Attachment 1.

Date Aug 4, 1981

ETHICAL REVIEW COMMITTEE, ICDDR,B.

6-8

Principal Investigator L. Gothefors

Application No. 81-033

Title of Study Further Identification of Colonization Factors (CFA) in E. coli and Assays of Antiboides to These Factors as well as to Enterotoxins

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)

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 N.A.
   (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 N.A.
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
   NA 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.

Barbara Stoll for L. Gothefors
Principal Investigator

Trainee

81-033
Rec'd 7/8/81

SECTION I - RESEARCH PROTOCOL

1. Title: FURTHER IDENTIFICATION OF COLONIZATION FACTORS (CFA) IN E. COLI AND ASSAYS OF ANTIBODIES TO THESE FACTORS AS WELL AS TO ENTEROTOXINS

2. Principal Investigator: Dr. L. Gothefors

   Co-Investigator: Dr. B. Stoll, Dr. Ahren, Dr. A.M. Svennerholm, Dr. M.I.Huq

3. Starting date: August 1981

4. Completion date: July 1982

5. Total direct cost: US $12,540

6. Scientific Profram Head:

This protocol has been approved by the Host Defence Working Group.

Signature of the Scientific Program Head: Mikey Rahman

Date: August 14, 1981

7. Abstract Summary:

This study, being a logical continuation of our pilot study (81-001P), is designed to give further information on the presence in faecal E. coli strains of different colonization factors as well as their production of enterotoxin. We will use fresh isolates from hospital patients as well as stocked strains from the surveillance study. The prevalence of toxin production and CFA will be evaluated versus the clinical features of the patients, including their capacity to produce systemic and local antibodies to these bacterial antigens.

8. Reviews:

a. Research Involving Human Subjects: _____

b. Research Review Committee: _____

c. Director: _____

d. B M R C: _____

e. Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objectives:

1.1. To study various E. coli strains for the presence of colonization factors (CFA) and their relation to toxin production and clinical picture.

1.2. To study serum and salivary antibodies to LT, ST and CFA in patients with diarrhoea caused by ETEC.

2. Background Information:

2.1. ETEC diarrhoea

In developing countries the incidence of diarrhoea caused by enterotoxigenic E. coli (ETEC) is highest in children under two years of age. Immunity is later acquired and the disease is generally rare in adult life.

The clinical illness caused by ETEC is largely the same whether the strains produce one or both of the enterotoxins, and ranges from mild diarrhoea to a severe cholera-like disease.

Merson et al (1) in the autumn 1976 studied patients admitted to the CRL Hospital in Dacca. ETEC was the only pathogen isolated in 54% of the 176 cases. Out of them 65% were LT/ST, 32% ST and 3% LT only. Later serotyping revealed that 86% of the LT/ST strains belonged to one of four O serogroups (2).

These results were confirmed in a village based study from Matlab in 1977-78 (3) where ETEC were the most frequently identified pathogens. Episodes of rotavirus diarrhoea, however, more often resulted in severe dehydration.

In their surveillance for ETEC in Matlab Hospital between February 1977 and January 1978, Black et al (4) identified the cause of diarrhoea in 85 per cent of all persons coming to the hospital. ETEC were very common in infants as well as in adults (estimated frequency = 25%). Expressed in another way the incidence of E. coli diarrhoea was 8.1 per 1000 persons compared with 3.7 for cholera. ST strains were most frequently found (incidence 4.2) followed by ST/LT (2.7) and LT (1.2) strains. Many enterotoxigenic E. coli, esp. ST/LT, were found to belong to relatively few serotypes.

2.2 Enterotoxins

To date only two well-recognized enterotoxins are known to be produced by E. coli, although others are suspected. These two enterotoxins, one heat-labile (LT) and the other heat-stable (ST), have been purified and their mechanisms of action have been well characterized. Both enterotoxins are known to be genetically controlled by DNA residing in transferable plasmids (13). ETEC strains however, vary in their ability to harbor the plasmids over long intervals: after storage and multiple transfers some strains lose their plasmids and thus the ability to produce enterotoxin.

2.3 Colonization factors

Bacterial surface structures characteristic of ETEC were first described in animal strains and are likewise plasmid-mediated. These fimbriae were shown to aid in colonization of the small intestine and subsequent production of disease. They are easily recognized by their ability to cause mannose-resistent haemagglutination (HA) of certain erythrocytes.

