22

Attachment 1. Date

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator P. Speelman
Application No. 81-003
Title of Study Giardiasis and Amebiasis among expatriates in Dacca

Trainee Investigator (if any)
Supporting Agency (if Non-ICDDR,B)
Project status:
(x) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

Circle the appropriate answer to each of the following (If Not Applicable write NA).

Source of Population:
(a) Ill subjects (Yes) No
(b) Non-ill subjects (Yes) No
(c) Minors or persons under guardianship (Yes) No

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)

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

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

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 Cttee. for review.

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

Principal Investigator

Trainee

81-003

recd 27/1/81

SECTION I - RESEARCH PROTOCOL

1. Title: Giardiasis and Amebiasis among expatriates in Dacca, Bangladesh. Clinical, epidemiologic, immunologic and treatment aspects, a prospective study.

2. Principal Investigator: Dr. Pieter Speelman

3. Starting Date: January 1981

4. Completion Date: January 1982

5. Total Direct Cost:

6. Scientific Program Head: Dr. W. B. Greenough

This protocol has been approved by the Pathogenesis to Zoo [illegible] Working Group.

Signature of Scientific Program Head: W D G

Date: 22.1.81

7. Abstract Summary:

A prospective study will be done among 200 expatriate people, living in Dacca for a prolonged period. This group will be followed for the occurrence of infections with Giardia lamblia or Entamoeba histolytica for at least one year. There will be two groups of about 100 people each.

1. Dacca veterans: living for > 1 year in Bangladesh
2. New arrivals

These subjects will be under clinical surveillance continuously and will have quarterly follow-up for periodic examination and laboratory

tests. As soon as amoebic- or giardia-cysts (or trophozoites) are found - during an episode of illness, on admission, or at quaterly check-up ___ patients will enter a treatment study.

Giardiasis will be treated with metronidazol, tinidazol, one single oral dose, or placebo (double blind). Non-dysentery intestinal amoebiasis will be treated with diloxanide furoate, metronidazol or placebo (double blind). Before, during and after treatment sera will be drawn for detection of serum-antibodies (ELISA and IF) and saliva will be tested for total and specific secretory IgA, as measurement for the local (gut) immune response. Stools will be collected for detection of parasite antigen. Entamoeba histolytica will be cultivated for iso-enzyme characterization.

Epidemiological investigation will be performed to identify other cases and clusters of cases

SECTION II- RESEARCH PROTOCOL

A. INTRODUCTION

1. Objective

The purpose of this prospective work is to study the population of patients with giardiasis or amoebiasis in our traveller's clinic and to explore the relationship with signs, symptoms, stoolpicture, immunoglobulins, serum antibodies and local antibodies. A double blind treatment-study will be performed in patients with Glamblia and E. histolytica infection (non-dysentery). Epidemiologic investigation will be performed.

2.. BACKGROUND

Giardia Lamblia is a cosmopolitan parasite with worldwide distribution. Incidences vary between 2 and 25 to 30 percent.[1] Frequently the incidence rate is under-estimated because only a single stool specimen is examined.

Most patients harbouring this parasite are asymptomatic; in tropical countries, but also in moderate climates.

Giardiasis has become a common parasitic cause of water-borne outbreaks of disease. Beavers have been incriminated as an animal reservoir. Food and drinks, prepared with contaminated water, are probably frequent sources of infection. High rates of infection are found among children in nurseries and institutions most likely a result of hand to mouth transmission.[2]

During the last years increasing clinical and epidemiological evidence has been produced that G. Lamblia may be an important pathogen in man. Why some people develop symptoms and others do not, is not understood.

The incubation period of symptomatic giardiasis is usually about 2 weeks. The most common complaint is diarrhoea (sometimes with explosive onset), or loose stools which may be bulky foul-smelling and are often passed only in the mornings.

