22

REVIEW BOARD ON THE USE OF HUMAN VOLUNTEERS Date 24th November 1
CRL

Principal Investigator Michael H. Merson M.D Trainee investigator(if any) _____

Application No 78-003 Supporting Agency(if Non-CRL) _____

Title of study Epidemiology of Rotavirus Diarrhea

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.

Michael H. Merson
Principal Investigator

_____
Trainee

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

Recd 14/12/77

78-003

Attachment A

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 address 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 amount of time required for the interview.

7. Assess the potential benefits to be gained by the individual subject or society, the benefits which may accrue or knowledge generated as a result of the planned work. Indicate how the person is compensated for 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.

78-003

SECTION I - RESEARCH PROTOCOL

1 Title: Epidemiology of Rotavirus Diarrhea.

2 Principal Investigator: Michael Merson. M.D.

Collaborating Investigator: Robert Yolken M.D.,
Laboratory of Infectious Diseases,
National Institute of Allergy and Infectious Diseases
National Institutes of Health,
Bethesda, Maryland, USA.

3. Starting Date: December 1 1977.

4. Completion Date: March 1 1978.

5. Total Direct Cost:

6. Abstract Summary This study will define the epidemiology of rotavirus diarrhea in rural Bangladesh. Fifty cases of rotavirus diarrhea presenting at the Matlab Hospital will be identified by the ELISA assay during the winter season. The clinical course of hospitalized cases will be documented. Breast milk from lactating mothers of hospitalized cases and nasal secretions from cases will be screened for rotavirus and rotavirus antibodies by the ELISA assay All household contacts and bari contacts age 5 or less of all index cases will be examined for rotavirus infection by stool examination and serology Stools from animals belonging to families of these cases will be examined for rotavirus and any rotavirus detected will be compared by RNA genotype analysis to rotavirus from cases. This study will provide information on the ratio of symptomatic/asymptomatic cases in rotavirus diarrhea, the transmission of the organism in the family and bari in man and animals and the role of serum antibody in protection from disease. Cases of rotavirus diarrhea will also be identified by stool examination and serology in a village that has ongoing surveillance for E. coli diarrhea and other pathogens.

SECTION II RESEARCH PLAN

A. INTRODUCTION

1. Objective

To define the epidemiology of diarrhea caused by rotavirus in rural Bangladesh.

2. Background

Studies conducted since 1973 have identified a newly recognized virus as an important cause of childhood diarrhea worldwide. This virus has been given various names and is now most commonly referred to as rotavirus. Most studies of this virus have been conducted in highly developed countries, especially England (1), Australia (2) the United States (3), and Japan (4) and have for the most part included only patients who have been hospitalized with dehydrating diarrhea. These studies have demonstrated that rotavirus is responsible for approximately 50-70% of hospitalized diarrheal cases occurring in the winter months in children 6 months to 4 years of age. The typical clinical picture includes diarrhea of acute onset with antecedent or subsequent vomiting and fever (5). Disease in adults is less common (6). Serologic studies using a human rotavirus or antigenically related Nebraska calf diarrhea virus (NCDV) complement fixing (CF) antigen have shown that by age four 50-70% of children have demonstrable antibodies, indicating that infection is very common in early life (7, 8).

Studies of rotavirus diarrhea in lesser-developed countries are comparatively few. Studies in Vellore, India (9, 10) have documented rotavirus to be a cause of febrile, dehydrating diarrhea. One study conducted at CRL in 1975 in Matlab indicated that rotavirus may be responsbile for as many as 55% of moderate to severely dehydrating diarrheal cases in children age 2 years or less seen at Matlab hospital. In that study rotavirus was found to produce a febrile diarrhea with vomiting lasting 9 10 days and resulting in more fluid loss than enterotoxigenic *E. coli* diarrhea (11). This study

included only 12 rotavirus patients and thus further clinical observations of the disease are needed. Additional evidence that rotavirus is an important cause of diarrhea in Bangladesh is provided by data from two additional as yet unpublished studies. In one by Black et al rotavirus was found responsible for diarrhea in 30% of children 0 2 years of age given care at the CRI OPD in the winter of 1976. Serologic studies revealed that 67% of children age 4 or less had demonstrable CF antibody to rotavirus In the second study by Merson et al of severely dehydrating diarrhea in adult males in the fall season of 1976 over 95% of patients were found to have CF antibody to rotavirus in their acute sera and 5% had rotavirus in their admission stool specimen. In that study five of nine patients with rotavirus infection were also infected with shigellae.

