Attachment 1.

8/6

REVIEW BOARD ON THE USE OF HUMAN VOLUNTEERS
CRL

Date 9-8-77

Principal Investigator Robert Black Trainee investigator(if any) None

Application No _____ Supporting Agency(if Non-CRL) _____

Title of study Epidemiology of Enterotoxigenic Escherichia coli

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 possibly 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
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 Board for review.

We agree to obtain approval of the Review Board on Use of Human Volunteers for any changes involving the rights and welfare of subjects before making such change.

Robert E Black
Principal Investigator

Trainee

Please return 2 copies of entire protocol to Chairman, Review Board on Use of Human Subjects.

Attachment 1a

INFORMATION TO INCLUDE IN ABSTRACT SUMMARY

The Board will not consider any application which does not include an abstract summary. The abstract should summarize the purpose of the study, the methods and procedures to be used, by addressing each of the following items. If an item is not applicable, please note accordingly:

1. Describe the requirements for a subject population and explain the rationale for using in this population special groups such as children, or groups whose ability to give voluntary informed consent may be in question.

2. Describe and assess any potential risks - physical, psychological, social, legal or other - and assess the likelihood and seriousness of such risks. If methods of research create potential risks, describe other methods, if any, that were considered and why they will not be used.

3. Describe procedures for protecting against or minimizing potential risks and an assessment of their likely effectiveness.

4. Include a description of the methods for safeguarding confidentiality or protecting anonymity.

5. When there are potential risks to the subject, or the privacy of the individual may be involved, the investigator is required to obtain a signed informed consent statement from the subject. For minors, informed consent must be obtained from the authorized legal guardian or parent of the subject. Describe consent procedures to be followed including how and where informed consent will be obtained.

   (a) If signed consent will not be obtained, explain why this requirement should be waived and provide an alternative procedure.

   (b) If information is to be withheld from a subject, justify this course of action.

6. If study involves an interview, describe where and in what context the interview will take place. State approximate length of time required for the interview.

7. Assess the potential benefits to be gained by the individual subject as well as the benefits which may accrue to society in general as a result of the planned work. Indicate how the benefits outweigh the risks.

8. State if the activity requires the use of records (hospital, medical, birth, death or other), organs, tissues, body fluids, the fetus or the abortus.

The statement to the subject should include information specified in items 2,3,4 and 7, as well as indicating the approximate time required for participation in the activity

Rcd 09 Aug 1977

77-012

SECTION I - RESEARCH PROTOCOL

1) Title: Epidemiology of Enterotoxigenic Escherichia coli Diarrhea.

2) Principal Investigator: Robert E. Black, M.D.

3) Starting Date: August 1, 1977

4) Completion Date: July 31, 1979.

5) Total Direct Cost: $ 80,039

6) Abstract Summary:

Diarrhea caused by enterotoxigenic Escherichia coli (ETEC) is probably an important cause of morbidity and mortality in developing countries. In previous studies in Bangladesh ETEC were isolated from 20 to 70% of patients with acute watery diarrhea without other known pathogen isolated.

Application of laboratory techniques for identification of ETEC currently available at the Cholera Research Laboratory to a sample of patients in Matlab, along with the demographic data currently available, will permit a study of the epidemiology of ETEC diarrhea in an endemic area. To obtain reliable information about age, sex seasonal and geographical incidence of ETEC diarrhea, the study will be conducted over a 2 year period (February 1977 to January 1979). Data tabulation, analysis and publication is expected to take an additional 6 months (until July 1979).

7) Reviews:

a) Research Involving Human Subjects: _____

b) Research Committee: _____

c) Director: _____

d) BMRC: _____

e) Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective: The objective of this study is a better understanding of the epidemiology of diarrhea caused by enterotoxigenic Escherichia coli in rural Bangladesh.

2. Background: Acute diarrheal disease is a major cause of morbidity and mortality in many developing countries. In Bangladesh watery diarrhea may be due to Vibrio cholera, Shigella, Salmonella or non-agglutinable vibrios; however, these organisms can be isolated in only 20 - 50% of cases coming to medical attention (1). Within the past several years techniques have become available to detect bacterial and viral agents which have heretofore been overlooked. The application of these techniques in Bangladesh and in other countries has led to recognition of enterotoxin producing E.coli as an important cause of diarrheal disease among residents and travellers in many developing countries (2-6). E.coli diarrhea in travellers is uncomfortable and inconvenient, but rarely life threatening; however, illness caused by enterotoxigenic E.coli may be an

important cause of death in residents of endemic areas such as Bangladesh (7).