More recently analogous colonization factors have been identified in human strains of ETEC, and these have been designated CFA/I and CFA/II (5). These factors are also plasmid-mediated and single plasmids have been identified that carry genes for both CFA/I and ST (6). Originally it was thought that CFA were present in the majority of ETEC strains of human origin, but it now appears that they are highly associated with certain serotypes (CFA/I with 078:H11 and 12, CFA/II with 06:H16), and thus their frequency depends mainly on the serotypes isolated. It is also now clear that these recognized colonization factors are not prerequisites of virulence, since strains not possessing them can cause diarrhoea in volunteers (7). Therefore, it seems almost certain that additional colonization factors from human strains will be identified.

Evans et al (5) reported that 25/29 (86%) strains from travellers diarrhoea in Mexico possessed CFA/I. In contrast Gross et al (8) examined 89 ETEC pathogens and found that < 10% reacted with antibody to CFA/I. Levine et al in a recent study (7) of ETEC strains used in volunteer challenge studies, reported that out of eight strains causing diarrhoea only two produced CFA. He suggested that many strains had lost their CFA plasmids prior to the time of testing.

At Dr. Bernard Rowe's laboratory 1000 ETEC strains have been tested. They were brought there from Dacca by Dr. M. Merson. According to personal communication, 40% of these strains had CFA/I, CFA/II or "CFA/III". CFA/III agglutinate as CFA/I but do not give identical immunodiffusion.

2.4. ETEC immunity

Natural immunity to ETEC infection occurs as persons remain at high risk for infection. An inverse relationship was found between geometric mean serum antibody titers to LT and the occurence of diarrhoea due to LT-producing E. coli (9). Determined by ELISA the titers of IgG anti-toxin antibodies declined from birth to three months and rose thereafter to three years of age.

In earlier studies it has not been possible to obtain antisera protecting animals against challenge with ST (10) and it was supposed that ST was non-antigenic. Takeda et al however, have been able to immunize rabbits with purified ST and found that at a dilution of 1/4 the sera of immune rabbits will neutralize 2 mouse units of purified ST (personal communication).

Immunity in ETEC infection may not only be of antitoxic character. Latin American students rarely excrete the organism in stool asymptomatically, in contrast to the common finding of asymptomatic excretion of ETEC in stool of US students (11).

These data suggest that an antibacterial immunity is operative. This could be represented by intestinal antibodies to somatic antigens, colonization factors etc. CFA antisera neutralize the colonization potential of human ETEC possessing the respective antigen as tested in an infant rabbit model.

The WHO Scientific Working Group on Immunity and Vaccine Development (WHO/DDC/78.2) felt that more research was needed to develop tools to "assay anti-bodies to various colonization factors..... They would also inlcude improved assay of antibodies to enterotoxins and their subunits... Simple assays for such antibodies in serum as well as intestinal fluid are needed so that sero-epidemiological studies can be carried out."

2.5. Preliminary studies

In an attempt to analyze the prevalence of CFA in Bangladesh, a limited protocol (81-001P) was developed not knowing that a similar, but much larger, study was undertaken in UK. We specifically wanted to see if examination of fresh isolates yeilded a higher number of CFA and enterotoxin-positive strains. Another aim was to specify "new" colonization factors by using an extended haemagglutination system. Thirdly we found it important to develop laboratory methods in an area where the incidence of ETEC is highest in the world.

So far we have analyzed fresh samples from 91 patients in the surveillance study and 31 hospital patients (dark-field and cholera-culture negative and with a clinical picture not suggesting dysentery). 20-30 samples from family contacts in the campylobacter study have also been studied.

Three separate colonies plus a pool of 10 colonies are examined without further subculturing and storage. As seen in Table I, 45% in the patient group have either CFA/I or CFA/II compared with 22% of the surveillance subjects. In both groups, 70% of ent$^+$ strains have CFA/I, CFA/II or an adhesin not yet defined. This figure is almost double that given by Drs. Merson and Rowe.

LT/ST producing strains usually have CFA, but the LT only and ST only strains are more often without CFA Table 2 . In the surveillance group we have tried to randomize by age.

Isolates, stocked on agar slants for 3-4 months, when retested for CFA showed identical reactions regarding degree of haemagglutination. Thus no major loss of CFA seemed to take place over that time. It must be remembered, however, that the original isolates never were repeatedly subcultured which may have a more devastating effect on the plasmids.