A paucity of data is available from any geographic location on the incidence of rotavirus diarrhea in a community setting or on the trans mission of disease among family contacts. In one study from Washington DC. 55% of adult contacts of children with rotavirus diarrhea had serologic evidence of infection but only 11% had diarrhea (12). The primary reason why such studies have not been carried out to date is that rotavirus has not been grown in tissue culture and electron-microscopy has been primarily used to identify rotavirus particles in stool. This technique requires a sophisticated laboratory and is very time consuming and does not lend itself to testing of a large number of stool specimens.

Recently numerous investigators have developed simpler assays for detecting rotavirus viral particles in stool. These include counter immuno electro phoreses (13) radio-immuno assay (14) and free viral immuno fluorescence (15). However these assays are either of limited sensitivity or also require sophisticated laboratory equipment. One very significant development

has been the development of an ELISA (enzyme-linked immuno-absorbent assay) test to detect rotavirus antigen in stool. This assay is simple, very sensitive, and is suitable for field laboratories (16). It has been set up at CRL in November, 1977. Initial results using this assay demonstrated that 9 of 19 children below age 3 seen at the CRL hospital with dehydrating diarrhea not caused by V. cholerae were infected with rotavirus compared with 1 of 29 persons age 3 or over.

Studies to date have questioned the role of serum antibody in conveying resistance to rotavirus infection (3). Soon to be published data from a small number of children in which the ELISA assay was used to measure antibodies to rotavirus suggests that detectable serum levels of anti-rotavirus IgG are not protective against symptomatic infection and that development of anti rotavirus IgM correlates with the presence of recent symptomatic infection (17). Additional studies are warranted to elucidate the role of immuno globulin specific immunity in sera and in intestinal secretions against rotavirus infection. Analysis of breast milk secretions is presently one of the easiest approach to investigate intestinal immunity

An additional area that requires study is the relationship between rotavirus infection in man and other animals. Rotavirus is a well-described cause of severe diarrhea in young calves (18) and lambs (19) and rotavirus antigen has been found by CF to be related to the Nebraska calf diarrhea virus (NCDV), the epizootic diarrhea of infant mice (EDIM) virus, the simian agent (SA 11) and the O'' (offal) agent which was recovered from mixed intestinal washings of sheep and cattle (20). The human, calf, lamb and the other viruses have been shown to share a common antigen probably situated within the inner capsid layer (21). What has not been clearly established is whether in nature rotaviruses from animals and man can produce illness in heterologous

species. Studies conducted in a laboratory setting have provided strong evidence that human rotavirus can cause diarrhea in gnotobiotic newborn calves (22), gnotobiotic newborn pigs (23), and colostrum deprived newborn rhesus monkeys (24). Studies are needed to determine whether animal strains are capable of causing diarrhea in humans.

3. Rationale

Diarrheal disease is a leading cause of mortality in Bangladesh in the first years of life. At present many diarrheal cases are of unknown etiology. This study will document the relative importance of rotavirus as a cause of diarrhea and will elucidate factors that play a role in transmission in rural family settings and between man and animals. If rotavirus is found to be an important cause of diarrhea in Bangladesh an intervention measure such as testing of a rotavirus vaccine which is currently being developed would be warranted.

B. SPECIFIC AIMS

1. To define the clinical spectrum of rotavirus diarrhea.
2. To determine the importance of rotavirus as a cause of severe dehydrating diarrhea in Bangladesh.
3. To elucidate transmission patterns of rotavirus in families and baris
4. To assess the infectivity of animal and human rotavirus strains to the heterologous species.
5. To determine the role of immuno-globulin specific serum and intestinal immunity in protection from rotavirus diarrhea.
6. To determine the incidence of rotavirus diarrhea in a village community
7. To determine the role of breast milk in protecting against rotavirus diarrhea

C. METHODS OF PROCEDURE

1. Family Study

Location - Matlab field trial areas; when possible the areas closest to Matlab.

Time Period - December 1, 1977 - March 1, 1978.

Study Subjects - Household members of families with index cases visiting Matlab Hospital with rotavirus diarrhea and children (age 5 or less) of families in the same bari as the index family.

a) Index Cases

(1) Definition - Index cases will be selected from patients treated as inpatients or outpatients at the Matlab Hospital and having the following characteristics:

(a) they are from the appropriate part of the field area.

(b) they have an illness characterized by watery diarrhea without blood and/or vomiting of 5 days or less duration with or without fever.

