985

(Conversion rate US$1 = Tk.24)

BUDGET SUMMARY

		Dollar
1.	Personnel Services	834.00
2.	Supplies & Materials	151.00
3.	Equipment	-
4.	Patient hospitalization	-
5.	Transport -	-
6.	Rent/Communication/Utilities	-
7.	Other contractual services	-
	Total US $:	985.00

(Conversion rate US$1 = Taka:24)