E.coli are known to cause diarrhea through the production of 2 distinct enterotoxins - a heat labile toxin (LT) and a heat stable toxin (ST). Since organisms may produce one or both toxins, tests must be performed that enable recognition of each type. Recent studies in Bangladesh have used a variety of laboratory techniques to look for enterotoxigenic E.coli in hospitalized patients and/or in outpatients with watery diarehea. During the 1974 cholera toxoid vaccine trial in Matlab (which utilized all females over 1 year old and all males 1-14 years old). Nalin et. al.(2) sought enterotoxigenic E.coli with the Chinese hamster ovary (CHO) assay and the dog jejunal loop using pooled E.coli. They found a case rate of 7 per 10,000 persons/year for LT E.coli associated illness and 4 per 10,000 persons/year for ST only E.coli associated illness from November to July. This study also found that 33% of inpatients, who were mostly adults, and 15% of outpatients who were mostly children, were infected with LT E.coli.

Disease associated with ST E.coli was studied only in adults inpatients who had no LT E.coli identified; 30% of patients were positive.

In November 1974 Sack et. al. (4) studies patients of any age with severe watery diarrhea admitted to the Cholera Hospital in Dacca during a 1 week period. Ten single E.coli isolates and pools of 10 isolates from each patient were tested in the CHO cell assay and the adrenal cell assay and pools were tested for ST in the infant mouse assay. E.coli from 23 (35%) of 65 patients were enterotoxigenic (16 ST/LT, 4 LT only, and 3 ST only). Although a few children were infected, most enterotoxigenic E.coli were isolated from adults.

Between December 1974 and January 1975 Ryder et. al. (3) studied hospitalized patients with watery diarrhea in Matlab. Ten pooled and individual E.coli colonies were tested in the Y1 - adrenal cell system using mini-cultures and 5 colonies were tested in the infant mouse assay. E.coli producing LT and ST were found in cultures of 7 (39%) of 18 adults and 1 of 30 children. No LT only or ST only organisms were found.

In January and February of 1976, Black et. al. (unpublished) studied the etiology of watery diarrhea among children less than 10 years in the outpatient clinic at the Cholera Hospital in Dacca. Laboratory techniques included the CHO cell assay for LT and the infant mouse assay for ST. Cultures from 115 children negative for other bacterial pathogens were studied using both 5 individual picks of E.coli and pools of 10 organisms. Seventeen (15%) children had ST only E.coli isolated and 10 (9%) had ST/LT organisms. Pooled E.coli alone identified 70% of the total positives including 3 cases that had 5 negative individual picks.

In late 1976 Merson et. al. (unpublished) studied enterotoxigenic E.coli in adults hospitalized in Dacca. Although the data is not yet available, they reportedly found that 60-70% of the adult inpatients with acute watery non-Vibrio cholera diarrhea had enterotoxigenic E.coli.

From these 5 studies, it appears that E.coli producing ST and/or LT infect both mildly and severely ill

children and adults. However, because of the use of various laboratory techniques and patient sampling procedures it is not possible to determine the relative importance of ST only, LT only, or ST/LT E.coli. Nor is it possible to determine the incidence of illness associated with these organisms in various age groups. In the studies by Sack et. al. (4) and Ryder et. al. (3) E.coli diarrhea was found in hospitalized adults but rarely in children, while in Black's study children 24% of outpatient children were infected with enterotoxigenic E.coli. This apparent conflict may be explained by 1) differences between children and adults in the severity of illness caused by enterotoxigenic E.coli infection (i.e. children may get less severe disease than adults) 2) differences in the proportion of ill children and adults coming to the clinic and/or in the criteria for admission to the hospital, 3) differences in the rate of E.coli illness by year or time of year (i.e. since hospital and OPD children and adults were not studied concurrently) 4) differences between children and adults or by time of year in the rates of diarrhea with other causes (i.e. since rates of E.coli

illness were not previously studied in children and adults, and increase or decrease in diarrhea of other causes would alter the proportion of total cases having E.coli, 5) differences in laboratory technique.