TABLE I

Production of Enterotoxins and CFA

	Surveillance n = 91	Patients n = 31
ent^+, CFA/I	10	11
ent^+, CFA/I?		1
ent^-, CFA/I	2	
ent^-, CFA/I?	7	
ent^+, CFA/II	1	1
ent^-, CFA/II		1
ent^+, adhesin unknown	3	2
ent^-, adhesin unknown	2	
ent^+, adhesin negative	6	7
ent^-, adhesin negative	48	8
ent^-, type 1 pili	12	

ent^+ : ST, LT or ST/LT

CFA/I : agglutination with specific antiserum

CFA/I? : typical haemagglutination pattern but no agglutination with specific antiserum

adhesin unknown : haemagglutination pattern not consistent with CFAI or II and negative in both antisera

adhesin negative : no haemagglutination at all in any of the six systems used

TABLE 2

Production of LT/ST and CFA in Relation to Age of the Patient

A. Surveillance 243725 - 255825
256050 - 262075

		<1	1-2	2-6	7-15	>15
LT+	NoCFA	2		1		1
ST+	CFA	1		2		
	NoCFA	2		3	1	
LT+/ST+	CFA	3	3	6	2	2
	NoCFA			1		
NonETEC	CFA[x]	4	3			1
	NoCFA	19	19	17	8	10

[x] except type 1 - pili

B. Patients 252582 - 259377 (inclusive of SB 127-131)

		<1	1-2	2-6	7-15	>15
LT+	NoCFA	1				
ST+	CFA		1	1		2
	NoCFA					
LT+/ST+	CFA	1				10
	NoCFA					2
NonETEC	CFA					1
	NoCFA	2		3		2

2.6. Overall design of proposed study

This study has two parts: one involving patients, one working with bacterial strains already in stock on agar slants.

2.6.1 We will expand the CFA studies described in 2.5. Especially the groups of hospital patients and non-diarrhoeal subjects must be enlarged in order to allow comparisions between the groups. For that part fresh stool samples only are needed.

2.6.2 In order to study the immune response to ETEC diarrhoea the following considerations are made:

In our laboratory we have experience of assaying antibodies in different body fluids to cholera toxin, closely related to LT. In collaboration with Dr. Evans, Houston, Texas, we may be able to determine levels of antiboides to CFA/I and CFA/II with ELISA. Dr. Takeda, finally, is interested to measure antibodies to ST with the test described (2.4.).

To evaluate the efficacy of these tests, we will use acute and convalescent sera from the patients where stool specimens already are analyzed for CFA and ST/LT production (2.6.1.). We will also take paired samples of saliva to see whether a local immune response can be confirmed that way.

2.6.3 The haemagglutination pattern seems to be relatively stable over a couple of months (2.5). It also turns out that it is above all the enterotoxin producing E. coli strains which have mannose-resistent adhesins on their surface. Against that background, we will study tox$^+$ strains from the surveillance study: 100 LT, 100 ST and 100 LT/ST from 1980. They will also be randomized by age of the patient.

Their pattern of haemagglutination and their reaction with specific CFA antisera will be determined as well as their serotype (in collaboration with Dr. Ørskov, International Escherichia and Klebsiella Centre, Copenhagen). These data will then be matched with the information we already have on the patient and his clinical picture.

3. Rationale:

The predominance of rotavirus and enterotoxigenic E. coli as causes of life threatening diarrhoea suggests that priority for vaccine development should be given to these two pathogens.

3.1. Regarding ETEC, characterization of their virulence properties will indicate the appropriate antigens that may eventually be included in future vaccine strains. The enterotoxins are now well defined and assays are available which - some of them - can be performed in laboratories of a developing country.

So far only a few laboratories in the world are capable of performing characterization of ETEC by their colonization factors. These factors may be even more important for virulence, as immunity seems to be anti-bacterial rather than antitoxic. Thus the prevalence of CFA in countries where diarrhoea is common must be known before a rational selection of candidates for development of new vaccines is made (12).

3.2. Another way to get information on the relative importance of the different bacterial somatic structures and extra-cellular products is to evaluate their respective capacity to induce an immune response, in serum as well as on the mucosal surface.

B. SPECIFIC AIMS

1. To study the distribution of colonization factors in E. coli causing diarrhoea from the Dacca area.
2. To specify "new" colonization factors by using an extended haemagglutination system.
3. To correlate the presence of toxin and CFA with serotype and clinical features.
4. To determine the local and systemic immune response to CFA and enterotoxin (LT as well as ST).

C. METHODS OF PROCEDURE

1. STRAINS

The study will deal with two sources of E. coli strains:

1.1. Fresh isolates from

1.1.1 hospital patients (n=30-50) with moderate severe watery diarrhoea of 36 hours duration, darkfield and cholera culture negative and with a clinical picture not suggesting dysentery.

