No. 20

REVIEW BOARD ON THE USE OF HUMAN VOLUNTEERS
CRL

Date _____

Principal Investigator David A. Sack Trainee investigator (if any) _____

Application No 79-002 Supporting Agency (if Non-CRL) _____

Title of study Travelers diseases in Project status:
Diarrheal epidemiologic, clinical, immunologic ( ) New Study
and vaccine aspects. (✓) 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) [illegible] sitive questions Yes No
   e) [illegible] to be [illegible] (Yes) No
   f) [illegible] 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.

David A Sack
Principal Investigator

_____
Trainee

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

79-002

Rec'd 4/11/79

SECTION I - RESEARCH PROTOCOL

1) Title: Travelers' diseases in Dacca: epidemiologic, clinical, immunological and treatment aspects.

2) Principle Investigator: David A. Sack, M.D.

Co-Invistigators: Ansaurudin Ahmed, M.B.B.S., Abu Eusof, M.B.B.S., Asma Islam, M.B.B.S.

3) Starting Date: February, 1979

4) Completion Date: December, 1980

5) Total Direct Cost: $ 115,477

6) Abstract Summary:

Two-hundred expatriate people of all ages living in Dacca for a prolonged period will be followed prospectively for the occurrence of diarrheal and other illnesses for one year. These subjects will be divided into two groups: 1) "Dacca veterans" (persons living in Bangladesh for more than one year), 2) "New arrivals." The subjects will be under clinical surveillance continuously and will have quarterly follow up for periodic examination and laboratory tests. Adults from the groups will be included in one of two subprotocols: 1) local immunity in travelers' diarrhea, 2) doxycycline treatment for travelers' diarrhea. The primary aim of the study is to define travelers' diarrhea, its immunology and treatment in Dacca with special reference to defining correlates of protection; however, we will also define other travelers' diseases. Expatriates offer a unique group for study for several reasons: 1) definition of both the primary and booster immune response to ETEC disease is possible, 2) before-illness specimens are possible to obtain, 3) they will act (at least for some diseases) as sentinel people since they are generally non-immune to many of the common pathogens found in Dacca, 4) long term expatriates are good controls for the "non-immune" newcomers.

7) Reviews:

a) Research Involving Human Subjects: _____

b) Research Committee: _____

c) Director: _____

d) BMRC: _____

e) Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objectives: The object of this study is to characterize common diseases of travelers' to Bangladesh, especially diarrhea, with reference to specific etiologic agents and the immune response to these agents. Secondary objectives include establishing the efficacy of doxycycline in the treatment of travelers' diarrhea, and defining other travelers' diseases other than diarrhea.

2. Background: Traveler's diarrhea (TD) (known locally as the Bangladash) is an acute watery diarrheal illness experienced by many travelers to developing countries. Studies from many geographic areas have characterized the clinical illness, its attack rate, and the causative pathogens.[1,2,3,4,5] It seems well established now that enterotoxigenic E. coli (ETEC) is the most common agent responsible for TD though Salmonellae and Shigellae may also occasionally be responsible. Strains of E. coli producing heat stable enterotoxin (ST), heat labile enterotoxin (LT) and both LT and ST have all been isolated from patients with TD and antibody responses to LT have been found in some, but not all patients with the disease. Many serotypes of ETEC have been associated with TD in Mexico, Kenya, and elsewhere, but these include the ETEC serotypes most commonly found in Dacca (06, 08, 078, 0115).[6,7] ETEC have usually been tetracycline sensitive (and multiply sensitive), even though non-ETEC in the same environment are often antibiotic resistant,

suggesting an incompatability between the ent+ plasmid and common R factors.[8] However antibiotic resistant ETEC do occur and in one paper from East Asia were very common.[9]

It is likely that individuals do have protection from ETEC diarrhea, at least on an immunological basis and perhaps through other mechanisms as well.[3,4,5] With attack rates of 60% in three weeks, it seems likely that virtually everyone is exposed to the ETEC but only some develop symptoms. This is supported by the finding that even individuals who remain asymptomatic tend to have small (but statistically significant mean titer) increases in anti-LT when they travel to developing countries.[5] The reason that these people did not get sick is still unknown, though inoculum size, gastric acid,[10] genetic suseptibility and local immunity may all play a role. Further evidence that immunity to ETEC diarrhea developes includes a lowered attack rate for TD in persons from developing countries compared to persons from North America and Northern Europe when they all attend an international conference.[11] Finally, in Bangladesh the incidence of ETEC diarrhea is highest in infants and falls to adult levels by age five,[12] suggesting an age acquired immunity.

When attempting to define the factors which lead to protection from ETEC diarrhea, travelers offer a unique group to study: 1) They have a predictable high attack rate (approximately 60% in three weeks), 2) Prospective study is possible during the time they are developing immunity, 3) The first attack should induce a primary type immune response; a late attack should induce a "booster" response, 4)

Travelers are generally highly motivated to remain healthy, and are likely to willingly volunteer for controlled studies. Travelers therefore should be ideal subjects for documenting correlates of protection from diarrheal disease.

Travelers are also a unique group to study for defining other diseases in a particular location since they act, in a sence like "sentinel" people. The use of travelers as sentinel individuals could be illustrated by mapping malaria episodes in expatriates not taking prophylaxis. Quickly one would know the geographic "hot spots" of malaria, the predominant species, and sensitivities. One could of course obtain the same information by studying local populations; but with expatriates relatively small numbers would be needed. The malaria experiment of course would never be done because effective prophylaxis is available. For most other diseases however, prophylaxis is not available.

During their stay in Bangladesh, the expatriates will come in contact with a host of pathogens which are new to their immunologic system, and so will likely suffer a high attack rate from these as well, (e.g. certain parasitic enteric infections, viral respiratory infections). Therefore it should be possible to define many of the common illnesses of Bangladesh by studying a small group of travelers. There are of course limitations to the use of travelers as good "sentinel" people since the habits and hygiene of expatirates are different from village Bangladeshi

people, they are not at risk of developing cholera, and are at a much lower risk of developing most parasitic infections. They are however exposed to viral respiratory infection, arthropod borne infections and enteric infections where the inoculum needed for infection is small. For the purpose of this study, a travelers' disease will be any infectious disease occurring significantly more frequently in newcomers than in Dacca residents.

Previous protocols have outlined our approach to the study of the local immune response.[13] At present there is no single assay to measure local antibodies; rather, we use a battery of immunologic tests using specimens from blood, saliva, breast milk, and intestinal lavage. The specific assays used include the elisa (Ig class specific) for binding of toxin,[14,15] Y_1 adrenal cell assay for neutralization of toxin,[16] and bacterial agglutination for antibacterial antibodies. For cholera we use an LPS elisa assay for binding antibodies to Inaba or Ogawa LPS.[14] (The methods for *E. coli* LPS elisa assay have not yet been developed but should depend only on the purification of the homologous *E. coli* LPS.)

Using these assays done serially in the same subjects over the course of a year it should be possible to characterize the antitoxin local immune response to ETEC infection and to correlate this immune response with subsequent attacks. It is known that multiple ETEC episodes occur in travelers,[5] so that the antitoxic response from a single infection is not sufficient for high level protection.