Laboratory techniques may differ not only in the tests utilized but also in the preparation of E.coli that is tested (e.g. 5 or 10 individual colonies of E.coli or pools of 5 or 10 colonies or both). Although testing pools of E.coli may be less sensitive than testing individual colonies, especially during recovery, testing pools is probably sensitive enough (70 - 90%) in acutely ill patients to justify use of the technique (Merson, Black, unpublished). The increased efficiency of testing pools instead of individual colonies will permit a study of rates of hospitalization and outpatient clinic visits due to enterotoxigenic E.coli in Matlab. Since factors such as criteria for hospitalization are difficult to control, parameters such as serum solids and weight at admission and discharge at the initial visit will be obtained and used retrospectively to estimate the degree of dehydration in children and adults.

The epidemiology of E.coli diarrhea is poorly understood in any area of the world. Lindenbaum et. al.(1) prospectively studied "non-vibrio cholera" or diarrhea in which no etiologic agent was identified for a one year period in East Pakistan. They observed that the "syndrome" was common from February till May but was relatively sporadic in other months. This was distinctly different from the seasonal pattern of cholera in the same area; the cholera peak during the last months of the year, co-incident with the nadir of the "non-vibrio cholera" cases. Although the number of cases in which no etiology for diarrhea can be found by routine methods varies from year to year, a similar seasonal variation has usually been observed in both Matlab and Dacca. It is now clear that the syndrome they were describing is caused by at least 2 agents - enterotoxigenic E.coli and rotavirus - and very likely, also by other currently unrecognized pathogens (2-4). Each of these diseases may have a seasonal pattern that will not be apparent until data are collected over a 1-2 year period. Furthermore a systematic sampling of patients in Matlab may provide information

on the geographical distribution of E.coli disease in the field trial area. This information on the seasonal and geographical patterns of E.coli diarrhea may assist in further epidemiological studies of the means of transmission and control of the illness.

A study designed to investigate these questions was hegun in Matlab in February 1977. A preliminary tabulation of results (February and March 1977) indicate that 39% of inpatients (Appendix A) and 30% of out-patients (Appendix B) were infected with enterotoxigenic E.coli. Since these data are incomplete no further analysis will be attempted at this time but it appears that a large proportion of the diarrhea cases at Matlab have enterotoxigenic E.coli identified by the current techniques. Thus it seems justified to continue the study for a 2 year period (18 months from the present) to obtain useful information on the epidemiology of illness related to enterotoxigenic E.coli.

3. Rationale: Now that we have laboratory techniques available to find the etiology of many cases of acute tropical diarrhea, it is possible to determine the epidemiology of diarrhea associated with enterotoxigenic E.coli in an endemic area. Use of these techniques to

identify the etiology of diarrhea in a sample of patients in Matlab, along with the demographic data currently available for that area, provides a unique opportunity to determine the incidence of illness associated with ST only, LT only and ST/LT E.coli in various age groups, and to study the seasonal and geographical distribution of illness.

B. SPECIFIC AIMS

1. To determine the incidence by age and sex and religion of diarrheal disease associated with ST only, LT only, or ST/LT E.coli.

2. To determine if the severity of diarrhea (degree of dehydration, stool volume etc) is influenced by age, sex, enterotoxin type, season, presence of intestinal parasites or nutritional status (additional aspects of these questions will be investigated in a study of family members of persons with E.coli diarrhea to be done in late 1977).

3. To define the seasonal pattern of enterotoxigenic E.coli disease in the Matlab area.

4. To define the geographical pattern of enterotoxigenic E.coli disease in the Matlab area.

C. METHODS OF PROCEDURE

1. Patient population.

The study population will be selected from Matlab Hospital inpatients and outpatients who have no pathogens found on routine bacteriology and who live in the field trial area. To study a sufficient number in each age group each month, the sample will consist of 1) all inpatients (estimated 40-150 per month), 2) all outpatients age 10 years or greater (estimated 50-100 per month) and 3) ½ of outpatients less than 10 years (select even OPD numbers for study - estimated 70-140 per month). If the number of patients in any of these 3 groups exceeds 100 per month, a sample of 100 patients will be selected from that group using a random number table. Thus a maximum of 300 patients would be tested per month.

