Attachment 1.

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. D. Mahalanabis

Trainee Investigator (if any) _____

Application No. 89-010

Supporting Agency (if Non-ICDDR,B) _____

Title of Study Small bowel microbial Ecology of Severe persistent Diarrhoea with particular reference to diarrhoeagenic E.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 damaging to subject or others Yes (No)
3. Does the study involve:
   (a) Use of records, (hospital, medical, death, birth or other) Yes (No)
   (b) Use of fetal tissue or abortus Yes (No)
   (c) Use of organs or body fluids (Yes) No
4. Are subjects clearly informed about:
   (a) Nature and purposes of study (Yes) No
   (b) Procedures to be followed including alternatives used (Yes) No
   (c) Physical risks (Yes) No
   (d) Sensitive questions Yes (No)
   (e) Benefits to be derived (Yes) No
   (f) Right to refuse to participate or to withdraw from study (Yes) No
   (g) Confidential handling of data (Yes) No
   (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure Yes No NA
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:
   - NA Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   - ___ Protocol (Required)
   - ___ Abstract Summary (Required)
   - ___ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
   - ___ Informed consent form for subjects
   - ___ Informed consent form for parent or guardian
   - ___ Procedure for maintaining confidentiality
   - ___ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

(PTO)

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

Mahalanabis
Principal Investigator

Trainee

DEC 11 1989

Title: Small bowel microbial ecology of severe persistent diarrhoea with particular reference to diarrhoeagenic E.coli: a descriptive study on pathogenesis and pathophysiology of severe persistent diarrhoea

Principal Investigator(s): Dr. Dilip Mahalanabis
Dr. S.Tzipori

Co-PI's: Pradip Bardhan, CSD
Khaleda Haider, LSD
M. John Albert, LSD
Rukhsana Haider, CSD
N.H. Alam, CSD
MA Wahed, LSD
Hamidur Rahman, LSD
Christina Wanke, ADDR, Boston

Budget - 41,322

Starting Date:

Ending date:

Signature of Division Head: .....
Dr. D. Mahalanabis

.....
Clinical Sciences Division

Date: October 03, 1989.....

A-033778

Abstract Summary:

The objective is to evaluate in clinically severe persistent diarrhoea in infants and small children the role of:

a) diarrhoeagenic E.coli in the small bowel particularly enteroadherent E.coli of the type with localised adherence and autoaggregative adherence.

b) bacterial overgrowth of small bowel with colonic type of flora, and,

Findings in infants and small children with severe clinical disease due to persistent diarrhoea will be compared with those with persistent diarrhoea of mild clinical severity and also with infants and children with acute non-bloody diarrhoea.

Review Committees:

Research Review Committee:.....

Ethical Review Committee:.....

Director: .....

SECTION II - RESEARCH PROTOCOL

Rationale

a. Persistent diarrhoea is an ill understood clinical syndrome and descriptive studies on pathogenesis and pathophysiology are needed to generate a hypothesis leading to more definitive studies. Some recent evidence suggests that certain diarrhoeagenic E.coli may be causally related to the pathogenesis of persistent diarrhoea (particularly enteroadherent E.coli).

b. Preliminary studies have shown some association of small bowel bacterial overgrowth of clonic flora, with persistent diarrhoea; a similar syndrome in adults i.e. tropical sprue has also been shown to have small bowel bacterial overgrowth.

c. Small bowel bacterial overgrowth could lead to intraluminal deconjugation of bile salts, which in turn could cause malabsorption, and controlled studies are required to evaluate the role of deconjugated bile salts in persistent diarrhoea.

d. These studies may also assist in improving case management of persistent diarrhoea; hyperimmune bovine colostrum produced by immunizing cows with cryptosporidium antigen was demonstrated to be an effective treatment for chronic cryptosposidiosis; bacterial strains from small bowel shown to be closely associated with severe persistent diarrhoea will be saved and used to produce

hyperimmune bovine colostrum against these strains for future therapeutic studies in persistent diarrhoea.