1.1.2 family contacts in the campylobacter study with no diarrhoea during the last few weeks (="negative controls")

1.2 300 strains-stocked on agar slants - from the surveillance study, already tested for LT- and ST- production.

2. SUBJECTS

see C 1.1.1.

3. CLINICAL SPECIMENS

3.1 Stool One sample will be obtained as early as possible and sent for culture. If culture is negative for cholera, salmonella and shigella, 3 single E.coli colonies plus a pool from the McConkey plate will be further analyzed (4.1).

3.2 Serum will be separated from blood samples. Fingerstick blood samples (volume 200 µl) will be taken from children (<15 years) and venepuncture blood samples (volume 5 cc) will be taken from adults. Two samples will be taken - the first at the same time as the stool sample is delivered, the second 14 days later.

3.3 Saliva will be collected twice: at the same time as the serum samples. The subject is asked to chew on parafilm for a few minutes while the saliva is put in a fecal cup.

4. LABORATORY INVESTIGATIONS

4.1 The fresh isolates will - without further storing and repeated sub-culturing be tested for

1. LT production - GM_1-ELISA
   - CHO-cell
2. ST - production - infant mouse assay
3. CFA - haemagglutination

The surveillance strains will be tested for CFA only.

4.2 Identification of colonization factors with haemgglutination

4.2.1 Media

Isolates are cultivated on CFA-agar (18 hrs, 37°C)

1%	casamino acids	Solve in H_2O, autoclave and pour in Petri dishes
0.15%	yeast extract	
0.005%	$MgSO_4$	
0.0005%	$MnCl_2$	
2%	agar	

Isolates are also cultivated on ordinary blood agar plates

4.2.2 Haemagglutination (HA)

HA is performed in blood with or without 1% mannose added. 15 µl of bloodsuspension (+4°C) is put on a glass slide. Colonies of the bacteria to study are harvested and carefully mixed in saline on the slide with the erythrocyte suspension. If positive: "true" agglutinates (different from what is seen with the bacteria in saline) should have been performed. From each patient 3 colonies and a pool will be tested.

Positive HA is graded 0,+,++ and +++.

4.2.3. Erythrocytes

We are using a battery of erythrocytes:
Human A, Bovine, Chicken, Goat, Rat and Guineapig. Blood is collected once weekly in heparinized tubes. The erythrocytes are spinned down and washed 3-4 times in PBS. The cells are then diluted in saline to 3% final concentration with or without 1% mannose added. Erythrocytes should befresh: not kept longer than 4-5 days (at +4°C).

4.2.4 Agglutination

For confirmation, haemagglutinating strains are agglutinated with antisera specific to CFA/I and CFA/II (produced by Drs. Svennerholm and Ahren).

4.3. Serology

4.3.1 Antibodies to LT will be determined with the ELISA. Serum specimen will be analyzed for specific IgA as well as IgG antibodies, saliva only for specific IgA.

4.3.2. The test for ST antibody will be performed by Dr. Takeda in his laboratory in Osaka, Japan. If useful, this technique will be set up at ICDDR,B in the future.

4.3.3. Purified CFA/I and CFA/II is available through collaboration with Dr. Evans. Her method (ELISA) for determination of antibodies to these antigens will be set up in our laboratory.

D. SIGNIFICANCE

This study will extend our knowledge on the frequency of various colonization factors in E.coli strains causing diarrhoea in Bangladesh. If these antigens are proven to be responsible for the colonization, they may have an important role as a component in a combined vaccine to prevent human diarrhoea due to ETEC.

The relation between the virulence factors known so far (ST- and LT-enterotoxin, CFA), the clinical picture in different age groups and the immune-response will also be investigated. Is ST-diarrhoea more severe in infant when CFA is present? Are there mucosal (=salivary) antibodies to CFA? Age differences? Can antibodies to ST be demonstrated?

E. FACILITIES REQUIRED

Patients will be treated in the TC or in the hospital depending on the severity of their diarrhoea.

A field worker has to accompany them to their home, in order to be able to return later for taking serum - and saliva samples.

Laboratory specimens will be handled by the Immunology and Microbiology laboratories. No new facilities are required.