(c) they are the only members of their family with diarrhea or they become ill at the same time (within 48 hours) as other family members when possible)

(2) Selection

(a) Each day stools from all persons visiting the Matlab Hospital will be examined. Two rectal swabs will be obtained. One will be plated on Monsur's, SS, and MacConkey agars and the plates incubated at 37°C for 18-24 hours. The second will be inserted into a vial containing Phosphate Buffered Saline (PBS) PH7.4. From all persons who remain at the hospital for initial intravenous rehydration

(inpatients) a second rectal swab will be obtained and placed in PBS on the day of admission. All PBS material will be examined for rotavirus.

(b) On the morning following admission the agar plates will be examined for vibrio, shigella and salmonella and the ELISA plates read for rotavirus

(c) Patients who are ELISA positive for rotavirus and who have characteristics 1a-1c above will be used as index cases (up to 2/day). From the MacConkey plate for each of these patients 5 lactose positive (LP) colonies that are typical of E. coli and a pool of 10 other LP colonies will be picked to nutrient agar slants. These will be stored for testing for LT testing by the CHO assay (25) and ST testing by the infant mouse assay (26).

(3) Clinical and Laboratory Evaluation

All rotavirus positive cases (index cases and other patients who would have been eligible as index cases but were not selected) who require hospitalization (i e. intravenous therapy) for treatment of their diarrhea will be studied as follows:

(a) Standard forms will be used to obtain a history and to record the clinical course of the patient's illness Feeding history and a history of any recent illness in mothers will be obtained. Stool volume, duration of all symptoms and intravenous and oral fluid requirement will be measured. Admission and discharge weight and discharge height will be recorded.

All patients will be discharged 24 hours after diarrhea has stopped.

(b) Two rectal swabs will be obtained on each hospital day and examined for rotavirus by the ELISA assay.

(c) Serum will be obtained on the second hospital day by finger stick (acute serum). Convalescent sera will be obtained by fingerstick at home 10 days and 21 days after admission. Acute and convalescent sera will be examined for IgG and IgM rotavirus antibody by the ELISA assay. (for index cases only).

(d) On the second hospital day nasal washings will be obtained and examined for rotavirus and IgG, IgM and IgA rotavirus antibody by the ELISA assay.

(e) On the second hospital day a breast milk sample (1 cc) will be obtained from lactating mothers of infant index cases and examined for rotavirus and immunoglobulin specific antibody to rotavirus by the ELISA assay. If possible a second milk sample will be obtained from the mothers 21 days after admission, and on any other study day on which the mother has diarrhea. (follow-up specimens for index cases only)

b) Family/Bari Contacts

After the index cases are selected, visits will be made to their family/bari and the following procedures carried out:

1) Day 1 house/bari visit

(a) On a standard form the name, age sex, and history of diarrhea for that day and the seven days prior to the visit will be recorded for every member of the household (defined as all people eating together and sleeping under

the same roof) and every child age 5 or less in the bari. These persons will be subsequently referred to as contacts.

(b) Information will be obtained on water sources used by each family member.

(c) A rectal swab will be obtained from each contact (and outpatient index cases) and placed in PBS. A second swab will be obtained and placed in Cary Blair transport media if the index case had shigellae, salmonellae or vibrio infection in addition to rotavirus infection. All swabs will be returned to the Matlab laboratory. If any contact has diarrhea a second rectal swab will be obtained when possible by the dai/later in the day (or female field worker) and preserved in PBS overnight.

(d) A fingertip blood specimen will be collected from each contact using a Natelson mico blood collection tube and will be spun at the Matlab lab.

(e) Stool cups will be provided for all contacts to use if diarrhea occurs. These stools will be examined on subsequent visits to document the character of the diarrhea.

(f) A 1 cc breast milk sample will be obtained when possible from lactating mothers of children age 5 or less in the bari. The mothers will also be questioned about feeding patterns.

(g) Swabs will be taken from cows and goats owned by the index case house and from all cows and goats in the bari with diarrhea and placed in PBS. Two swabs will be collected daily from animals with diarrhea.

(h) Water samples (100 cc) will be taken from drinking water in the index case home, from the source of that water, and from any surface water source used for cooking, bathing or washing.

2) Day 2 9 house/bari visit

(a) History of diarrhea from each contact will be obtained at each visit.

(b) Rectal swabs will be obtained from each contact at each visit as in b1)(c).

(c) On day 2 and 3 repeat animal cultures will be obtained as in b1)(g).