In the small number of repeat infections which have been documented, the serotype of the subsequent infection was different from the first, so that serotype specific protection may develop more rapidly than antitoxic protection.

Further questions relating to ETEC include management of individual diarrheal episodes, with regard to the use of antibiotics. Doxycycline taken prophylactically markedly decreases the attack rate for TD.[17] Anecdotal evidence suggests that doxycycline is also effective in shortening episodes of TD[18] (when they occur without antibiotic prophylaxis). Controlled studies of the efficacy of a tetracycline or doxycycline in the treatment of TD have not been reported though one is being carried out in Central America. Besides the question: is doxycycline effective?, is the question: if it is effective in shortening the diarrhea, does it lessen the immune response and make a person more suseptible to subsequent attacks? This is not important for short term travelers since they are at risk for only a short time; however, for residents (both expatriates and local people), the answer to this might be important in determining proper management of individual episodes as well as in further understanding of the immune response to enteric infections. It has been noted that patients with mild or antibiotic-treated cholera have a poorer serum antitoxic response than patients treated without antibiotics.[19]

The definition of the local immune response to ETEC diarrhea is important in the development of a vaccine, especially an oral

vaccine which could act by stimulating the local immune system. Short term (up to one year) local antitoxin protection is possible in dogs challenged with cholera vibrios when immunized by local antigens and it would seem feasible that -- given the proper antigens, in proper dosage, over a proper interval, perhaps with the proper adjuvant -- an oral vaccine could be developed which would be protective against E. coli diarrhea. While the present study focuses primarily on the antitoxic response, the specimens collected will also be suitable for studies of anti LPS antibodies and anti-colonization factor antibodies when those techniques become available. In order to develop such a vaccine the basic methodology of quantitating the local response must be developed, and it would seem that the response to the natural disease would be the best "yardstick" against which to compare a vaccine response.

The development of a study population of expatriates would seem appropriate for further studies as well. Certainly when an ETEC vaccine does become available, this group would be a logical group for a field trial. With a known high attack rate, the efficacy of the vaccine could be established quickly with a relatively small group of subjects. The study population could also be used in the future for other antibiotic trials either of ETEC diarrhea or shigellosis. Doxycycline is certainly not the final solution for travelers diarrhea.

3. Rationale: Travelers are a unique group of people for studying the natural history and immunology of enterotoxigenic E. coli diarrhea, and are likely to be a valuable group in defining some of the other pathogens in Bangladesh by serving as "sentinel" people. The results of this study should be important in: 1) managing patients with travelers' diarrhea and travelers' medical problems in general; 2) the study of local immunity to ETEC diarrhea -- and this study will hopefully lead eventually to a vaccine for the disease; 3) defining travelers' diseases other than ETEC diarrhea.

B. SPECIFIC AIMS

1. Characterize travelers' diarrhea (TD) in Bangladesh.

   a. Define the clinical disease in Dacca.

   b. Define the attack rates in "immune" and "non-immune" individuals.

   c. Define the frequency of episodes of TD over time.

   d. Define the etiologic agents of TD in Dacca.

   e. Compare the "travelers' ETEC" with ETEC isolated from hospitalized cases of diarrhea -- by serotype, antibiotic sensitivity, toxin type.

   f. Compare the travelers' ETEC with travelers' non-ETEC by serotype and antibiotic sensitivity.

2. Define the immune response in (LT-producing) ETEC diarrhea.

   a. Compare the serum immune response in the primary and late ETEC infections.

   b. Compare the local immune response in the primary and late ETEC infections.

c. Define the correlation between systemic and local anti-LT immunity and subsequent illness with LT-ETEC.

d. Define other epidemiologic correlates of protection -- age, sex, place of residence, home country, water purification practices, food buying, cooking and storage practices.

3. Determine the efficacy of doxycycline in the treatment of TD in Dacca newcomers and long term residents, and determine if the practice of shortening diarrheal episodes with doxycycline is associated with increased risk in the future.

4. Determine if Dacca newcomers are at higher risk of respiratory and other febrile viral diseases. Besides travelers' diarrhea, are there other travelers' diseases?

5. Define the common illnesses in travelers to Dacca.

C. METHODS OF PROCEDURE

The subjects in this protocol will be in two groups (see Table 1).

Group A: Dacca Expatriate Residents: This group of $\geq$ 100 people from North America or Northern Europe or Australia who have lived in Dacca for more than one year will be assumed to have been exposed to the common enteropathogens of Dacca and, at least to some extent, be "immune." The group will be stratified according to three age groups: less than three years ($\geq$20 subjects), three to ten years ($\geq$20 subjects), greater than ten years ($\geq$60 subjects). A subgroup of 20 adults will be selected for more extensive local immune studies.

Group B: Dacca Expatriate Newcomers: This group of ≥ 100 people from North America , Northern Europe or Australia who have not lived outside their home country during the last ten years will be recruited during their first week in Dacca. We will assume that this group is not immune to the common enteropathogens of Dacca. Age stratification will be the same as Group A. A subgroup of 20 adults will be selected for more extensive local immune studies.

Table I

Subjects to be Included in Protocol

	Group A	Group B
Total	≥100	≥100
Age Stratification		
< 3	20 (designated AI)	20 (designated BI)
3-10	20 (designated AC)	20 (designated BC)
> 10	60 (designated AT or AS)	60 (designated BT or BS)
Study Subgroups		
Treatment Study	40 adults (designated AT)	40 adults (designated BT)
Special Immunological Studies	20 adults (designated AS)*	20 adults (designated BS)*

* If subjects are lactating they will automatically be asked to be in the special studies groups, otherwise patients will be assigned on the basis of willingness and qualifications to participate in special studies.

Both groups will be recruited from the expatriate community of Dacca primarily through organizations which have no specific medical care plan for their employees. In general we will attempt to recruit entire families so that the lower age groups can be filled. In return for participating in the study we will provide routine medical care for the subjects, including immunization, routine examinations, and treatment for acute illnesses; and advice and referral for more complex medical problems.

On admission into the study, the study will be explained and the following baseline data will be obtained.

1. Complete history and physical examination. This will be recorded on a standardized form which will be suitable for coding.
2. The following laboratory tests will be done:
   a. Chest X-ray
   b. PPD
   c. Blood-CBC, Sera for Serology (multiple alequots)
   d. Throat Swab - Viral Culture
   e. Stool - Culture, Rotavirus (Elisa), Viral Culture, save alequot for IEM, Microscopy
   f. Special Immunological Studies Subgroups - Intestinal Lavage, Saliva and milk in lactating mothers.

Following this initial session each subject will keep a health diary in which subjects will record illnesses. This will be recorded weekly if no illnesses but daily during illnesses (see enclosure). Most often the recording of "no illnesses" can be made over the telephone. Illnesses however will require either a visit by the nurse supervisor, or a clinic

visit. A set of algorithms will be developed for each symptom set for both clinical and research purposes in an attempt to diagnose, by etiologic agent each infectious illness.