F. COLLABORATIVE ARRANGEMENTS

1. Dolores G. Evans, The University of Texas Medical School at Houston, Texas, USA
2. Ida and Fritz Ørskov, statens Seruminstitut, Copenhagen, Denmark
3. Yoshifumi Takeda, Research Institute for Microbial Diseases, Osaka University, Japan

REFERENCES

1. Merson MH, Sack RB, Islam S, et al. Enterotoxigenic Escherichia Coli (ETEC) disease in Bangladesh Clinical, therapeutic and laboratory aspects. Proceedings of 13th Joint Conference on Cholera 1977.

2. Merson MH, Ørskov I, Sack RB, Huq I, and Koster FT. Relationship Between Enterotoxin Production and Serotype in Enterotoxigenic Escherichia coli. Infect Immun 1979;23:325-329.

3. Black RE, Merson MH, Huq I, Abdul Alim ARM. Epidemiological importance of diarrhoea agents in rural Bangladesh. Nobel Symposium 1980.

4. Black RE, Merson MH, Rowe B, et al. Epidemiology of enterotoxigenic Escherichia coli in rural Bangladesh. Symposium on Cholera, Karatsy 1978.

5. Evans DG, Evans DG, Tjoa WS and DuPont HL. Detection and characterization of colonization factor of enterotoxigenic Escherichia coli isolated from adults with diarrhoea. Infect Immun 1978;19:727-36.

6. Smith HR, Cravioto A, Willshaw GS, et al. A plasmid coding for the production of colonization factors antigen I and heat -stable enterotoxin in strains of Escherichia coli serogroup 078. Federation of European Microbiological Societies Letters 1979;6:255-260.

7. Levine MM, Rennels MB, Daya V, Hughes TP. Haemagglutination and colonization factors in enterotoxigenic and enteropathogenic Escherichi coli that cause diarrhoea. J Inf Dis, 1980;141:733-737

8. Gross RJ, Cravioto A, Seohand SM, Cheasty T, Rowe B. The occurence of colonization factors (CF) in enterotoxigenic Escherichia coli. Federation of European Microbiological Societies Letters 1978;3:231-233.

9. Evans DJ, Ruiz-Palacios G, Evans DG et al. Humoral immune response to the heat-labile enterotoxin of E. coli in naturally acquired diarrhoea and antitoxin determination by passive immune hemolysis. Infect Immun 1977;16:781-88.

10. Robertson DC, Alderate JF. Chemistry and biology of the heat-stable E. coli enterotoxin. In:Ouchterlony O, Holmgren J, eds. Cholera and Related Diarrhoea (43rd Nobel Symposium). Basel:S Karger 1980:115-26.

11. Pickering LK, DuPont HL, Evans DG et al. Isolation of enteric pathogens from asymptomatic students from the United States and Latin America. J Inf Dis 1977;135:1003-05.

12. Sack RB. Enterotoxigenic Escherichia coli: Identification and characterization. J Inf Dis 1980; 142:279-786.

13. Smith HW, Linggod MA. The transmissible nature of enterotoxin production in a human entero-pathogenic strain of Escherichia coli. J Med Microbiol 1971; 4:301-5

ABSTRACT SUMMARY

1. The population to be studied includes patients coming to the hospital with diarrhoea, presumably caused by ETEC. Even young infants will be involved as ETEC diarrhoea is very common in that age group.
2. The risks are minimal. Fingerstick blood samples (volume 200 ul) will be taken from children (<15 years) and venepuncture blood samples (volume 5 cc) will be taken from adults.
3. See para 2.
4. Research records are kept in a locked filing cabinet in the investigators office. Samples and records will be identified by code number.
5. Signed informed consent will be obtained, if applicable from the parents.
6. No interview.
7. The individual will benefit from the treatment of his illness and from the check-up after 2-3 weeks by a fieldworker. The society will benefit by the development of a successful vaccine to E. coli diarrhoea.
8. The research will use medical records, blood, stool and saliva.

SECTION III - BUDGET

A. DETAILED BUDGET

1. Personnel Services:

	Effort	Time	Annual Salary	Taka	Dollars
L. Gothefors	20%	3 months	40,000	-	2,000
B. Stoll	10%	6 months			1,000
I. Huq				No Cost	
Field worker		4 months		8,000	-
Lab staff Microbiology	100%	5 months		18,000	-
Immunology - one for		5 months		24,000	-
two for		2 months			
			Sub Total :	50,000	3,000

2. Supplies and Materials

Plastics, glassware (plates, pipette tips, tubes)		-	2,000
Reagents (antisera, conjugates, enzyme)		-	1,000
100 ST-test		600	-
100 LT-test	(CHO-cell)	2,000	-
100 LT-test	(GM_1, ELISA)	1,500	-
Blood for HA	(Tk.50/week)	1,500	-
	Sub Total :	5,600	3,000