Background

Intestinal Microflora and Persistent Diarrhoea

Most enteropathogens that cause acute diarrhoea have also been associated with persistent diarrhoea, notable exceptions being Vibrios and rotavirus. Those organisms that are isolated with about equal frequency from episodes of acute and persistent diarrhoea include non-typhoid Salmonella, enterotoxigenic E.coli, Campylobacter jejuni, and Aeromonas hydrophila. Their continued presence in episodes of persistent diarrhoea probably reflects an impaired ability of the host to terminate infection. Organisms isolated with greater frequency from episodes of persistent diarrhoea include Shigella, enteroadherent E.coli (EAEC) enteropathogenic E.coli (EPEC) and cryptosporidium (Black, Penny - Unpublished; Bhan et al - Unpublished).

Epidemiological studies at ICDDR,B suggest (Black et al) that 7% of episodes of acute diarrhoea associated with enterotoxigenic E.coli persists for longer than 3 weeks compared with 3% of episodes of rotavirus diarrhoea.

The association of EAEC with persistent diarrhoea is of particular

interest and requires further investigation. Enteroadherent strains, some of which belong to EPEC serotypes are characterised by their capacity to adhere to cells in tissue culture (eg Hep-2 cells). E.coli with localised and autoaggregative types of adhesion are believed to be associated with persistent diarrhoea. The latter type which usually does not belong to EPEC serotypes may play an important role in persistent diarrhoea. In India, for example, they were isolated from 2% of healthy controls, 9% of children with acute diarrhoea and 26% of children (under age 3 years) with persistent diarrhoea (Bhan et al - Unpublished).

In mst studies less than half the children with persistent diarrhoea have recognized enteric pathogens in their faeces. However, a few studies such as the one in Peru (Penny et al) have shown that patients with persistent diarrhoea have an increased number of aerobic fecal bacteria in the small bowel compared with healthy controls from developed countries in whom the upper bowel only contains very small numbers of oral type commensal bacteria. However, studies in children with acute diarrhoea and in locally recruited healthy controls in Lima showed similar results as those in persistent diarrhoea patients, raising doubts as to their significance in relation to the pathogenesis of persistent diarrhoea. These children with persistent diarrhoea had mild clinical disease. Finally, preliminary data from culture of small bowel fluids in infants and children with persistent diarrhoea admitted to ICDDR,B treatment centre showed predominantly E.coli and Klebsiella.

Tropical Sprue

This syndrome of chronic diarrhoea of unknown etiology, with intestinal malabsorption, and often multiple nutritional deficiencies in adults and older children is endemic in this subcontinent. These patients usually respond to a course of antibiotic therapy, mainly to Tetracycline (French et al; O'Brien et a, Shefy et al; Klepstein et al). although small bowel overgrowth with enteric flora has been demonstrated in them, a convincing difference from the controls taken from the same socioeconomic group could not be demonstrated (Gorbach et al and Bhan et al).

Diarrhieagenic E.coli and Persistent Diarrhoea

Bray and Beaban suggested in 1945 that certain number of E.coli were associated with infectious diarrhoea. However, advances in our understanding of basic mechanisms made only in the last decade, have finally established this charismatic group of microorganisms as a cause of several diarrhoeal syndromes. Enterotoxigenic, enteropathogenic and enteroadherent E.coli are associated with acute watery diarrhoea and persistent diarrhoea, enteroinvasive E.coli with dysentery and enterohaemorrhagic E.coli with haemorrhágic colitis.

Enterotoxigenic E.coli in Persistent Diarrhoea

The term enteropathogenic E.coli was coined in the 1950's to refer to E.coli of O serogroups including 026, 055, 086, 0111, 0119, 0125, 0127 and 0128 that were isolated more frequently from children with diarrhoea compared with controls. They do not produce CFA's, LT or ST neither do they show Shigella like invesiveness of enteroinvasive E.coli. Examination of electron micrographs of jejunal and colonic biopsies from infants with oersistent diarrhoea associated with enteropathogenic E.coli gave insights into mechanisms of pathogenicity. At sites of mucosal attachment of enteropathogenic E.coli the brush border was destroyed (effaced) and the E.coli was closely attached to cup like projections (pedestals) of bare plasma membrane. The bacterial determinants of this pathognomic process have been studied recently using Hep-2 and HeLa cells in tissue culture. Typically enteropathogenic E.coli induce acute self limiting watery diarrhoea associated with fever and vomiting in infants. In some the illness may become persistent.