Quarterly each subject will visit a clinic specially set up for certain routine follow-up examinations including verification of weekly health diaries, detailed histories of illnesses not recorded, and a screening physical exam and routine lab tests. The quarterly lab tests will be the same as the initial except the X-ray will be annual and the PPD biannual. Immunizations and gamma globulin will be kept up to date at the time of the quarterly visits. The subsample designated for special studies will also have a saliva sample and an intestinal lavage during their quarterly follow-up visits, however, only ten from Group AS and ten from Group BS will have a lavage at any given follow-up. (That is, one cohort will have lavage on admission, at three months, and at nine months. The other cohort will have lavage on admission, at six months, and at 12 months.)

When illnesses occur the subjects will be instructed to visit the clinic or notify the nurse during the first day of illness for special studies, diagnosis and treatment. It is anticipated that those illnesses will be primarily: 1) diarrheal disease, 2) fevers, 3) respiratory infections; though of course other problems will also be evaluated and treated, or referred.

1. Patients with diarrhea will have a standardized history, physical exam, and the following lab tests: stool ME, stool culture, stool for rotavirus, stool saved for other viral studies and ten day convalescent sera for serological tests. All patients will be kept

and blood for bacteriologic culture. In addition a throat swab and stool culture will be obtained for viral culture, and a ten day convalescent serum will be collected for viral serology. (Acute serum will be the previous quarterly serum.) Treatment of fevers without obvious site of infection will be treated according to best clinical judgement.

3. Patients with respiratory infection will have a standardized history and physical exam and will have a throat and/or sputum culture as indicated, a throat-swab for viral culture and a convalescent serum. Patients whose illness suggests a lower respiratory illness will also have a chest x-ray, and a PPD if more than three months since the last PPD. Treatment appropriate to the clinical situation will be given.

4. Other illnesses will be managed according to usual medical practice.

Double-Blind Treatment Study

Adults in Group AT and BT will be asked to participate in a double-blind study of the efficacy of doxycycline used in the treatment of travelers' diarrhea. This should include at least 40 adults from Group A and Group B. Patients with an allergy to tetracycline, pregnancy or lactation will not be included. Since in previous studies, travelers' diarrhea lasts for a mean of four to five days and since, if it is effective, doxycycline should shorten the disease to one day after beginning therapy, a significant difference should be seen with as few as 20

well hydrated with oral sugar-electrolyte solution. If they have watery diarrhea and are in Group AT or BT they will be included in a double-blind treatment trial of doxycycline for the treatment of traveler's diarrhea (a description of this trial is included later in the protocol). For dysentery they will be treated with Ampicillin if the clinical diagnosis is shigellosis (Septra in penicillin allergic individuals) and Flagyl if the clinical diagnosis is amebiasis.

Patients in the special studies subgroups (Groups AS, BS) will not be treated with antibiotics for watery diarrhea and may be selected for intestinal lavage though not all episodes of diarrhea from this subgroup will be studied. For Group B (newcomers), fifteen first episodes occurring within a month after arrival in Dacca will be studied and another group of fifteen episodes occurring after at least six months will be studied. If possible the same individuals will be studied during two episodes (early and late). For Group AS, fifteen episodes will be studied at any time they should occur.

The special studies will include the following on day five, nine, and 17 after the onset of symptoms: saliva, serum, intestinal lavage, (and if nursing - breast milk daily).

2. Patients with fever will have a history and physical exam. If the clinical information strongly suggests the site of infection (e.g. middle ear infection, urinary infection, etc.) appropriate cultures will be done and specimens will be obtained from throat, stool, urine

episodes per group. It is possible however that episodes occurring in Group A (the immune group) will be of shorter duration (less than four days mean) and in this "immune" group, it may be difficult to show a statistically significant effect from doxycycline treatment (just as it is difficult to show efficacy of tetrycycline in Bangladeshi patients). Also, it is possible that doxycycline therapy by shortening the illness will lessen the immune response in Group BT, making subjects taking doxycycline more suseptible to repeated bouts of diarrhea? In hopes of answering this subquestion regarding antibiotic treatment, we would plan that each patient would continue to receive the same drug with each episode so that the frequency of episodes during the year could be determined in the treated versus placebo group.

Other tropical, but more chronic enteric diseases may also be affected by the intermittent use of doxycycline including sprue and amebiasis. The numbers may not be large enough to detect protection from these diseases by doxycycline but by consistently giving either drug or placebo to the same individual, we may see a difference in attack rates.

Assignment to either the drug or placebo will be done in a double blind manner. Each patient will have his own vial of coded capsules (kept at the office). The vials will be distributed randomly among the study numbers by drawing the vials from a box. Stratification will be by Group A and B, each with $\geq$ 40 individuals. A single dose of 200 mg (two capsules) will be given as treatment and this will be given at the office after the necessary laboratory work is completed. Diarrhea will

be defined in Groups AT and BT as the occurrence of ≥ 3 watery stools per day or ≥ 2 watery stools with other symptoms, e.g. fever or abdominal cramps.

The patients will be evaluated clinically by their diaries which will note stool frequency, stool character, cramps, fever and other symptoms daily. The primary determinant of success of therapy will be the duration of diarrhea. A significant treatment effect will be determined both by T test (mean duration of diarrhea), by Chi-square (numbers of episodes persisting more than 24 hours after capsules given), and by KS Test. The possibility that the treatment will interfere with the immune response will be determined by Chi-square test (mean number of episodes of diarrhea per year or per quarter).

Laboratory Studies: Bacteriology

All stool specimens will be tested for Salmonellae, Shigellae, Vibrio, Aeromonas, enterotoxigenic E. coli (five colonies for Y_1 cells, two colonies for infant mice) and for rotavirus antigen. Serotyping and antiboitic sensitivities of enterotoxigenic E. coli and a matched non-enterotoxigenic E. coli selected from the last routine stool culture from the same subject will be carried out. Antibiotic sensitivities will be done by the Kirby-Bauer method. Serotyping will be done by Drs. Frits and Ida Ørskov in Copenhagen.

"Bangladeshi ETEC" will be collected concurrently for stool culture collected from age matched patients admitted to the treatment center with watery diarrhea, without cholera.

Bacteriologic cultures from other sites will be tested appropriately for that specimen. Throat cultures will be screened for Group A β hemolytic streptococci, (and H. influenzae in children less than five years). Urine cultures will be quantitative, from clean catch specimens.

Parasitology

Stool microscopy will be performed on fresh wet mount preparations. In addition an alequot of stool will be preserved in formal saline and merthiolate-iodine-formaline (MIF) for confirmation, concentration and quantitation. The parasitology work will require one technician full time.

Immunology

All patients will have multiple serum specimens obtained. These will be alequoted, undiluted (four alequots of 1 cc each). One set for antitoxin neutralization, antitoxin elisa, antibacterial agglutination, rotavirus serology and ameba serology. One set for respiratory viral serology. Two sets for storage (potential uses include stool immune electron microscopys hepatitis antibody, giardia, or bovirus serology as well as anti E. coli LPS elisa and anticolonization factor elisa).

The special studies will also have saliva, intestinal lavage, and breast milk specimens (if lactating). These will be tested for antitoxin neutralization, antitoxin elisa, bacterial agglutination, (and perhaps LPS elisa in the future) according to the schedule outlined on Table II.