3. Equipment

0

4. Hospitalization costs

0

5. Outpatient care

0

		Taka	Dollar
6.	ICDDR,B transport		
	7.5 miles x 2 x 2 per patient 80 patients = 2,400 miles	4,800	-
	Sub Total :	4,800	0
7.	Travel		
	One round-trip ticket (to see the collaborative laboratives)	-	2,000
	Sub Total :	0	2,000
8.	Transport of things		
	Samples to Japan and Europe	-	400
	Sub Total :	0	400
9.	Rent, communication		
	0		
10.	Printing		
	Forms, stencil, xerox	1,000	-
	Publication costs	-	300
	Sub Total :	1,000	300
11.	Contractual service		
	0		
12.	Construction		
	0		
	Grand Total :	61,400	8,700

Or US Dollars - 12,540.00

(16 Taka = 1 US Dollar)

B. BUDGET SUMMARY

	US-Dollar
1. Personnel Services	6,127.00
2. Supplies and Materials	3,350.00
3. Equipment	-
4. Hospitalization costs	-
5. Outpatient care	-
6. ICDDR,B Transport	300.00
7. Travel	2,000.00
8. Transport of things	400.00
9. Rent, Communication	-
10. Printing	363.00
11. Contractual service	-
12. Construction	-
Total US $:	12,540.00

PERMISSION FORM - STUDY OF ANTIBODY RESPONSE TO ENTEROTOXIGENIC E.COLI

The International Centre for Diarrhoeal Disease Research, Bangladesh is carrying out research in order to find a vaccine to diarrhoea caused by E. coli. We are trying to understand which factors in the bacteria are important in causing disease. We would like you to participate in this study. If you decide to participate, you can expect the following:

1. We will collect one sample of stool.
2. We will also take two samples of saliva and blood: the first during your stay in hospital, the second 2 weeks later when a field worker will visit your house.
3. None of the tests are harmful to your health. Blood drawing - finger stick for children, venepuncture for adults - is only somewhat uncomfortable.
4. You do not have to join the study. If you decide not to join, you will still be elligible for care at ICDDR,B. You may also decide to withdraw after entering the study and this will not affect any medical care you might receive now or later on.
5. Your medical records will be kept confidential.

If you decide to join the study, please sign here -

_____

Patient or Guardian

_____

Investigator

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র
বাংলাদেশ

সম্মতি পত্র

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র বর্তমানে উদরাময় নিয়ন্ত্রণের "হুইলেরী" নামক একটি জীবাণু প্রতিষেধক তৈরী আবিষ্কারের চেষ্টা করছে। আমরা এই রোগজীবাণুটি নিস্তেজ করতে পারি তার অনুসন্ধান কার্য চালিয়ে যেতে উদরাময় দ্বারা আক্রান্ত রোগীর সাহায্য প্রার্থনা করছি। যদি আপনি আমাদের এই গবেষণায় অংশ গ্রহণ করতে ইচ্ছুক হোন তবে নিম্নলিখিত পদ্ধতিগুলি অনুসরণ করুন।

১। আপনার পায়খানার নমুনা সংগ্রহ করবো।

২। দুইবার আপনার মূত্র ও রক্ত সংগ্রহ করবো। প্রথমবার যখন আপনি হাসপাতালে থাকবেন তখন, এবং দ্বিতীয়বার - ২ সপ্তাহ পর তখন একজন পল্লী কর্মী আপনার বাসায় যাবেন।

৩। এগুলো আপনার কোন ক্ষতি করবে না। শুধু রক্ত নেবার জন্য আপনার শরীরে সুঁচ দেবার সময় সামান্য বিরক্তি বোধ করবেন।

৪। এই গবেষণায় অংশগ্রহণ না করলেও আপনার চিকিৎসা করা হবে। আপনি ইচ্ছা করলে যে কোন সময় গবেষণা পরিত্যাগ করতে পারেন, তাতে চিকিৎসার কোন তারতম্য ঘটবে না।

৫। আপনার চিকিৎসা সম্পর্কিত সকল তথ্য গোপন রাখা হবে।

এই গবেষণায় অংশগ্রহণে রাজী থাকিলে আপনি এইখানে টিপসই দিন -

অনুসন্ধানকারীর স্বাক্ষর

স্বাক্ষর / টিপ সহি