2. Specimen collection.

Each patient will have a stool or rectal swab taken during the clinic visit or upon admission to the hospital. The swab will be plated on MacConkey, Salmonella-Shigella, and TTGA agars. If there are no suspicious non-lactose fermenters or vibrios after 18-24 hours, pools of 10 E.coli organisms will be made from the MacConkey plate. If suspicious organisms are present, the MacConkey plate will be stored at 4°C for 24 hours until other bacterial pathogens are investigated. If shigella salmonellae, or vibrios are not found E.coli pools can be prepared. The pools will be prepared on infusion agar slants, stored at room temperature, and sent to Dacca for testing as soon as possible.

3. Tests for enteroxigenicity.

a. Each pool will be tested for the presence of LT by the Chinese hamster ovary tissue culture system at CRL in Dacca. (8).

b. Each pool will be tested for ST production using the infant mouse assay (9).

4. Patient care.

a. Patient care will be supervised by the medical staff at Matlab and routine care will not be altered by the bacteriologic sampling for E.coli. Since tests for E.coli enterotoxin will not be performed until several weeks after the illness, therapy directed specifically at E.coli related illness will not be possible. Clinical information (duration of diarrhea, symptoms, degree of dehydration etc.) will be recorded.

b. Each patient at the time of first visit will have a plasma specific gravity performed and a stool specimen will be examined for parasites. Parasitic infections will be treated if appropriate.

5. Data analysis.

Information will be obtained from the census records concerning the age and village of residence of each study patient. Additional information needed for the epidemiologic analysis includes 1) the number of patients by age living in the VTS area who were seen

in the OPD or hospitalized each day, and in whom no pathogens were found on routine bacteriology

2) the population at risk in various age groups for each village in the Matlab area.

The incidence of OPD and/or hospital associated E.coli diarrhea would be calculated by:

$$\text{Incidence} = \frac{\text{Numbers of E.coli: positive patients} \times \frac{\text{Number of persons without pathogen found who are from VTS}}{\text{Number of persons tested for E.coli}}}{\text{Population at risk in VTS area.}}$$

The incidence of illness would be calculated by age, sex, month, year village of residence and by each of these factors for each enterotoxin type if permitted by an adequate number of positive patients. Computer analysis will be needed and data forms will be devised to enable immediate key punching. It is recognized that the incidence calculated from hospital or clinic visits will be affected by factors such as the ability or desire of persons to make a visit to Matlab. This may have little influence on some areas of study such as seasonality; however, other areas such as geographic and age

and sex specific incidence may be to a greater degree . Because of this potential problem will be combined with other E.coli studies that are community based.

6. Schedule.

The study will be conducted over a 2 year period beginning in February 1977 and ending January 1979. Laboratory testing and data tabulation will probably require 3 months after January and data analysis an additional month. Preparation of the manuscript for publication should take 2 months after data analysis is completed.

D. SIGNIFICANCE

Enterotoxigenic E.coli are probably an important cause of morbidity and mortality in many developing countries; however, many aspects of the epidemiology of E.coli diarrhea are unknown. If illness is to be controlled either by environmental (water, sewage etc.) or host (vaccines) manipulation, these epidemiological characteristics must be defined. The field trial area, which apparently has endemic E.coli diarrhea and which has accurate demographic data for

residents, is an ideal area to study these important questions.

E. FACILITIES REQUIRED

1. Office Space - already exists in CRL, Matlab.
2. Laboratory Space - already exists in CRL, Matlab.
3. Hospital Resources - None required.
4. Animal Resources - 11,000 suckling mice.
5. Logistical Support - none required (specimens can be sent to Dacca in routine trips during the 2 year period).
6. Major items of equipment - none required.
7. Other - none required.

F. COLLABORATIVE ARRANGEMENTS

All collaborators in the study will be within CRL. It is expected that authorship of any publications desired from this study will include the following persons : Robert Black, Michael Merson, Mizanur Rahman, William Spira, Golam Kibriya, K.A. Al-Mahmud, George Curlin.