(d) Lactating mothers of children in the bari age 5 or less with diarrhea will be queried about feeding histories as in b 1)(f).

3) Day 10 house/bari visit

(a) History of diarrhea during the previous day will be obtained from each contact.

(b) Rectal swabs will be obtained from each contact as in b 1)(c).

(c) A fingertip blood specimen will be obtained from each family contact only as in b 1)(D).

(d) Each contact will be asked to record any diarrhea during the next eleven days. The village dai will also be asked to visit the contacts daily to record episodes of diarrhea.

4) Day 21 house/bari visit

(a) History of diarrhea during the previous eleven days will be obtained from each contact.

(b) Rectal swabs will be obtained from each contact as in b 1)(c).

(c) A fingertip blood specimen will be obtained from each contact as in b 1)(d).

5) Control environmental/animal cultures

On day 1-3 visits will also be made to the nearest adjacent bari and a house randomly selected. If the occupants of the house deny diarrhea in the previous week, the house will be selected as a control house. If diarrhea has occurred, other families will be questioned until a suitable control house is found. Then water cultures (for control home) and animal cultures (for control home and bari) will be taken as described for index cases in b 1)(g) and b 1)(h).

6) Laboratory procedures

(a) All PBS suspensions containing rectal swabs will be examined for rotavirus antigen by the ELISA assay. Rotavirus detected in stools from humans and animals in the same household or bari will be studied by the ELISA blocking test to determine their similarity. In selected instances the specific RNA genotype patterns will be determined.

(b) All swabs in Cary Blair will be examined for vibrios, shigellae and salmonellae by routine CRL methods.

(c) All sera will be examined for IgG and IgM rotavirus

antibody by the ELISA assay.

(d) All breast milk samples will be tested for rotavirus antigen and immunoglobulin specific rotavirus antibody

(e) All water specimens will be passed through an Amicon filter and the filter then examined for rotavirus by the ELISA assay. Coliform counts will also be determined.

Sample Size We estimate that the study sample should include 250 persons in families of index cases. This means that we would like to study 50 families and baris. To do this the following laboratory tests are required:

a) Antigen Detection

1) To find 50 index cases (assuming 1 in 20 patients screened are positive and 1/3 of patients screened are hospitalized) we would need to test 1665 stools Hospital follow-up cultures from stool, breast milk and nasal secretions from inpatients would require about 200 tests.

2) Contact studies would require 3300 tests for family members (6 members per family) 3950 tests for bari members (7 children/bari) and 2000 tests for animal and environmental cultures.

b) Antibody determinations

1) Index case studies will require 100 antibody determinations.

2) Contact studies will require 1750 antibody determinations.

Data Analysis From the contact study the proportion of family members and children less than age 5 in their bari becoming ill or infected with rotavirus will be determined and the spread within the

family and bari will be studied. The rates of severe and mild diarrhea and asymptomatic infection will be calculated. The occurrence of symptoms will be correlated with serum immunoglobulin levels. The frequency of rotavirus isolation from animals and water in the study and control household will be compared. Transmission of rotavirus from animal to man will be assessed. The role of breast milk in protecting against rotavirus infection will be determined.

From the hospital study the clinical picture of severe rotavirus diarrhea including duration of virus excretion will be described. The importance of respiratory transmission will be assessed by the nasal washings. Breast milk antibody determinations will elucidate the relationship between the quantity of intestinal antibody and disease occurrence in mothers of ill children and disease severity in their children.

2. Community Study of Rotavirus Epidemiology

Location - Since October 1977 surveillance of enterotoxigenic E. coli and rotavirus diarrhea has been conducted in a village in Matlab with 1200 residents.

Time Period - Surveillance for these two diseases will continue in this village until at least March 1, 1978.

Study Subjects Procedures used in this village for selection of subjects are described in protocol 77-025 (Dr. R.E. Black investigator).

Laboratory Tests - To study rotavirus in this village two laboratory tests will be added:

a) A second rectal swab will be obtained from each case and control and will be preserved in a vial of PBS and the suspension tested for rotavirus by the ELISA assay.

b) When rotavirus infection is documented in the village, and 2 months

later a fingerstick blood specimen will be obtained in a Natelson tube from all members of 50% of the village families and will be examined for IgG rotavirus antibodies. At the time of the second bleed Td inoculation will be offered.

This work will require testing of 1170 stools for rotavirus antigen and 1200 sera for rotavirus antibody.