The stools may contain mucus, but blood and pus are absent. Other symptoms include abdominal distention and discomfort (upper epigastric

cramps), weakness anorexia, nausea, vomiting, flatulence, weightloss, depression and in children failure to thrive. The acute stage may last from a few days to several months. Some people may have subacute symptoms lasting for months or years.[3]

Parasitologic confirmation of the diagnosis may be difficult. Stool examination can give false negative results. The presence of G. Lamblia - cysts or trophozoites may be irregular and unpredictable.[4]

Intermittent passage of parasites in the stool may be related to periods of active multiplication.[5] Probably related to the intermittent passage of parasites is the fact that examination of stools on alternate days has provided an increased yield of positive specimens. Stool examination using a concentration method will kill the trophozoites. A false negative result can be obtained if the stool contains these forms only.

One stool examination only is diagnostic in 50[6]-76[5]%. Utilizing direct smeer and formol-ether concentration-tests 76% were positive on the first specimen, 90% were confirmed with two specimens and 97% of the cases were determined to be positive with three specimens[5][6] Delays of several months between the onset of symptoms and the diagnosis are common.[7]

Studies from a number of centers have reported malabsorption with giardiasis d-Xylose and fat-malabsorption have been reported from India[8][9] and North America [10] [11] [12] [13].

In overland travellers with symptomatic giardiasis impaired Xylose and fat absorption has been shown in 58% and 38% of patients but unlike other gruoups, they found abnormal absorption of Vit B_{12} in 50% of patients.[14)]

Abnormalities of the jejunal mucosa are found in association with malabsorption.[15)]

The extent to which secretory immunoglobulin contributes to eradication is not known, but it has been recognized for some time[16)] that deficiency of humoral activity is associated with an increased prevalence of giardiasis, suggesting that immunoglobulin is important.

However in generally healthy individuals, giardiasis is not etiologically related to relative immunoglobulin deficiencies.[17)] Hypochlorhydria or achlorhydria have been shown to facilitate Giardia infections.[18)]

In giardiasis, like in tropical sprue, colonization of the small intestine with enterobacteria has been found.[22)]

Serum antibodies to G. lamblia have been found [15) 20) 21)]. Probably this shows that parasite antigen is absorbed through the intestinal epithelium. However, there is still doubt if and how Giardia invades the tissue. Because Giardia are found in the intestinal lumen, secretory antibody would be expected to play a role. Although a reduced level of secretory IgA has been reported in patients with giardiasis, this was confirmed by others. Circulating IgE has also been found to be normal is giardiasis.[63)]

.../6A

Immunofluorescent labelling of lymphocytes and plasma cells in the intestinal wall in giardiasis showed initial proliferation of cells bearing IgM and later IgG and IgA.[21)]

Cellular immune mechanisms have been studied using athymic nude mice In immunologically competent mice, Giardia are cleared from the stools within 28 days after infection.[64)] In nude mice and neonatally thymectomized mice, infection persisted up to six months[65)] unless their immune system was reconstituted with cells from normal mice, expecially donors with prior Giardia infection.[66)]

Normal mice ususally show resistance to reinfection, but thymusdeficient mice do not. There is an increased incidence of infection in patients with blood group A.[67) 68)]

During the last ten years many workers have recommended quinacrine as the treatment of choice for giardiasis. It was found to be highly effective. Excellent results have been obtained in those children who tolerate it, but quinacrine proved to be tolerated very poorly by young children.[5)] Nowadays metronidazol is recommended by many authors. Several studies have been performed to compare quinacrine and metronidazol.

In 1970 Bassily[23)] treated 4 groups of 20 patients with quinacrine, metronidazol, furoxone and placebo. The cure-rates were respectively 100-95-80 and 0%. Considering the side-effects of quinacrine he concluded that metronidazol was the drug of choice. In 1977 Wright[3)] S.Q. et al found that metronidazol 2.0 g as a single daily dose on 3 successive

days, produced a parasitological cure rate of 91%. In contrast, the standard course of mepacrine, 100 mg thrice daily for 10 days eradicated the parasite in only 63% of patients. Kavousi[24] compared in a prospective study metronidazol and quinacrine. Considering the low failure rate, the less severe side-effects and the relatively more tolerable flavor, he found out that metronidazol was preferable above quinacrine in the treatment of giardiasis.