Table II

Immunological Tests for Studies of Travelers' Diarrhea

	Serum[a]	Lavage[b]	Saliva[b]	Milk[b]
Y_1 adrenal LT neutralization	+	+	+	+
Elisa anti-LT:				
IgA/total IgA	+	+	+	+
IgM/total IgM	+	+		
IgG/total IgG	+	+		
Bacterial agglutination	+[c]	+	+	+
Antirotaivrus antibody	+			
Ameba serology (HAI)	+[d]			

[a] Obtained quarterly and ten days following an episode of diarrhea.

[b] Special local immune studies only.

[c] Paired sera - previous quarterly serum and ten day convalescent serum.

[d] On last serum only, quarterly sera assayed if last serum is positive.

Lavage Procedure

Because lavage procedure is the only non-standard procedure done it will be described in more detail. Fasting patients are asked to drink an isotonic, balanced salt solution (Na, 141; K, 10; Cl, 121, HCO_3, 30 mEq per liter) at the rate of 250 ml every ten minutes for the entire procedure. Within 90 minutes this rapid ingestion of fluid induces a watery

diarrhea, the contents of which are a simple washing of the lumen. The procedure proceeds until a two hour collection of diarrheal stool is obtained. The watery stool is immediately measured, inactivated (56°C x 15 minutes) to destroy proteolytic enzymes, and then pushed through a gauze pad. After centrifugation (10,000 x g for 30 minutes) the supernate is passed through a millipore filter (0.45 um). The filtrate is then concentrated (approximately 50 x) by negative pressure dialysis, alequoted and frozen at -70°C until assay.

The lavage procedure which was first developed as an easy method for cleansing the bowel prior to barium enema, is quite painless and easy to perform. Patients will of course notice a full abdomen and very mild cramps but the diarrhea is painless. Because of the salt load it will be done only with patients between the ages 15-40 and who have no hisotry of, or physical examination evidence of, hypertension, heart disease, epilepsy, or chronic renal disease. Most persons will experience about a one Kg. increase in weight during the procedure; however, this is of course temporary.

Analysis of Data

The specific aims (see p. 7-8) were designed so that a particular Table could be constructed from each aim. These tables would be as follows (the numbers by each table refer to the list of aims):

Table 1a

Comparison of travelers' diarrhea in Dacca newcomers with Dacca long-term residents.

	Dacca Newcomers	Dacca Residents
1. Percent of subjects with episode of diarrhea during first 3 mths of study.	____%	____%
2. Total no. of episodes of diarrhea.	____	____
3. Mean duration of TD (days)	____±SE	____±SE
4. Mean No. diarrheal stools/ episode	____±SE	____±SE
5. Percent with vomiting	____%	____%
6. Percent requiring change in plans	____%	____%
7. Percent with fever >101°F	____%	____%

Table 1c

Comparison of TD frequency during year in Dacca.

	1st Quarter		2nd Quarter		3rd Quarter		4th Quarter	
	No. of persons with diarrhea	No. of episodes	No. of persons with diarrhea	No. of episodes	No. of persons with diarrhea	No. of episodes	No. of persons with diarrhea	No. of episodes
Dacca Newcomers								
Dacca Residents								

Table 1d

Etiologic Agents Detected in Persons with Diarrhea.

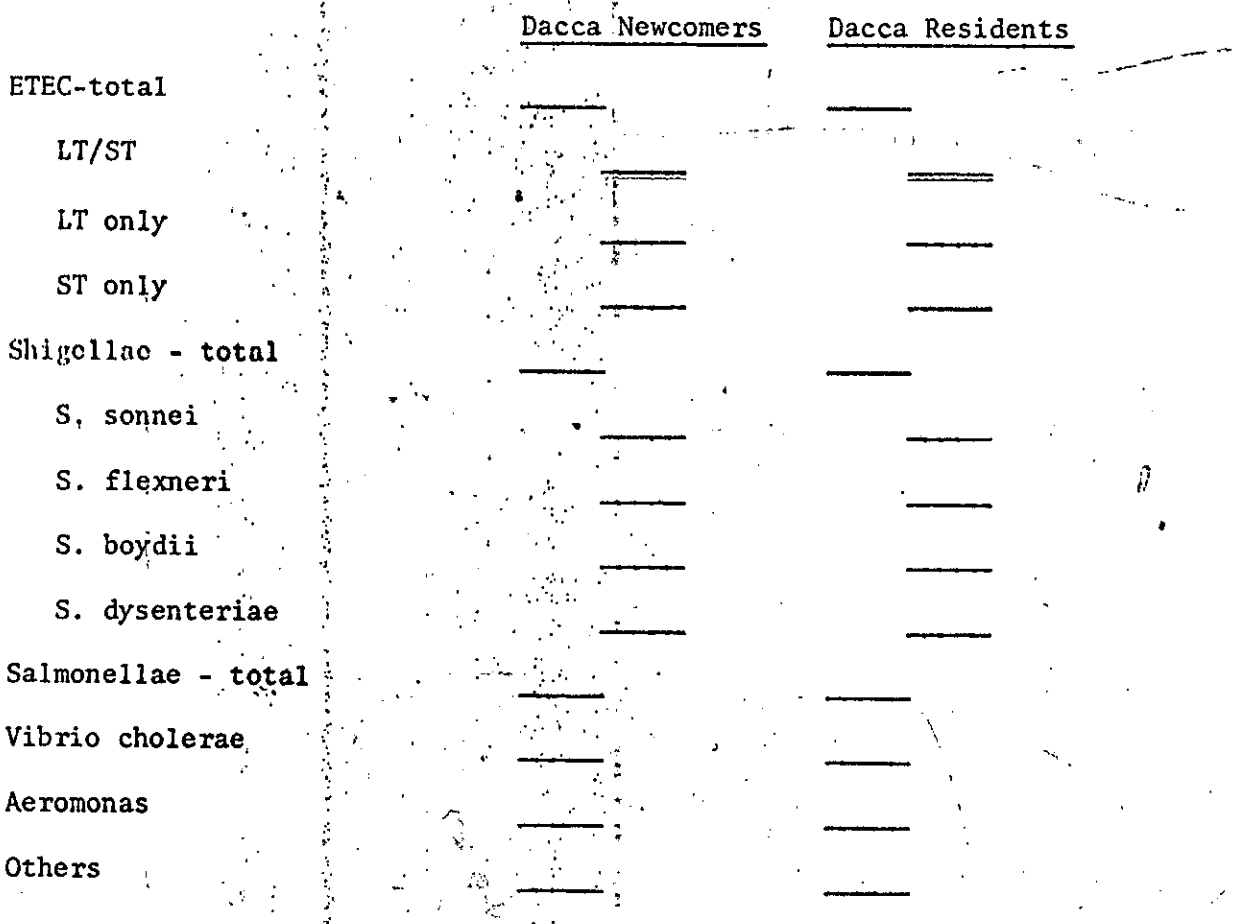

Table 1e

Comparison of "travelers' ETEC" with "Bangladeshi ETEC" isolated from hospitalized cholera-like diarrhea patients.

	Travellers ETEC	Bangladeshi ETEC
Serotype		
06	____ %	____ %
08	____ %	____ %
078	____ %	____ %
0115	____ %	____ %
Others	____ %	____ %
Percent sensitive to tetracycline	____ %	____ %
Percent sensitive to all antibiotics	____ %	____ %
Toxin Type		
LT/ST	____ %	____ %
LT only	____ %	____ %
ST only	____ %	____ %

Table 1f

Comparison of "travelers' ETEC" with travelers' non-ETEC.