Data Analysis In this study the rates of symptomatic and asymptomatic rotavirus infection during the winter season will be established by age. The frequency of antibody response in ill and well infected persons will be compared. The relationship between serum titer and occurrence of symptoms after infection will be assessed.

D. SIGNIFICANCE

This study will provide information on the importance of rotavirus as a pathogen and the spectrum of rotavirus diarrhea in rural Bangladesh and will define transmission patterns of the disease in the family and community setting. The data will be important for determining the age groups at greatest risk of developing serious disease and thus will indicate who would benefit from rotavirus vaccine or other intervention measures. The immunologic studies will provide information on the role of serum and intestinal antibodies in protection from rotavirus infection. The information obtained may provide information about immunity against other enteric pathogens. The animal studies will provide needed data on the pathogenises of human and animal rotaviruses in heterologous species. This information also may have relevance toward development of intervention measures, especially if animal strains are found to be capable of causing human disease. Secondarily, these studies will also indicate the role of rotavirus as a cause of animal diarrhea in rural Bangladesh.

In addition to these items the study will serve the very useful purpose of establishing the ELISA assay at CRL. This assay will undoubtedly be useful to detect other antigens and measure antibodies to other pathogens.

E. FACILITIES REQUIRED

Office space - no additional space required.

Laboratory space - half a Revco freezer will be needed for storage of specimens.

Hospital resources - a study ward will be needed in Matlab for 10 beds.

Animal resources - 600 infant mice.

Logistical support none required.

Equipment - none.

F. COLLABORATIVE ARRANGEMENTS

Dr. Robert Yolken, LID, NIAID NIH, USA visited CRL in November, 1977 to set up the ELISA assay for rotavirus antigen detection and rotavirus antibody measurement. He will provide consultative advice throughout the study and will carry out some of the laboratory procedures at his laboratory These will include RNA genotype analysis of select animal and human strains, ELISA blocking antibody tests with human and animal strains, and immunoglobulin specific rotavirus antibody determinations in nasal secretions breast milk, and sera. RNA analysis will be performed by Anthony Kalica, Ph.D who is at the same laboratory as Dr. Yolken.

SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

Name	Position	% or # of days	Annual Salary	Project Requirement Taka	Project Requirement $
Dr. Michael Merson	Investigator	25%	$ 42,000		10,500
Dr. Robert Black	Co-investigator	10%	$ 46 000		4.600
Dr. Phil Taylor	Co investigator	25%	$ NA		
Dr. Mizanur Rahaman	Co-investigator	10%	Tk 70,331	7033	
Dr. Md. Yunus	Co-investigator	10%	Tk 54,682	5468	-
Mr. Ansurradin Ahmed	Co-investigator	5%	Tk 72.708	3635	
To be named(Matlab)	Sr. Ward Nurse (3)	45d	Tk 15 818 x3	5932	
To be named (Matlab)	Cleaners (3)	15d	Tk 7 095 x2	591	
To be named(Matlab)	Pathology Sr.Lab Tech	90d	Tk 16,605	4151	
To be named(Matlab)	Field Assistant (3)	30d	Tk 18 739 x 3	4685	
A.R.M.A. Alim	Sr.Research Assist.	45d	Tk 30,420	3803	
To be named	Bact.Sr.Research Tech	25d	Tk 23 830	1986	-
To be named	Bact.Sr.Research Tech.	25d	Tk 23,830	1986	-
Mr. Azizul Huq(Matlab)	FSA	80d	Tk 28.061	7015	
To be named(Matlab)	Field Assistant (13)	80d	Tk 18 739x13	60902	-
Mr. S. Huda	Sr.Research Tech.	180d	Tk 22,483	11242	-
Mr. J. Gomes	Sr. Laboratory Tech.	60d	Tk 22 108	3685	
Mr. Golam Kibriya	Sr. Research Assist.	7d	Tk 41,933	806	-
Dr. Al Mahmood	Head, Animal Resources	14d	Tk 45,470	1748	
To be named(Matlab)	Country Boatman	80d	Tk 11/d	880	
To be named(Matlab)	Dais (6)	80d	Tk 6/d	2880	-
To be named	Statistical Assist.(1/6)	30d	Tk 19 681	2236	
			Sub total	130,667	15,100