REFERENCES

1. Lindenbaum J, Benenson A.S., Rizvi S, et. al. Non-vibrio Cholera Lancet 1: 1081-1083, 1965.

2. Nalin D.R., McLaughlin J.C., Rahaman, M. et. al. Entero-toxigenic Escherichia coli and idiopathic diarrhea in Bangladesh. Lancet 2: 1116-1119, 1975.

3. Ryder R.W., Sack D.A., Kapikan A.Z. et. al. Enterotoxigenic Escherichia coli and reovirus like agent in rural Bangladesh. Lancet 1: 659-662, 1976.

4. Sack D.A., McLaughlin J.C., Sack R.B. et.al. Enterotoxigenic Escherichia coli isolated from patient at a hospital in Dacca. J. Infect. Dis. 135: 275-280, 1977.

5. Guerrant R.L., Moore R.A., Kirschenfeld P.M. et.al. Role of toxigenic and invasive bacteria in acute diarrhea of child-hood. N. Engl. J. Med. 293: 567-573, 1975.

6. Merson M.H., Morris G.K., Sack D.A. et.al. Traveller's diarrhea in Mexico. A prospective study at physicians and family members attending a congress . N. Engl. J. Med. 294: 1299-1305, 1976.

7. Sack, R.B., Gorbach S.L., Banwell S.G. et.al. Enterotoxigenic Escherichia coli isolated from patients with severe cholera - like disease. J. Infect. Dis. 123: 378-385, 1971.

8. Guerrant R.L., Brunton L.L., Schnaitman T.C. et.al. Cyclic adenosine morophosphate and alterations of Chinese hamster assay cell morphology: A rapid, sensitive in vitro assay for the enterotoxins of Vibrio cholerae and Escherichia coli. Infect. Immun. 10: 320-327, 1974.

9. Dean A.G., Ching Y.C., Williams R.G. et.al. Test for Escherichia coli enterotoxin using infant mice: Applications in a study of diarrhea in children in Honolulu. J. Infect. Dis. 125: 407-411, 1972.

APPENDIX 'A'

EPIDEMIOLOGY OF ENTEROTOXIGENIC E. COLI STUDY

Inpatients - Matlab

	February					March				
Age	ST	LT	ST/LT	NEG	TOTAL	ST	LT	ST/LT	NEG	TOTAL
< 2				14	14	3	3	6	42	54
2-4				3	3	1			4	5
5-9			1	1	2	3		2	6	11
10-19				5	5	2		9	5	16
20-29	2	1	1	1	5	3	1	5	9	18
30 +			3	7	10	6	4	22	27	59
Total	2	1	5	31	39	18	8	44	93	163

Incomplete

APPENDIX 'B'

EPIDEMIOLOGY OF ENTEROTOXIGENIC E. COLI STUDY

Outpatients - Matlab

Age	February					March				
	ST	LT	ST/LT	NEG	TOTAL	ST	LT	ST/LT	NEG	TOTAL
< 2	3	2	2	33	40	22	13	12	101	148
2-4		3		5	8	1	2	6	27	36
5-9			1	3	4	3		2	5	10
10-19	2	1	2	8	13	6	3	7	15	31
20-29			2	3	5	1	2	2	16	21
30 +	1	1	3	17	22	5	1	11	48	65
Total	6	7	10	69	92	38	21	40	212	311

Incomplete

SECTION - III BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES:

Name	Position	% or No. of days	Annual Salary	Project Requirement Taka	Dollars
Dr. Robert Black	Investigator	15%	$ 46,000	-	6,900
Dr. Michael Merson	Co-investigator	5%	$ 46,000	-	2,300
Dr. Mizanur Rahman	Co-investigator	10%	Tk.60,252	6,025	-
Dr. William Spira	Co-investigator	5%	$ 39,732	-	1,987
Mr. Golam Kibriya	Sr.Res.Assistant	45 d	Tk.35,820	6,200	-
Dr. Al-Mahmud	Head, Animal Resources	60 d	Tk.38,380	8,972	-
Mr. Rezaur Rahman	Pes.Tech.	80 d	Tk.20,484	6,303	-
Mr. Anisur Rahman	Glasswasher	60 d	Tk. 9,696	2,238	-
To be named(Matlab)	Sr.Lab. Tech.	130 d	Tk.16,056	8,028	-
Mr. K.Akhand	Sr.Field Asst.	260 d	Tk.23,004	23,004	-
To be named(Matlab)	Sr.Field Asst.	260 d	Tk.23,004	23,004	-
To be named(Matlab)	Field Assistant	260 d	Tk.16,056	16,056	-
To be named(Matlab)	Field Assistant	260 d	Tk.16,056	16,056	-
To be named(Matlab)	Field Assistant	260 d	Tk.16,056	16,056	-
To be named(Matlab)	Filed Assistant	260 d	Tk.16,056	16,056	-
To be named(Matlab)	Field Assistant	260 d	Tk.16,056	16,056	-
To be named(Dacca)	Key Punch Operator	10 d	Tk.11,448	440	-
To be named(Dacca)	Computer Programer at Grade C-1	15 d	Tk.42,000	2,423	-
				153,166	11,137