Enterodherent E.coli

The Hep-2 adhesion assay has been exploited to identify strains of E.coli which do not produce LT or ST and whose serotypes may differ from enteropathogenic E.coli. EAEC with localised adhesion (LA) usually belong to enteropathogenic serotypes.

Autoaggregative (AA) EAEC do not belong to usual pathogenic serotypes. Such strains were first identified in travellers diarrhoea. Unpublished epidemiological data (Bhan et al), as stated earlier, suggest a role for enteroadherent E.coli in acute and specially persistent diarrhoea. A cross-sectional age cohort of 580 children below 3 years of age residing in a village in North India were seen at weekly intervals for 12 months, faecal samples were available for 346 of 565 diarrhoeal episodes, seventy of which were persistent. Enteroadherent E.coli were isolated from 2.4% of controls, 9.2% of patients with acute diarrhoea and 26% of children with persistent diarrhoea. The majority had watery or loose stools but 11,6% had bloody stools, raising the possibility of relationships between some strains of enteroadherent and enterohaemorrhagic E.coli.

Design and Methodology

Patient population at the Clinical Research Centre of ICDDR,B will be used. Infants and children with diarrhoea lasting for more than 14 days are usually admitted either to the observation (short stay) ward or into the general ward depending on initial evaluation of severity. For this study the patients will be drawn from the following sources:

a. Patients from two studies to commence soon (e.g. TRial of

coconut oil based chicken meat diet in persistent diarrhoea in children and Evaluation of trimethoprim-sulphamethaxazole in the treatment of infants and children with perssistent diarrhoea) which includes patients with severe persistent diarrhoea (control only); the former is under consideration for funding by WHO and a copy of the latter is being sent to WHO. The patients with persistent diarrhoea who routinely receive intubation procedures for diagnostic purposes at ICDDR,B will also be included.

In this study patients with severe clinical disease due to persistent diarrhoea will be compared with a) patients with mild persistent diarrhoea and b) patients admitted with acite watery diarrhoea in the same age group.

Inclusion criteria:

1. Infants and children aged 3 months to 3 years with diarrhoea of more than 14 days but less than 6 weeks of duration, with watery/loose stool of four or more in number during previous 24 hours will be included in the study.

Exclusion criteria:

Patients with complications e.g. pneumonia, meningitis, convulsions, etc., will be excluded. Patients with severe marasmus (weight for age less than 55% NCHS) or with kwashiorkor will also be excluded.

Definition of severe persistent diarrhoea

1. Patients requiring prolonged IV maintenance (i.e. more than 48 hours).
2. Stool output of more than 100ml/kg/day during initial 48 hours of observation.
3. Duration of diarrhoea after admission more than 6 days inspite of supportive treatment and diet manipulation (but without antimicrobials used).

Patients with all three above will be considered to have severe persistent diarrhoea.

Mild persistent diarrhoea

1. Duration more than 14 days but less than 22 days.
2. Did not require intravenous maintenance beyond first 24 hours.
3. Diarrhoea did not last beyond 4 days after admission on supportive treatment and diet manipulation (but without antimicrobial therapy).

Patients with all three above will be considered to have mild persistent diarrhoea.

Patient Population and Controls

One problem in designing such studies is the difficulty in obtaining data from suitable controls. It is proposedmthat patients who gave a history of persistent diarrhoea of relatively short duration (i.e. of more than 14 days but less than 22 days) and settles during initial few days of observation in the hospital will act as controls. We postulate that these patients are on the tail-end of normal distribution of patients with an acute attack of diarrhoea. In addition, for each patient with persistent diarrhoea of any severity in the study an age matched child admitted with acute watery diarrhoea will be identified concurrently and included in the acute diarrhoea controls; these patients will have history, physical examination, stool microscopy and culture performed in the same way as in the persistent diarrhoea patients. They will also have a breath hydrogen test and an intestinal permeability test (with lactulose and manitol ingestion and their excretion in the urine) - the two non-invasive small bowel function tests.