2. SUPPLIES AND MATERIALS

Items	Unit Cost	Amount Required	Project Requirement Taka	$
Rectal Swab for SSV	Tk 7.6	2400	18,240	-
Stock vials	Tk 0.5	300	150	-
CHO Assay	Tk 3.0	300	900	-
ST Assay	Tk 3.1	300	930	-
ELISA Assay -virus(includes vials)	Tk 1.5	12285	18,428	-
ELISA Assay - antibody	Tk 2.0	3050	6,100	-
Natelson tubes	$ .13	2950	-	383.50
1 dram vials (serum storage)	Tk 0.6	2950	1,770	-
Stool cups	Tk 0.5	200	100	-
100 cc milk dilution bottles	Tk 15	200	3,000	-
Coliform Count	Tk 1.5	200	300	-
Plasma SG	Tk 0.25	50	13	-
Misc. Costs (pens, pencils)				50.00
Multivit capsules, candy				
		Sub total	49,928	425.50

3. EQUIPMENT None.

4 PATIENT HOSPITALIZATION

Estimated 100 patient days		13,000
	Sub total	13,000

5. OUTPATIENT CARE None

6 CRL TRANSPORT

Dacca/Matlab/Dacca 2 per week for 15 weeks		9,000
Water transport speed boat (98.84 per hr)		59 304
Land transport (1.4 per mile)		5,250
Country boat		3,000
	Sub total	76 554

		Project Requirement Taka	$
7. TRAVEL AND TRANSPORTATION OF THINGS			
Local travel none			
International travel Meeting		-	2,500
	Sub total		2 500
8. TRANSPORTATION OF THINGS			
Local Shipments (to Matlab) 4 trips		1 200	-
Shipment of 3000 vials to NIH			2,000
	Sub total	1,200	2 000
9 RENT COMMUNICATION AND UTILITIES			
Postage		500	200
Telephone (to NIH)		-	
Rent - none	Sub total	500	200
10. PRINTING AND REPRODUCTION			
Forms		3,000	-
Publication cost		-	400
	Sub total	3,000	400

1. CONTRACTUAL SERVICES None

2. CONSTRUCTION None

B BUDGET SUMMARY

		Taka	Dollars
1.	Personnel	130,667	15 100,00
2.	Supplies	49,928	423,50
3.	Equipment	–	–
4.	Hospitalization	13,000	–
5.	Outpatients	–	–
6.	CRL Transport	76 554	–
7.	Travel Persons	–	2 500,00
8.	Transportation Things	1,200	2.000,00
9.	Rent/Communication	500	200,00
10.	Printing/Reproduction	3,000	400,00
11.	Contractual Services	–	–
12.	Construction	–	–
	Total	274.849	20,623.50

Dollar Total (15.5 Tk/dollar) = 38.358

Abstract Epidemiology of Rotavirus Diarrhea

This study will define the clinical spectrum and transmission patterns of rotavirus diarrhea in a rural environment. Using an ELISA assay cases of rotavirus diarrhea will be identified at the Matlab hospital. The clinical course of hospitalized patients will be carefully documented. Nasal excretions from these cases and breast milk from lactating mothers will be examined for rotavirus antigen and immunoglobulin specific antibody. Visits will be made to the household and bari of all cases. Stool specimens will be obtained from each household family member and each bari member age 5 or less for 10 consecutive days and 21 days after the index case is identified, and will be examined for rotavirus. Fingerstick blood specimens will be obtained from index cases and household contacts on day 1, day 10 and day 21 and from bari children age 5 years or less on day 1 and day 21, and will be examined for immunoglobulin specific rotavirus antibody. To study the relationship between human and animal rotavirus diarrhea, stool from animals belonging to the household and animals in the bari with diarrhea will be examined for rotavirus.

A village presently under surveillance for E. coli diarrhea will also be studied for cases of rotavirus diarrhea. Stool will be obtained from persons with diarrhea and well persons in the village for a 5 month period and examined for rotavirus. When cases of rotavirus are detected in the village, fingerstick blood will be obtained on 2 occasions 3 months apart to measure immunoglobulin specific rotavirus antibody.

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 significant risks to persons in this study. Only rectal swabs finger tip blood specimens, nasal secretions and breast milk will be collected.
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 locked in a 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.

5. A signed consent will not be obtained since the risks of the study are minimal; 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. A short interview will be conducted with each family member (young children excluded). This will question certain exposures such as drinking water and will record the occurrence of diarrhea.

7. Individual patients may benefit in that the cause of their illness may be determined. Furthermore we will provide rehydration therapy or hospitalization for cases as appropriate.

   The potential future benefit of the study to society could be the control or prevention of rotavirus diarrhea. Any future environmental (water sanitation etc.) or host (vaccine) manipulation to attempt to control rotavirus 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.