	ETEC	non-ETEC
Serotype		
06	____ %	____ %
08	____ %	____ %
078	____ %	____ %
0115	____ %	____ %
Antibiotic Sensitivity		
Tetracycline sensitive	____ %	____ %
Multiply sensitive	____ %	____ %

Figure 2a

Comparison of serum antibody response:
primary ETEC infection with late ETEC infection.

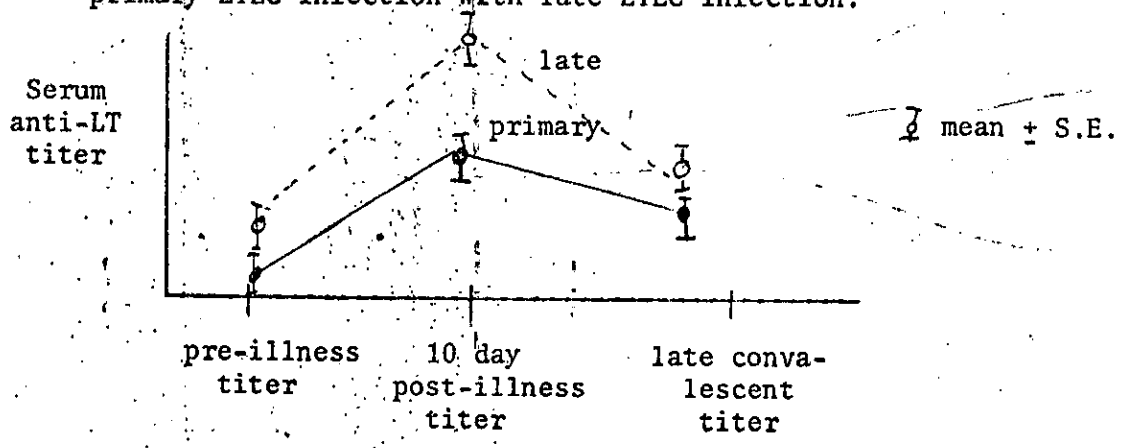

Figure 2b

Comparison of intestinal IgA antibody response:
primary vs late ETEC infection.

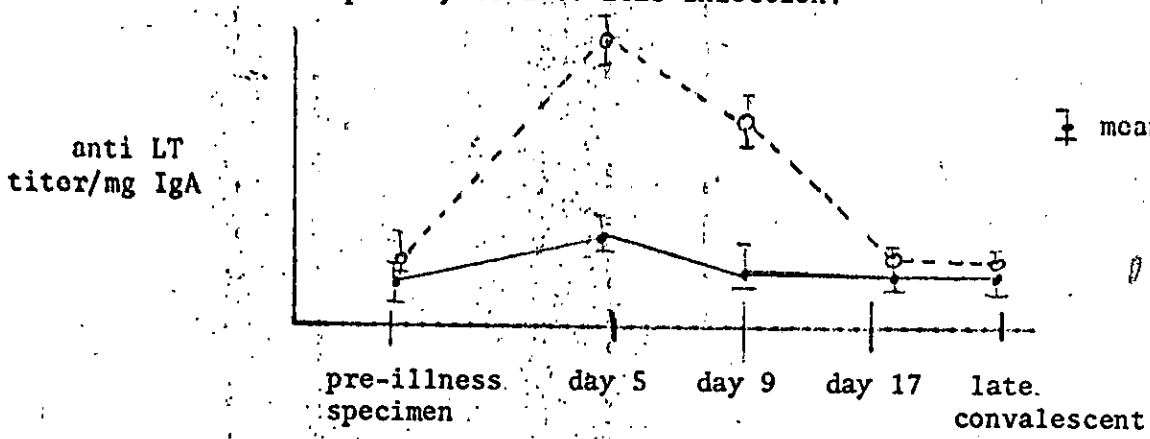

Table 2c

Relation between quarterly serum anti LT titer and risk of diarrhea during subsequent 3 month period.

Titer	Quarterly attack rate all T.D. 1st Quarter D.N.*	D.R.*	2nd Quarter D.N.	D.R.	3rd Quarter D.N.	D.R.	4th Quarter D.N.	D.R.
0-5 units								
5-25 units								
25-125 units								
> 125 units								

Note: Table 2c will be repeated with Quarterly attack rate for LT only-ETEC diarrhea.

Table 2c'

Relation of quarterly anti LT titer in intestinal lavage with risk during subsequent 3 month period.

Titer	Quarterly attack rate all T.D. 1st Quarter D.N.*	D.R.*	2nd Quarter D.N.	D.R.	3rd Quarter D.N.	D.R.	4th Quarter D.N.	D.R.
upper 3rd								
middle 3rd								
lower 3rd								

* D.N. = Dacca Newcomers.
D.R. = Dacca Residents.

Table 3a

Duration of diarrhea in Persons Treated with doxycycline or placebo for T.D.

	Doxycycline		Placebo	
	D.N.*	D.R.*	D.N.	D.R.
Mean Duration (days±S.E.)	____	____	____	____
No. of episodes > 24 hrs after medication	___/___	___/___	___/___	___/___

Table 3b

Number of episodes (quarterly) of T.D. in persons taking doxycycline or placebo for each episode of diarrhea.

		Doxycycline		Placebo	
		D.N.*	D.R.*	D.N.	D.R.
First Quarter	Persons				
	Episodes				
Second Quarter	Persons				
	Episodes				
Third Quarter	Persons				
	Episodes				
Fourth Quarter	Persons				
	Episodes				

* D.N. = Dacca Newcomers.
D.R. = Dacca Residents.

Figure 3b

Number of episodes of diarrhea quarterly in persons taking either doxycycline or placebo.

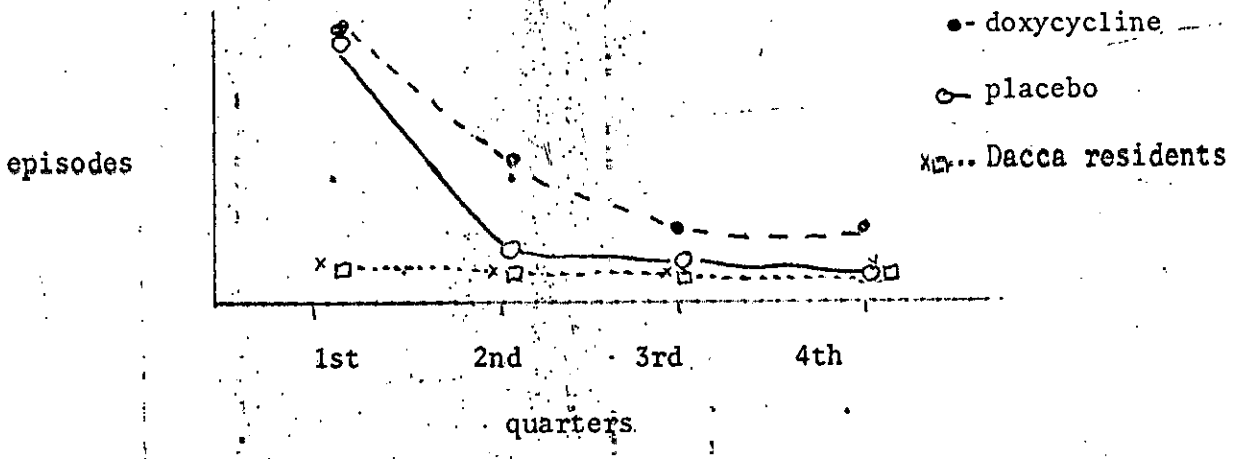

Table 4a

Number of episodes of febrile episodes quarterly in Dacca newcomers and residents.