Summary of Procedures

1. History and physical examination including nutrition anthropometry will be performed on admission and recorded on pretested forms.

2. Stoolmicroscopy on admission for cryptosporidium, G. lamblia, E. histolytica, other protozoa and for helminths.

3. Stool culture on admission for E.coli (strains will be saved) Klebsiella pneumoniae (strains will be saved) Salmonella, Shigella species V.cholerae o1, Aeromonas hydrophila, Plesiomonas shigelloides; stool test for rotavirus and other viruses; and anaerobic bacteria (Bacteroides fragilis, clostridium perfringens & clostridium difficile). E.coli strains will be tested for pathogenesis as described below for small bowel samples. Stool will be saved for viruses.

4. Blood for haemoglobin, WBC total and differential count, plasma total protein and albumin, haematocrit and plasma total solids.

5. Small bowel intubation during initial 48 hours of observation (procedures for intubation and sample processing including tests are given below).

6. Recording of progress including faecal volume and IV needs.

7. Lactulose/manitol permeability test and breath hydrogen test during initial observation period.

Data management and analysis

Patients will be classified into mild and severe disease according to criteria described earlier and without the knowledge of

laboratory results by the investigators directly involved in patient care.

Quantity of E.coli, Klebsiella pneumoniae and other enteric bacteria isolated will be compared between severe persistent diarrhoea and mild self-limited persistent diarrhoea and acute diarrhoea. Stool pathogens will also be compared among the three groups.

Enteroadherent E.coli isolation rates will be compared among the three groups first by combining those with localised adhesion and autoaggregative adhesion and then individually; diarrhoeagenic E.coli of any type isolated in the stool and/or small bowel samples will be compared among the three groups; cryptosporiduum detection rate in the stool and/or in the small bowel samples will be compared among the three groups. Enteropathogenicity of E.coli in RITARD rabbit model will be compared among the groups.

Sample size

Preliminary evaluation at ICDDR,B in a group of infants with severe persistent diarrhoea indicated that most of them had high counts of E.coli in their upper small bowel and about 90% of the isolates exhibited one type of adhrence or other in tissue culture experiments. We therefore expect about 80% of the patients with severe persistent diarrhoea to have enteroadherent E.coli in their upper small bowel as against less than 30% in mild persistent

diarrhoea (comparable to those seen in the field studies by Bhan et al in New Delhi). If we take a conservative estimate of 70% of severe persistent diarrhoea patients to have enteroadherent E.coli of one type or the other in their upper small bowel and 30% of the patients with mild persistent diarrhoea to have enteroadherent E.coli in their upper small bowel and with =.05 and =.2 the sample size required to detect this difference would be 21 patients in each group.

Sample size for significant small bowel contamination with colonic flora.

We assume that at least 60% of patients with severe persistent diarrhoea will have a high count (more than 10^6 per ml) of one or other type of enteric bacteria (combined). Whereas we expect not more than 30% of the patients with mild persistent diarrhoea to have such a high count. To detect this degree of difference with =.05 and =.2 the sample size required in each group is about 40 patients.

Some procedures

1. Intubation

This procedure is routinely performed at ICDDR,B for persistent diarrhoea patients. Intubation of the duodenum will be carried out with a sterile double lumen polythelene tube (diameter 1.5mm)

with a small mercury-filled tip. The definitive sampling lumen will be closed from distal and with sterile melted agar; rest of this lumen will be filled with boiled water. The tube will be introduced per orally. The passage of the tube through the stomach and pylorus into the duodenum will be facilitated by changing the position of the child from left to right side and vice versa. The final position of the tip of the tube will be confirmed by checking the colour and the pH of aspirate through the open lumen, which is usually above 6.5 in duodenum and upper jejunum. The sample will be examined microscopically for parasites, particularly G. lamblia and S. Stercoralis and cryptosporidium. Definitive sample for processing will be collected through the agar sealed tube after breaking the seal by pushing with nitrogen from a syringe, allowing time for the intestine to empty.