A HOSPITAL PATIENTS

(To patient or parent or guardian)

The doctors at the Cholera Hospital are studying a recently discovered cause of diarrhea and wish to determine if your diarrhea has been caused by this organism. We would like to take from you 2 rectal swabs each day you have diarrhea and one fingerstick blood sample and one sample of your nasal secretions.

(To lactating mother)

We think your child's diarrhea is caused by a recently discovered organism and would like to obtain a small amount of your breast milk now, and in 3 weeks to determine if it contains this virus or a substance that will help your child fight the infection with this organism.

(to both)

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

B. FAMILY STUDY

The doctors at the Cholera Hospital are studying a recently discovered infectious cause of diarrhea and they think that your family member who was treated at Matlab Hospital had illness caused by this infection. They are trying to find out if other persons in the family (or bari) also have this infection. To do this we will do rectal swabs each day for 10 days and take fingertip blood samples now in 10 days (deleted for bari) and in 21 days. These will be tested at the Cholera Hospital. Persons who are ill will be given appropriate medicine for their illness or taken to the hospital if needed.

(To lactating mothers in bari)

We would also like to obtain a very small sample of breast milk to determine if it contains this virus or a substance that will help protect your child from infection.

You can ask any question 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 persons treated here.

C. COMMUNITY STUDY

The doctors at the Cholera Hospital are studying a recently discovered infectious cause of diarrhea. They are trying to find out at what age people are affected and how the disease spreads in a village

1. For ill person.

   We will do a rectal swab and test it for the cause of your illness. You will be provided appropriate medicine for your illness or taken to the hospital if needed.

2. For well person

   We will do a rectal swab and test it for bacteria that cause illness in some people. The results of your test will be used to help find out what is causing 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 persons treated here

If a serologic survey is done the following statement will be made:

We would like to obtain a fingerstick blood sample from you to determine if you have been infected with an organisms in your stool that we know has recently been present in some members of the village. 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 and the doctors will combine it with other information from persons treated here.

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Rotavirus Bari Study - Lactating Mothers

no. Index case name H or O H or OPD # VTS Number

Village Bari

ld Name TS No.	Mother Name & VTS No.	Milk ✓	Diet Day 1 Food	Duration	If diarrhea - ask question. In the 7 days prior to your child getting diarrhea, did he/she receive less breast milk?
					Yes: No:
					If YES, why?
					Yes: No:
					If YES, why?
					Yes: No:
					If YES, why?
					Yes: N No:
					If Yes, why?
					Yes: No:
					If YES, why?
					Yes: No:
					If YES, why?
					Yes: No:
					If YES, why?
					Yes: No:
					If YES, why?
					Yes: No:
					If YES, why?

Rotavirus Study - Index Case Outpatient Form

Study No: ____ ____ Date at OPD: ____ ____ ____ (day month year) OPD No: ____ ____

Name: ____ ____

VTS No: ____ ____ ____ ____ ____ ____ ____ ____ ____ ____

Village: ____ ____ Bari:: ____ ____

Age: ____ ____ years ____ ____ month Sex: M F

DIARRHEA: Time of onset: ____ ____ (day month) Time: ____ : ____ No. times: ____

CHARACTER OF STOOL: Watery: Yes No Mucoid: Yes No Bloody: Yes No

SYMPTOMS: Abdominal Pain: Yes No Chills: Yes No
Fever: Yes No Vomiting: Yes No

VOMITING: Time of onset: ____ ____ (day month) Time: ____ : ____ No. times: ____

ANTIBIOTIC TAKEN FOR PRESENT ILLNESS: Yes No

Name: ____ ____

Dose: ____ Number times taken: ____

IF AGE 5 OR LESS ASK MOTHER FOLLOWING QUESTIONS:

1. WHAT IS CHILD'S DIET?

(a) Only breast milk ____

(b) Breast milk with food (including liquid) supplement ____ ____

(c) Only food - no breast milk ____ ____

2. IF CHILD HAS BEEN TAKING FOOD, INDICATE WHICH FOODS AND DURATION TAKEN:

Food	Duration Taken		
Jau	____ ____	____	____ ____
Mori	____ ____	____	____ ____
Rice	____ ____	____	____ ____
Banana	____ ____	____	____ ____
Dahl	____ ____	____	____ ____
Cow's milk	____ ____	____	____ ____
Water	____ ____		

IN THE 7 DAYS PRIOR TO YOUR CHILD GETTING DIARRHEA, DID YOUR CHILD RECEIVE LESS BREAST MILK?