In 1969, Howes et al[26] and Tailor et al [27] reported their experiences with tinidazol. They demonstrated the high antiflagellate activity and the low toxicity of tinidazol. It was found to be more effective than metronidazol against Trichomonas. No serious side-effects were reported. Several authors[28-32] have reported their experiences with tinidazol in the treatment of giardiasis. With doses 2dd 150 mg/7 days, 1000 mg and 2000 mg as a single oral dose, cure rates of 90-100% were obtained.

Levi G.C. et al[25] compared furazolidone, nimirazol metronidazol, tinidazol and placebo in the treatment of giardiasis. The cure-rates were 72, 94, 87, 97 and 34% with no side-effects in the tinidazol and placebo-group. Jokipii[33] compared tinidazol 2dd 150 mg/7 days and 2000 mg as a single oral dose. Cure-rates were 74 and 92%. Recently Jokipii[34] compared in a comparative trial 2, 4 g metronidazol, either once or on two successive days with 2, 0 g of tinidazol in the treatment of giardiasis. The rates of success were 50, 77 and 93%. Tinidazol seems to be more active[35] and more effective drug in the treatment of giardiasis.

AMEBIASIS:

Amebiasis denotes the condition of harbouring Entamoeba histolytica with or without clinical manifestation (WHO 1969). Although multiple organs may be involved, the colon is the usual site of initial disease. The manifestations of illness may vary from the asymptomatic carrier state to a severe fulminating illness with mucosal inflammation and ulceration. There are numerous species of amoeba which inhabit the human intestinal tract, however, with rare exceptions E. histolytica seems to be the only variety pathogenic for man.

The worldwide average incidence of amebiasis has been estimated at 10 %, however there is a wide variation of incidence figures, depending upon the population studied.[36] Amoebiasis is also found in temperate climates, for instance in the United States, where 5% of the untraveled population is infected with E. histolytica.[37] Ninety per cent of this group are asymptomatic cyst passers, making detection and accurate epidemiologic study difficult.[38]

Asymptomatic patients with no evidence of tissue invasion harbor only cysts in their stools. However, they are of the most concern epidemiologically. The disease can be transmitted by individuals who are unaware of their infective potential. On the other hand, there are patients with gastrointestinal complaints of non-amebic origin who harbor cysts in their stools, but who are living in a peaceful symbiotic relationship with the organism. These patients are often erroneously diagnosed as having symptomatic amebiasis.

The most common manifestation of infection with E. histolytica is a completely normal, healthy asymptomatic person. There is much debate over whether these so-called "healthy carriers" have in their intestinal tract a commensurate microbe or whether they undergo small, easily controlled insults to the bowel mucosa. However, all agree that these persons are symptomless. The life cycle of E. histolytica is not complex, involving only the encystment of a trophozoite, followed by release of the trophozoite from the cyst when conditions are appropriate. The trophozoite undergoes encystment only in the bowel, usually associated with conditions in the lumen which are not ideal for continued activity of the trophozoite.

The conditions that allow these amebas to become invasive are not clear. However, it has been shown in germ-free animals that the virulence of E. histolytica is in some ways dependent upon the presence of bacteria and this fact is the basis for the rationale of using antibiotic therapy in the early stages of treatment. Experimental work in rats and guinea pigs suggests that an excess of cholesterol or carbohydrate in the diet provides a suitable environment for invasion by trophozoites.[40] Virulence has been promoted in experimental animals by irritating the bowel with abrasives, chemicals or invasive bacteria.[41] [42] Virulence can apparently be increased in certain conditions; in the Durban area a gain in virulence and hence in the frequency of invasion, was linked with rapid transmission.[43] Neal[40] found that patients with significant clinical symptoms harboured E. histolytica virulent to rats and had positive serological tests. In infected persons without clinical symptoms, serum

antibodies were found only occasionally, but the virulence of the strains they harboured was generally low. The mechanism of invasion is also not clearly understood. It has been postulated that toxic products are liberated from amebae. The released toxic products provoke a generalized inflammatory response. Trophozoites hove been shown to break down cement substance between epithelial cells and to gain access to the submucosal region by moving between these cells.[44)]