2. Breath hydrogen test

After a 4-hour fast (sips of plain water will be allowed), a fasting breath sample will be collected. Then lactose (2g/kg) will be given as a drink to the child and thereafter intermittent breath samples (end expiratory) will be collected every 30 minutes and after 3 hours into a syringe through a nasal prong. Hydrogen concentration in ppm will be determined with a gas chromatograph after calibrating with a commercial standard of 96 ppm hydrogen in air. A rise in hydrogen concentration of 20 ppm over baseline will be considered as indicative of lactose malabsorption. End

expiration samples will be collected by allowing the child to breathe through a nasal prong with a side tube for collection.

3. Permeability test

Small intestinal mucusal damage will be assessed by a noninvasive permeability test. Patients will be offered a freshly prepared drink containing 5g lactulose with 0.5g lactose (Duphalac 7.5ml) and 1g manitol in 20ml of 1% chloroform water. NO fasting is necessary; rather breastfeeding and fluid intake will be encouraged. urine will be collected for 5 hours in oaediatric urine collection bags. One drop of 20% chlorohexidine gluconate will be added to each bag before collection. Urine volume will be measured and recorded. Lactulose and mannitol will be measured using an automated enzyme assay system utilising Cobas-bio discrete centrifugal analyser. Results will be expressed as lactulose/mannitol excretion ratio, higher the value more extensive is the apparent "damage" to small bowel mucosa. Normal ranges are available from ongoing studies at ICDDR,B.

Small bowel intubation sample processing methods

Samples obtained from children under study will be processed immediately or stored at - 40 ^{o}C for processing within 2 hours. Anaerobiosis of sample will be maintained by sterile paraffin over the sample. Samples will be divided as follows:

a. wet mount: one to two drops examined for parasites including cryptosporidium, Giardia; for inflammatory cells and for presence of bacteria (indicative of more than 10^5 CFU bacteria per ml).

b. quantitative culture: to be done aerobically and anaerobically, 100 micro litres will be serially diluted and plated on blood agar and MacConkey agar. Isolates will be identified and quantified.

c. viral agents (rotavirus, adenovirus, etc.): 100 micro litres saved for viral culture or immune electron microscopy when electronmicroscope becomes available at ICDDR,B. Rotavirus ELISA will be performed if no alternative methods available.

Strains

Four strains of each E.coli and a single strain of Klebsiella isolated will be identified and quantified. If group D Steptococci are noted they will also be stored for further study. Any Candida isolated will be noted and quantified.

Secretory toxins LT and ST will be identified by Y1 adrenal cell assay and infant mouse assay. Adherent E.coli will be identified by examination in tissue culture (HeLa and Hep-2 cells) adherence assay, looking for localised, diffuse or aggregative adherence. The presence of cytotoxin will be noted; a cell-free supernatant of broth culture will be innoculated into HeLa cell monolayers and

toxic effects determined after 48 hours.

E.coli strains which are negative for LT/ST amd do not possess localised adherence on HeLa/Hep-2 cell tissue culture will be tested in the reversible ileal tie rabbit model (RITARD) using post-weaned (1 kg) rabbits to determine ability of these presumed non-pathogenic strains to cause diarrhoea in an animal.

E.coli strains from patients' stools will be examined for the same virulence trains using the same methods.

Klebsiella spp isolated from the small bowel, will be examined in tissue culture for adherence. The effects of cell free supernatants will also be examined on tissue culture (Y1 or Hela) cells for evidence of secretory- and cyto-toxins.

Method for reversible ileal tie in rabbits

The reversible ileal tie was first described by Sack and Spira in adult rabbits and was used for the production of antibody to colonizing bacteria. Later this method was used by Wanke and Guerrant in weaninf rabbits to evaluate the association between the small bowel colonization and the production of diarrhoea by nontoxigenic E.coli with colonizing ability. 70% of rabbits given the colonizing non-toxigenic E.coli developed watery diarrhoea. Any rabbit that did develop diarrhoea were colonized with at least 10^8 of the same E.coli per sq.cm of mucose. Any