	1st Quarter		2nd Quarter		3rd Quarter		4th Quarter	
	D.N.*	D.R.*	D.N.	D.R.	D.N.	D.R.	D.N.	D.R.
No. of persons								
age 0-3								
3-10								
> 10								
No. of episodes								
age 0-3								
3-10								
> 10								

* D.N. = Dacca Newcomers.
D.R. = Dacca Residents.

Table 4b

Types of Febrile Non-diarrheal Illnesses in Dacca Newcomers and Residents

	Dacca Newcomers			Dacca Residents		
age	0-3	3-10	> 10	0-3	3-10	> 10
Site of Infection						
Otitis Media						
Pharygitis						
U.R.I.						
Lower respiratory Infection						
Urinary Tract Infection						
Meningitis						
Hepatitis						
Malaria						
No Site Found						
Others						
Bacterial Pathogens Isolated*						
ß hemolytic strep						
H. influenzae						
S. pneumoniae						
Escherichia coli						
Klebsiella						
Other gram neg						
Staph aureus.						
Other						
Viral Agents Cultured						
Viral Agents - Titer Rise						

* From specimens obtained during illness. Isolates thought to be offending agent.

Table 4c

Prevalence of common enteric parasites detected in Dacca Newcomers and Residents during year.

	Dacca Newcomers	Dacca Residents
A.histolytica - cysts		
- trophs		
G. lamblia		
Trich hominis		
Ascaris		
Hookworm		
Whipworm		
Others		

The observations made during the study may of course suggest other data comparisons, but at least these will be made.

D. SIGNIFICANCE

This study will define the syndrome of travelers' diarrhea in Dacca, its bacteriology, immunology and treatment. It should also add to the basic understanding of the local immune system of the gut and should develop a study population of expatriates which could be useful in future enteric studies. Finally it will explore the possibility that travelers' are good sentinel people to detect common pathogens in the community.

E. FACILITIES REQUIRED

1. Office space is provided for the invistigator; however an office with attached examining room and bathroom will be needed for the study nurse. This will require renovation of space.
2. Laboratory space is already provided.
3. Hospital resources: Hopefully none will be needed. Rarely a severe medical illness will require treatment in the hospital.
4. Animal resources: Infant mice for ST assay.
5. Logistic support: We will need help from the following branches.
   a. Community studies - one field worker to work with study nurse in maintaining surveillance.
   b. Transport - one vehicle three hours, 3 times a week.
6. Major items of equipment: a mini computer (e.g. Tektonix or Hewlett-Packard would greatly facilitate the study. Approximate cost $10,000. This could be used by other branches also.
7. Other specialized requirements: A general clinic would need to be maintained with the usual clinical supplies for general practice. Examining table, suture equipment, vaccines, drapes, etc.

F. COLLABORATIVE ARRANGEMENTS

1. Virus studies will be carried out in collaboration with Dr. Arnold Monto at the University of Michigan School of Public Health.
2. Immune Electron Microscope studies on selected specimens from "no-pathogen diarrhea" will be done either at the University of Michigan or at NIH (Dr. A.Z. Kapikian).
3. Some of the elisa assays will be confirmed by Dr. Jan Holmgren as part of ongoing collaboration with him in studies of local immunity.
4. Drs. Frits and Ida Ørskov will serotype the ETEC.

The ICDDRB will bear the cost of shipping the specimens to the collaborators but not of the actual assays run elsewhere.

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ABSTRACT SUMMARY

1. 200 expatriate residents of Dacca will participate in the study including 40 infants less than 3, 40 from 3 to 10 and the remainder above 10 years. Infants and children are included in the surveillance because they are a major group at risk.

2. Risks of the proposed are minimal. Venepuncture (3 ml) will be done quarterly and 10 days post illness on all subjects. Adults will be included either in a local immunity subgroup or in a doxycycline treatment subgroup. The ones in the local immunity subgroup will have periodic intestinal lavage which is associated with minimal discomfort, a 1 kg. temporary weight gain and temporary watery diarrhea. The treatment subgroup will be treated with either a plaubo or doxycycline. This drug is an established drug with a known low risk of toxicity and a high likelihood of shortening the diarrhea. Possible adverse effects from the drug include allergic reactions, photosensitivity reactions, vomiting, diarrhea, vaginitis, and teeth straining in children. (It will not be used in children.) The risk of any adverse effect is less than 1%.

3. All patients will have complete histories taken and physical examination. Patients with any evidence of heart disease, hypertension, renal disease, epilepsy will not be included in the local immunity subgroup. Patients who are lactating, pregnant, or who are allergic to tetracycline will not be included in the treatment subgroup.

../2.

4. All clinical records will be maintained in a locked file in the clinic office. All specimens for research will be coded and the link between specimen and person will be kept in a locked file. At the conclusion of the study this link will be destroyed.

5. Signed informed consent will be obtained from adults and from children's parents.

6. Medical histories will be obtained.

7. The subjects will benefit from the clinic facilities which will include diagnosis and treatment of acute and chronic illnesses. Society in general should benefit from the knowledge gained as to the treatment and ultimate prevention of diarrheal disease.

8. The planned research will use the clinic records (which are being collected as research records) and will use blood, saliva, breast milk and stool.

SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

Name	Position	Percent of effort or number of days	Annual Salary	Project Requirements TAKA	DOLLARS
David A. Sack, MD	Investigator	50%	$34,750		17,375
Abu Eusof, MBBS	Co-investigator	20%	Tk40,000	8,000	
Asma Islam, MBBS	Co-investigator	20%	Tk30,000	6,000	
A. Ahmed, MBBS	Co-investigator	15%	Tk60,000	9,000	
To be named*	Study Nurse	100%	$10,000		10,000
To be named	Secretary	75%	Tk36,000	27,000	
Mr. Neogi	Head Study Tech.	50%	Tk30,000	15,000	
Mr. Gomes	Immunology Tech.	100%	Tk25,000	25,000	
To be named*	Clinical path-parasitology Tech.	100%	Tk25,000	25,000	
To be named	Bacteriology Tech. 2 Tech. - each 50%		Tk25,000	25,000	
To be named	Animal handler	20%	Tk20,000	4,000	
Dr. Mahmoud	Veterinarian	10%	Tk40,000	4,000	
To be named	Field Assistant	100%	Tk25,000	25,000	
To be named	Cleaner - office and lab	100%	Tk20,000	20,000	
			Subtotals:	170,500	27,375

* New positions for CRL.