2. SUPPLIES AND MATERIALS:

Items	Unit Cost	Amount required	Project Requirement Taka	Project Requirement Dollars
F-12 (3 liter=1 packet)	$ 5.45	1		5.45
Micro mate (50/box)	$ 47.25	2		94.50
Micro mate lid (50/box)	$ 14.60	2		29.20
Culture Flask (500/box)	$ 109.80	0.5		54.90
Disposable pipette(500/box)	$ 59.65	2		119.30
Disposable pipette(1000/")	$ 19.32	1		19.32
Trypsin 1:25	$ 5.80	1		5.80
Hepes buffer	$ 6.70	1		6.70
Fetal calf serum	$ 8.80	1		8.80
Penicillin+Streptomycin	$ 4.05	1		4.05
Mice	Tk. 3	11,000	33,000	-
Tuberculin syringe(100/box)	$ 5.26	40		210.40
Needle 27 g. (100/box)	$ 4.09	40		163.60
Ether	$ 3.60	10		36.00
Tape	$ 0.91	24		21.84
7 X - 1 gal.	$ 2.00	1		2.00
Rectal swab for V.cholera	Tk. 3.28	4000	13,120	-
Rectal swab for Salmonella/ Shigella	Tk. 2.5	4000	10,000	-
Biochemical tests for Salmonella/Shigella	Tk. 1.02	4000	4,080	-
Biochemical tests for Vibrios	Tk. 0.8	2000	1,600	-
Stock vials	Tk. 0.50	4000	2,000	-
Stool Routine M/E	Tk. 2.0	4000	8,000	-
Plasma Sp.Gr.	Tk. 0.25	4000	1,000	-
TSB broth with yeast extract (liter)	Tk.10.00	20	200	-
		Sub Total:	73,000	781.86

3. EQUIPMENT:

Item	Unit Cost	Amount Required	Project Requirement Taka	Project Requirement Dollar
Filing Cabinet	1,100	1	1,100	-
		Sub Total:	1,100	-

4. PATIENT HOSPITALIZATION:

Number of patient days = 1200 (@ Tk.130)		156,000	-
	Sub Total:	156,000	-

	Project Requirements	
	Taka	Dollars
5. OUTPATIENT CARE:		
Number of patient days = 2400 (@ Tk.50)	120,000	-
Sub Total:	120,000	-
6. CRL TRANSPORT:		
Dacca/Matlab/Dacca (1 month @ Tk.300 round trip)	3,600	-
Sub Total:	3,600	-
7. TRAVEL AND TRANSPORTATION OF PERSONS:		
Local Travel - none	-	
International Travel (attendance at meeting)	-	2,000
Per diem expenses (14 days @ $ 36 per day)	-	504
Sub Total:	-	2,504
8. TRANSPORTATION OF THINGS:		
Import of supplies (25% of $781.86)	-	196.47
Local shipments (supplies to Matlab) 4 trips	1,200	-
Sub Total:	1,200	196.47
9. RENT, COMMUNICATION & UTILITIES:		
Postage	500	-
Telephone - none	-	-
Rent - none	-	-
Sub Total:	500	-
10. PRINTING AND REPRODUCTION:		
Special reproduction, publications figures, forms	7,500	-
Sub Total:	7,500	-

11. OTHER CONTRACTUAL SERVICES:

	Project Requirements	
	Taka	Dollars
Computer time (6 hr at Tk.600/hr.)	3,600	-
Sub Total:	3,600	-