rabbit that was colonized with 10^8 of organisms developed diarrhoea. Under general anaesthesia the blind caecum of the weanling rabbit will be ligated. 10cm approximal to the ileal caecal junction a slip knot will be placed around the small bowel. 10cc of test E.coli strain at 10^8 organisms per cc in broth culture will be innoculated into the proximal ileal lumen, the abdominal incision will be lightly closed and anaesthesia maintained for 4 hours. After 4 hours the slip knot will be removed from the terminal ileum, abdominal incision completely closed and the rabbit monitored for the development of diarrhoea. At 72 hours or if the rabit becomes debilitated from diarrhoea the animal will be sacrificed and quantitative cultures of 1cm of small bowel mucosa will be performed by serial dilution to determine the extent of colonization.

Feasibility

1. Patient population

Patients with severe persistent diarrhoea will be enrolled from the two approved studies which will commence soon. Patients with mild persistent diarrhoea will be enrolled from patients undergoing evaluation from enrollment into the above two approved studies. Acute diarrhoea patients will be enrolled after each persistent diarrhoea (mild or severe) patient has been enrolled from among patients admitted to the treatment centre for the same age group (stratified into two strata, 3m to 11m and 12m to 36m).

2. Intestinal function tests

They form part of the two approved studies from which some persistent diarrhoea patients will be enrolled. Mild persistent diarrhoea patients will require lactulose/manitol permeability test and breath hydrogen test to be performed. Acute diarrhoea patients will also receive these two tests. Both tests are being done at present for other research projects.

3. Enteroadherenty E.coli

techniques for detecting enteroadherent E.coli have recently been established (using both Hep-2 and HeLa cells now being carried out for other protocols).

Assays for LT and ST are routine in the lab.

(If financial support is not required)	Time devoted to project	Year 1 US$	Year 2 US$	Year 3 US$	Total US$
Professional scientific staff (functional title and name - if available)					
1. D. Manalanansis Associate Dir. LSD	10	-	-		
2. B. Reiport (PI) Associate Dir. LSD	5	-	-		
3. M. Haider Microbiologist	20	1950	970		
4. C. Wanke Consultant	5	-	-		
Technical staff (functional title)					
5. F. Bardhan Senior Med. Officer	20	2300	1200		
6. R. Haider Senior Med. Officer	20	2300	1200		
7. M.H. Alam Senior Med. Officer	20	-	-		
8. A.A. Waned Biochemist	5	-	-		
Other staff (functional title if available)					
1. Technician (1) Gr.IV	30	1130	600		
2. Senior Health Asstt. (1)	50	2112	1060		
3. Hospital Attendant (2) (Ayah)	100	1200	600		
Sub-total (list in Budget Summary, item 2 at end of this section)	-	10992	5630		

Chemicals				
Supplies				
Glassware		2000	1000	
List minor equipment[1]				
Gasoline or petrol				
Equipment maintenance				
Data ~~analysis~~ management/analysis		500	500	
Other operating expenditures (specify) - stool pathological tests, blood biochemical tests, biochemical tests for intestinal function studies - Stool microbiology, small bowel fluid microscopy and microbiology entero-adherence tests (tissue culture) - ritard model tests (rabbits)		2000 3000 3000	1000 1500 1500	
Sub-total (list in Budget Summary, item 1 at end of this section)		10500	5500	

[1] These are items costing US$100-1000 each. For items ordered from abroad, include shipment and freight insurance costs, usually approximated as 20% of catalogue price if no better estimate is available. Consolidate items costing less than US$100 as one entry: "miscellaneous" and indicate the total amount.

Budget item	Year 1 US$	Year 2 US$	Year 3 US$	Total US$
Transportation				
Other (specify) 50% of hospital admission cost for 40 patients with mild persistent diarrhoea (PD) for 3 days each, 40 patients with severe PD for 8 days each at $30/day.	4000	2600		
Sub-total[1]	4000	2600		

1.4 Animals

Type of animal		Year 1 US$	Year 2 US$	Year 3 US$	Total US$
Rabbits costs covered under item 1.2 (operating expenditure)	Purchase				
	Maintenance				
	Purchase				
	Maintenance				
	Purchase				
	Maintenance				
Sub-total[1]					

1.5 Travel (specify)

Destination and purpose	Year 1 US$	Year 2 US$	Year 3 US$	Total US$
Sub-total[1]				

[1] (list in Budget Summary, item 2, at end of this section).