2. SUPPLIES AND MATERIALS

a. Clinical Supplies

Vaccines, clinical medications	1000
Other clinic supplies - drapes, syringes, needles, cups, plastic and glassware	1000

b. Lab Supplies			
Plastics, glassware, media, reagents			10,000
c. Lab Tests			
CBC #1500 tests @7.30		10,950	
Stool examinations #2000 @4.75		9,500	
Bacteriologic cultures #2000 @15.00		30,000	
Chest X-rays #120 @25		3,000	
	Subtotals:	53,450	12,000
3. EQUIPMENT			
Mini computer			10,000
	Subtotal:		10,000
4. PATIENT HOSPITALIZATION			
Estimated approx. 30 days during year @150/day		4,500	
	Subtotal:	4,500	
5. OUTPATIENT CARE			
This will be done through special clinic			
6. CRL TRANSPORT			
Mileage-Dacca: 1,000 miles estimated (30/mile)		3,000	
	Subtotal:	3,000	
7. TRAVEL AND TRANSPORTATION OF PERSONS			
International Travel to present paper at International Meeting			2,500
	Subtotal:		2,500

8. TRANSPORTATION OF THINGS			
Import of Supplies			3,000
Import of Equipment			3,000
Local Shipment - 0			
Transport of Specimens			1,000
	Subtotal:		7,000
9. RENT, COMMUNICATIONS & UTILITIES			
Postage			100
Telephone (for study nurse)		1,200	
Cables			100
	Subtotals:	1,200	200
10. PRINTING AND REPRODUCTION			
Printing forms		7,500	
Special Reproduction - 0			
Publication costs (3 publications likely)			1,000
Xerox		10,000	
	Subtotals:	17,500	1,000
11. OTHER CONTRACTUAL SERVICES			
Consultant fees - 0			
Patient payments - 0			
Others - 0			
12. CONSTRUCTION, RENOVATION, ALTERATIONS		15,000	
	Subtotal:	15,000	

Justification for Incremental Costs

1. Expatriate Nurse. The person filling this position would coordinate the clinical studies and collect the specimens needed. An expatriate would best relate to the expatriate community and since we will be asking for maximal cooperation, excellent communications with our study population is essential.

2. Parasitology Technician. In order to secure reliable parasitologic data, we must put special emphasis on this. Well trained technicians are available and could be incorporated into the clinical pathology laboratory. Routine wet mount examination of stool, as is currently being done in clin path will not be acceptable for this study.

3. Minicomputer. The study will not make-or-break on the availability of this item. It would however be very helpful in data storage and analysis, and would be used not only in this protocol but also in the bacteriologic, immunology and biochemistry laboratories to store and analyze laboratories records. In trying to evaluate the need for a mini computer in light of the intention to buy an IBM 34, it is felt that the mini will continue to be very useful for smaller operations and will be especially useful in expressing data as graphics. That is, the IBM 34 has very limited graphics capabilities, whereas, graphics is the strong feature of the mini. By connecting the IBM to the mini, the mini can then be used as a "graphics" terminal.

4. Construction. A clinic room will have to be set up to manage the patients for both routine visits and emergency visits. The site has

not yet been decided upon but the basic requirements would be a clinic office - waiting room with ajoining examination room and bathroom. It should have a sink, electrical connections and cabinets. It should be out of the main stream of traffic. One possibility would be to convert Dr. Greenough's office into the clinic room. A second would be to construct a room at the west end of the study ward.

B. BUDGET SUMMARY

	Year 1		Year 2	
	TAKA	DOLLARS	TAKA	DOLLARS
1. Personnel	170,500	27,375	93,775	28,743
2. Supplies	53,450	12,000	26,500	
3. Equipment		10,000		
4. Hospitalization	4,500		3,000	
5. Outpatients				
6. CRL Transport	3,000		3,000	
7. Travel Persons		2,500		
8. Transportation Things		7,000		
9. Rent/Communication	1,200	200	1,200	200
10. Printing/Reproduction	17,500	1,000	2,000	1,000
11. Contractual Service				
12. Construction	15,000			
Subtotal:	265,150	60,075	129,475	29,943
Total $:	77,181		38,296	
Final Total:	115,477			

Conversion Rate $ 1.00 = Tk. 15.5

FEVER FORM

Name _____ Study Number _____

This form is designed to record your symptoms daily during an illness with fever.

	Date				
Sore throat (yes/no)					
Head ache (yes/no)					
Runny nose (yes/no)					
Cough (Y/N)					
Sputum production "					
Chest pain "					
Abdominal pain "					
Pain on urination "					
Skin sores or boils "					
Pains in muscles "					
Pains in Joints "					
Highest temperature					
Chilly feeling (Yes/No)					
Shaking chills "					
Sweats "					
Changed plans because of illness "					
Stayed in bed because of illness "					

DIARRHEA DIARY

Name_____ Study Number_____

This form is designed to record your symptoms daily during an episode of diarrhea. Please call Ms if you have any questions about how to fill in the form.

	Date						
Nos. of stools in 1 day							
Description of stool*							
Cramps (Yes/No)							
Vomiting (no. of times)							
Feverish feeling (Yes/No)							
Highest temperature							
Changed plans because of illness (Yes/No)							

* 1 - Just like Watery

2 - very loose

3 - soft

4 - hard

5 - bloody

WEEKLY HEALTH SURVEILLANCE FORM

Name _____ Study Number _____

During the past week:

	Date			
1. Were you healthy?				
2. Did you miss any work or school for health reasons?				
3. Did you have any vomiting?				
4. Did you have any diarrhea? (3 or more loose stools in one day)				
5. Did you have any "cold" symptoms?				
6. Did you have any fever?				
7. Did you have any other symptoms?				

* If you develop any illness, please contact Ms _____ the same day.

PERMISSION FORM

Travelers' Diseases in Dacca
(Adult Treatment Group)

The International Center for Diarrheal Disease Research, Bangladesh (ICDDRB) (formerly Cholera Research Laboratory) is carrying out research into the cause, prevention and treatment of diarrheal disease. As you may know diarrheal diseases are quite prevalent in Bangladesh and are a major health problem. Expatriates who live in Bangladesh also frequently develop diarrheal illnesses which are generally caused by the same germs as cause illness in Bangladeshi people (except that cholera is almost never seen in expatriates).

The ICDDRB under supervision by Dr. David A. Sack is planning to carry out a study of specific illnesses of expatriates living in Dacca. The main reason for doing the study is to determine the way which persons develop protective immunity to these illnesses. Based on studies like this it is hoped that a vaccine will be developed for the most common causes of diarrhea of travelers and Bangladeshis.

We invite you to participate in this study of travelers' disease in Dacca. If you decide to enter the study you can expect the following:

1. A Clinic will be availab for your use during the next year (the duration of the study). This can be used for all your medical problems. The clinic will provide (free of charge) routine examination, immunizations, laboratory work, minor surgery (e.g. suturing small uncomplicated lacerations) and will recommend referral for more complex or serious medical problems such as major surgery or problems for specialists in other areas.

2. You will be able to read and examine the research protocol describing the study. This is available on loan from Dr. Sack. Also you will be able to see your clinic and research records should you desire, though of course you would not be able to see anyone elses records.

3. You would be expected to come to the clinic every three months for certain routine tests and examinations. These quarterly examinations would include obtaining samples (5 ml) of blood, throat secretion and stool.

4. During the year that you are enrolled in the clinic we want to keep in touch with you weekly to learn of any illnesses. This will be done by filling out a weekly health surveillance form and reporting to the clinic. Usually this surveillance can be done by phone.