12. CONSTRUCTION,ETC. - NONE

B. BUDGET SUMMARY

Category	Year 1 Taka	Year 1 Dollars	Year 2 Taka	Year 2 Dollars
1. Personnel	153,166	11,187	85,280[1]	11 187
2. Supplies	73,000	782	36,500[2]	391[2]
3. Equipment	1,100	-	-	-
4. Hospitalization	156,000	-	78,000[2]	-
5. Outpatients	120,000	-	60,000[2]	-
6. CRL Transport	3,600	-	1,800[2]	-
7. Travel Persons	-	2,504	-	2 504
8. Transportation Things	1,200	196	600[2]	98[2]
9. Rent/Communication	500	-	500	-
10. Printing/Reproduction	7,500	-	7,500	-
11. Contractual Service	3,600	-	3,600	-
12. Construction	-	-	-	-
Total:	519,666	14,669	273,780	14,180
Total $:	48,196		31,843	

Conversion rate $ 1.00 = Tk. 15.5

Footnotes

1 Personnel for 6 months only except data coder, keypunch operator, computer programmer and investigators.

2 For 6 months only.

Abstract - Epidemiology of Enterotoxigenic Escherichia coli Diarrhea

From the study we will determine the proportion of diarrhea treated at Matlab Hospital and Clinic that is caused by enterotoxigenic Escherichia coli. Furthermore, we will study the incidence of E. coli diarrhea by age, sex, religion, month, year and village of residence and the relationship between severity of diarrhea and nutritional status and parasitic infection. For the study, patients treated at Matlab Hospital and Outpatient Clinic will have enterotoxigenic E.coli sought from routine rectal cultures for an 18 month period. By using the results of routinely performed tests (serum solids, rectal culture, parasite examination), clinical examination and demographic data which have been and continue to be collected in the field trial area, we can perform the appropriate analysis.

1) Since the study seeks the incidence of illness in various age groups including infants and children, patients of all ages must be studied.

2) There are no risks to patients from this study. Since a rectal culture is performed on patients (from the field trial area) as part of normal patient care at Matlab the

study would not do a separate culture but would seek E.coli from the MacConkey plate of the routine culture. The stool specimen is also routinely examined for parasites while the patients are in the hospital or clinic; we will use the results of these examinations for our analysis. A finger tip blood specimen, tested for serum solids, is part of routine clinical evaluation at Matlab. Again, we will use the results of these clinical studies for our analysis.

3. There are no risks, therefore, this section is not applicable.

4. Confidentiality of data collected will be ensured. The names of individual patients will not be recorded. Although the VTS number will be recorded, it will be used only to obtain necessary information such as age and village of residence for the patient and to facilitate tabulation. The original data will be kept in a locked file in the custody of the principal investigator who will restrict its use to himself and the other investigators. Published data will not identify individuals by name or description. After the study the data will be destroyed.

5) A signed consent will not be obtained; however, all appropriate information will be provided to the patient and a verbal consent will be obtained. For minors informed verbal consent will be obtained from the authorized legal guardian or parent of the child. In each case the information provided will include a) the nature and purpose of the study b) the procedures to be used c) the physical risks d) the benefits to be derived e) the right to refuse to participate and f) the confidential handling of data.

6) Since no interview is required this section is not applicable.

7) Individual patients will not directly benefit from the study. On the other hand the study will not interfere with normal clinical care and therapy.

The potential future benefit of the study to society could be the control or elimination of enterotoxigenic E.coli diarrhea. Any future environmental (water, sanitation etc.) or host (vaccine) manipulation to attempt to control E.coli diarrhea must be based on accurate epidemiological information about the disease. This study should provide

some of that information.

8) The study requires the use of Matlab hospital and clinic records and census records.

Statement to be read to Subjects
when verbal consent is obtained.

The doctors at the Cholera Hospital are studying a recently discovered cause of diarrhea. They are trying to find out at what age people are affected, at what time of year it is most common, and in what villages it is most common. At this hospital tests are done to find the cause and severity of your illness, the study will not add any tests or interfere with treatment of your illness.

You can ask any questions you want and you are not required to take part in the study. Information collected will not be given to anyone other than yourself, and the doctors who will combine it with information from other patients treated here.