Budget item	Year 1 US$	Year 2 US$	Year 3 US$	Total US$
Communication, utilities, printing, publication, xerox, telex, medical illustration, files and file cabinets etc.	1500	500		
Sub-total[1]	1500	500		

1.7 Major equipment (specify)[2]

Budget item	Year 1 US$	Year 2 US$	Year 3 US$	Total US$
Sub-total[1]				

[1] Enter in Budget Summary, item 2, at end of this section.

[2] List items costing more than US$1000. For items ordered from abroad, include shipment and freight insurance costs, usually approximated as 20% of catalogue price, if no better estimate is available.

2. BUDGET SUMMARY

Budget item	Year 1 US$	Year 2 US$	Year 3 US$	Total US$
Personnel (1.1)	10992	5330		
Operating expenditures (1.2)	10500	5500		
Patient costs (1.3)	4000	2600		
Animals (1.4)	-	-		
Travel (1.5)	-	-		
Other expenditure (1.6)	1500	500		
Sub-total of items above (recurring costs)				
Major equipment (1.7)				
GRAND TOTAL (should agree with figures in Part I, Section 1.4)	26992	14230		

3. FINANCIAL OFFICER AND FINANCIAL ARRANGEMENTS

Name of Officer:

Title:

Name, address and number of institutional bank account for cheques and transfers:

Briefly relate each item in the budget (personnel, supplies, equipment, animals, travel, etc.) to the activities outlined in the research proposal, Part II. Items not adequately justified may not be allowed.

Item	Justification
1.1. Personnel	
- Drs. K. Haider, I. Albert	Supervise and perform microbiology, EAEC (tissue culture) diagnosis.
- Drs. C. Wanke, H. Rahman	Will assist with EAEC diagnosis (C.M.) and perform RITARD Model tests.
- Drs. Bardhan, R. Haider, N.H. Alam	Will carry out clinical work.
- M.A. Wahed	Will supervise biochemical tests.
- Technician	Will assist with microbiology.
- Senior Health Asstt. & Hospital Attendants	Will assist with patient care and patient procedures.
1.2. Operating Expenses	
Glassware etc.	Essential for lab. work.
Data Management and analysis	Needed for data management and analysis.
Stool tests, blood tests, intestinal function tests	Consolidated estimated cost of these tests.
Microbiology	Consolidated estimated cost of supplies, reagents, storage costs etc.
1.3. Patient Cost	50% of actual hospitalisation cost to the study ward. 50% will be charged to other accounts.
1.4. Rabbits	Needed for RITARD Model work.
1.6. Other items	Based on estimated need of the project.

(USE ADDITIONAL PAGES IF NECESSARY)

REFERENCES

1. Black RE et al. Effects of diarrhoea associated with specific enteropathogens on the growth of children in rural Bangladesh. Pediatr 1984; 73:799-805.

2. Black RE, Merson MH, Rahman SMM, et al. A two-year study of bacterial, viral, and parasitic agents associated with diarrhoea in rural Bangladesh. J Inf Dis 1980; 142:5:660-664.

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4. Black RE, Brown KH, Becker S. Malnutrition is a determining factor in diarrhoeal duration,but not incidence, among young children in a longitudinal study in rural Bangladesh. Amer J Clin Nut 1984; 37-87-94.

5. Bhan ML, Arora NK, Ghai OP, et al. Major factors in diarrhoea related mortality among rural children. Indian J Med Res 1986; 83:9-12.

6. Candy D et al. Increased adhesion of Escherichia coli to mucosal cells from infants with protracted diarrhoea: a possible factor in patghogenesis of bacterial overgrowth and diarrhoea Gut 1983; 24:538-541.

7. Chowdhury MK, Razzaque A, Mostafa T, et al. Demographic Surveillance System - Matlab Volume 10, ICDDR,B Scientific Report, No. 58, November, 1982.

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