...2/

5. If you become ill we want to know about your illness early in the illness so would ask you to visit the clinic on the first day. At some times house calls can be arranged. Reporting early is important so that accurate samples can be obtained and proper treatment given.

6. For most illnesses you will be treated for your illness with standard medical treatment (that is, no investigational treatment); however, if you get an episode of watery diarrhea, you would be included in a treatment study and would receive a medication for diarrhea consisting either of doxycycline 200mg or a placebo (sugar pill). This double blind treatment study is being done to determine if doxycycline is effective in shortening any episode of "travelers' diarrhea." Doxycycline is an antibiotic closely related to tetracycline and is a safe drug for nearly all people. Side effects do occur rarely (less than 1% incidence) and these consist of nausea, vomiting, diarrhea allergic reactions, photosensitive reactions, vaginitis. Since you would be taking on a single dose of the medication the incidence of side effects should be extremely rare.

   Doxycycline is a standard antibiotic that is, it is not a new or investigational drug. It is however being used in a new way, to treat an illness which at present has no effective antibiotic treatment.

7. We need a detailed record of your illnesses; therefore, during illnesses you would be expected to complete a daily record of your symptoms.

8. Ten days after your illness we will obtain a blood sample (5ml).

9. Your medical records will be kept confidential.

10. You are free to leave the study at any time.

If you agree to join in the study please sign your name here.

_____
Name

_____
Date

PERMISSION FORM

Travelers' Diseases in Dacca
(Local Immunity Studies Group)

The International Center for Diarrheal Disease Research, Bangladesh (ICDDRB) (formerly Cholera Research Laboratory) is carrying out research into the cause, prevention and treatment of diarrheal disease. As you may know diarrheal diseases are quite prevalent in Bangladesh and are a major health problem. Expatriates who live in Bangladesh also frequently develop diarrheal illnesses which are generally caused by the same germs as cause illness in Bangladeshi people (except that cholera is almost never seen in expatriates).

The ICDDRB under supervision by Dr. David A. Sack is planning to carry out a study of specific illnesses of expatriates living in Dacca. The main reason for doing the study is to determine the way which persons develop protective immunity to these illnesses. Based on studies like this it is hoped that a vaccine will be developed for the most common causes of diarrhea of travelers and Bangladeshis.

We invite you to participate in this study of travelers' disease in Dacca. If you decide to enter the study you can expect the following:

1. A Clinic will be available for your use during the next year (the duration of the study). This can be used for all your medical problems. The clinic will provide (free of charge) routine examination, immunizations, laboratory work, minor surgery (e.g. suturing small uncomplicated lacerations) and will recommend referral for more complex or serious medical problems such as major surgery or problems for specialists in other areas.

2. You will be able to read and examine the research protocol describing the study. This is available on loan from Dr. Sack. Also you will be able to see your clinic and research records should you desire, though of course you would not be able to see anyone elses records.

3. You would be expected to come to the clinic every three months for certain routine tests and examinations. These quarterly examinations would include obtaining samples (5ml) of blood, throat secretion and stool.

4. During the year that you are enrolled in the clinic we want to keep in touch with you weekly to learn of any illnesses. This will be done by filling out a weekly health surveillance form and reporting to the clinic. Usually this surveillance can be done by phone.

...2/

5. If you become ill we want to know about your illness early in the illness so would ask you to visit the clinic on the first day. At some times house calls can be arranged. Reporting early is important so that accurate samples can be obtained and proper treatment given.

6. You will be included in a group of people in whom the local intestinal immunity to the diarrheal agents is being studied. To do the special studies of the local immunity there will be tests done three times during the next year to sample the antibodies being produced in the intestine. If you get diarrhea, these same tests would be done three additional times following the illness.

   The test that is done to sample these antibodies is called intestinal lavage. In this test you will be asked to drink a large volume of (one glass every ten minutes) salty water over a period of about three to four hours. Drinking this solution will cause you to have watery diarrhea; when you stop drinking the solution the diarrhea will also stop. The diarrheal stool that you pass will contain the antibodies and so this stool will be collected and tested.

   Side effects from the test will consist of a full feeling in the stomach from drinking so much fluid and you will have about 1 kg increase in weight temporarily. There are not however any serious side effects.

   During and after your diarrheal illnesses we would also collect a sample of blood (5ml) on day 5, 9, and 17 after the beginning of your illness.

7. We need a detailed record of your illnesses; therefore, during illnesses you would be expected to complete a daily record of your symptoms.

8. Ten days after your illness we will obtain a blood sample (5ml).

9. Your medical records will be kept confidential.

10. You are free to leave the study at any time.

If you agree to join the study please sign your name here.

_____
Name

_____
Date

PERMISSION FORM

Travelers' Diseases in Dacca
(Children's Group)

The International Center for Diarrheal Disease Research, Bangladesh (ICDDRB) (formerly Cholera Research Laboratory) is carrying out research into the cause, prevention and treatment of diarrheal disease. As you may know diarrheal diseases are quite prevalent in Bangladesh and are a major health problem. Expatriates who live in Bangladesh also frequently develop diarrheal illnesses which are generally caused by the same germs as cause illness in Bangladeshi people (except that cholera is almost never seen in expatriates).

The ICDDRB under supervision by Dr. David A. Sack is planning to carry out a study of specific illnesses of expatriates living in Dacca. The main reason for doing the study is to determine the way which persons develop protective immunity to these illnesses. Based on studies like this it is hoped that a vaccine will be developed for the most common causes of diarrhea of travelers and Bangladeshis.

We invite you to enter your child in this study of travelers' disease in Dacca. If you decide to do this you can expect the following:

1. A clinic will be available for his use during the next year (the duration of the study). This can be used for his medical problems. The clinic will provide (free of charge) routine examination, immunizations, laboratory work, minor surgery (e.g. suturing small uncomplicated lacerations) and will recommend referral for more complex or serious medical problems such as major surgery or problems for specialists in other areas.

2. You will be able to read and examine the research protocol describing the study. This is available on loan from Dr. Sack. Also you will be able to see his clinic and research records should you desire, though of course you would not be able to see anyone elses records.

3. You would be expected to bring him to the clinic every three months for certain routine tests and examinations. These quarterly examinations would include obtaining samples (5ml) of blood, throat secretion and stool.

4. During the year that he is enrolled in the clinic we want to keep in touch with you weekly to learn of any illnesses. This will be done by filling out a weekly health surveillance form and reporting to the clinic. Usually this surveillance can be done by phone.

...2/

5. If he becomes ill we want to know about his illness early in the illness, so would ask you to bring him to the clinic on the first day. At some times house calls can be arranged. Reporting early is important so that accurate samples can be obtained and proper treatment given.

6. He will be given the best medical treatment available and he will not be included in any research treatment study. The purpose of including children in this study is for descriptive purposes only.

7. We need a detailed record of his illnesses; therefore, during illnesses you would be expected to complete a daily record of his symptoms.

8. Ten days after his illness we will obtain a blood sample (5ml).

9. His medical records will be kept confidential.

10. He is free to leave the study at any time.

If you agree to enroll him in the study please sign your name here.

_____ _____

Child's Name Parent's Name

_____

Date