Yes: ____ No: ____ If YES, why?: _____

SINCE YOUR CHILD DEVELOPED DIARRHEA:

(a) Have you offered breast milk to your child? Yes: ____ No: ____

If YES. Have you offered breast milk as often as when the child is well?

Yes: ____ No: ____

Has the child suckled more or less eagerly than usual?

Less: ____ More: ____ Same: ____

Estimate the child's intake as compared to a normal day's intake

More: ____ Same: ____ Little less: ____ Much less: ____ None: ____

(b) Have you given the child any other food or liquid during his illness?

Yes: ____ No: ____ If YES, indicate which: _____

ECIMENS TO COLLECT:

Breast milk (if lactating mother) day 1 _____ day 21 _____

Throat Swab: _____

Nasal Swab: _____

Rotavirus Study Case and Control Inpatient Form

Hospital No: _____ Diagnosis: _____

Study No: (if applicable) _____ _____ Date of Admission: __ __ __ __ __ __
day month year

Name: _____

VTS No: __ __ __ __ __ __ __ __ __ __

Village: _____ Bari: _____

Age: __ __ year __ __ months Sex: M F

AGE 5 OR LESS ASK MOTHER TO ASNWER FOLLOWING QUESTIONS:

WHAT IS CHILD'S DIET?

(a) Only breast milk _____

(b) Breast milk with food (including liquid) supplement _____

(c) Only food - no breast milk _____

IF CHILD HAS BEEN TAKING FOOD, INDICATE WHICH FOODS AND DURATION TAKEN:

Food	Duration Taken
Jau	_____
Mori	_____
Rice	_____
Banana	_____
Dahl	_____
Cow's milk	_____
Water	_____
_____	_____
_____	_____
_____	_____

IN THE 7 DAYS PRIOR TO YOUR CHILD GETTING DIARRHEA, DID YOUR CHILD RECEIVE LESS BREAST MILK?

Yes: _____ No: _____ If YES, why?: _____

SINCE YOUR CHILD DEVELOPED DIARRHEA:

(a) Have you offered breast milk to your child? Yes: _____ No: _____

If YES. Have you offered breast milk as often as when the child is well?

Yes: _____ No: _____

Has the child suckled more or less eagerly than usual?

Less: _____ More: _____ Same: _____

Estimate the child's intake as compared to a normal day's intake

More: _____ Same: _____ Little less: _____ Much less: _____ None: _____

(b) Have you given the child any other food or liquid during his illness?

Yes: _____ No: _____ If YES, indicate which. _____

SPECIMENS COLLECTED:

Sera (only if study case) _____

Nasal washing _____

Throat Swab _____

Breast milk (if lactating mother) _____

Rotavirus Bari Study: House Visit and Specimen Form
for Children age 5 or less

St no _____ Index case name _____ H or O _____ H or OPD # _____ VTS Number _____

Village _____ Bari _____

SSV Y N

rrhea coded Unk( ): none 0: present, char.unk 1; loose 2: watery, no dehyd. 3; watery, mild dehyd.4 watery, mod.dehyd.5; watery, sev.dehyd.6 bloody 7 other

me	VTS No	Age	Sex	7	1	2	3	4	5	6	7	8	9	10	11-21	BI
		y m	m f	DL V	DL V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	F T
		y m	m f	D V	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	F T
		y m	m f	D V	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	F T
		y m	m f	D V	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	F T
		y m	m f	D V	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	F T
		y m	m f	D V	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	F T
		y m	m f	D V	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	F T
		y m	m f	D V	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	F T
		y m	m f	D V	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	F T
		y m	m f	D V	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	F T
		y m	m f	D V	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	D V R	F T

Indicate with letter B if any child is breast feeding

Rotavirus Family/Bari Study Environmental Culture Form

St no _____ Index case name _____ Village and Bari _____ Village # _____ Bari # _____ Family # _____

	Water Type	Specific Source
Drinking	_____	River Canal Tank Ditch Tubewell Other
Cooking	_____	River Canal Tank Ditch Tubewell Other
Bathing	_____	River Canal Tank Ditch Tubewell Other
Washing	_____	River Canal Tank Ditch Tubewell Other

Culture Data

Index Household	Day 1	Day 2	Day 3
Source water (A) water (AA) water (AAA) water (AAAAA)			
se Drinking Water (B)			
Animals (C)			
Bari Animals (D)			