Sargeaunt and coworkers have studied the isoenzyme pattern of various strains of E. histolytica using starch gel electrophoresis. They found distinct electrophoretic mobility patterns of the three enzymes glucose phosphate, isomerase, phosphogluconentase and $NADP^+$ oxireductase in all strains from patients with invasive amoebiasis. This finding indicates that parasite genotypic characteristics might determine invasiveness of parasites. Since preliminary studies by Glass, Ljungström and Huldt suggest high prevalence of serum antibodies to E. histolytica in children and adults in Bangladesh, indicating presence of invasive strains it is considered of interest to examine Bangladeshi strains for their isoenzyme pattern.

The diagnosis of intestinal amebiasis is made by identifying E. histolytica in the stool. Finding either trophozoites or cysts confirms the diagnosis of intestinal infection. However, it must

be considered that laboratories vary tremendously in their ability to diagnose amebiasis. The success of identifying amebas depends on several factors, for instance, whether concentration techniques are used to find cysts, the average specimen load on the laboratory, and the turnover rate of the technicians. It has often been stated that because of intermittent shedding of amebas into the stool, three specimens should be submitted before excluding the diagnosis of amebiasis.

A variety of immunodiagnostic tests are available. A positive immunological test indicates past or present contact with amoebae. A negative sensitive test, like ELISA, practically eliminates the possibility of invasion amoebiasis. Antibodies probably arise only as a result of parenteral contact with amoebae. When amoeba confine their activity to the bowel lumen, negative results are expected. However, using the ELISA Bos et al[56] found titers between 20 en 500 in 50% of symptomless carriers, significantly different from the titers of lower than 20 in stool negative persons. It is apparent to clinicians that patients may have repeated reinfection with E. histolytica resulting in colitis, however reinfection is very rare after successful treatment for an amoebic liver abscess. Axenic amoebic antigen, chromatographed on Sephadex G-200, can be divided in three fractions. Fraction I proved to be highly immunogenic compared to other fractions.[57] Immunization of animals with this high molecular weight fraction (I) can protect 92% of them.[58] Cellular immunity may also play a part in the control of the infection. It has been shown

that cellular immunity is altered in patients with amoebic liver abscess. However Jain[59] recently observed that the cellular immunity was not markedly altered in experimentally infected guineapigs with only caecal lesions. Harris[60] reported almost identical findings in clinical cases of intestinal amoebiasis.

The question of whether patients with intralumenal infection should be treated at all has been raised repeatedly. In areas of high endemicity the argument is usually not to treat, unless invasive disease is documented. In other places, such as the US and Europe, all patients are candidates for therapy, for the transmission rates are low. Textbooks made the following recommendations for the treatment of asymptomatic intralumenal infection: Cecil-Beeson-Mc Dermott 1979: diiodohydroxyquin. Mandell-douglas-Bennett 1979: tetracycline 4 dd 250 mg/7 days followed by or diiodohydroxyquin or diloxanide furoate. Conn, current therapy 1980: diloxanide furoate. Sleisinger-Fordtran 1979: diiodohydroxyquin.

B. SPECIFIC AIMS

1. To study among expatriates the population of patients with giardiasis and non-dysenteric intestinal amoebiasis and to explore the relationship with signs, symptoms, stoolpicture and immunoglobulins.

2. To perform immunologic studies in these patients:

   a. Serum-antibodies against G. lamblia or E. histolytica will be measured before, during and after infection.

   b. The local immune-response will be studied through detection of total and specific secretory IgA in saliva, before, during and after infection.

   c. We will try to detect parasite antigen in the stools of these patients.

3. To study the iso-enzyme pattern of E. histolytica in amoebic cyst-passers.

4. To perform some epidemiologic studies of giardiasis and amoebiasis.

5. To study the results of different treatments in giardiasis and non-dysenteric intestinal amoebiasis in double blind trials.

C. METHODS OF PROCEDURE

At least 200 expatriate people of all ages, living in Dacca for a prolonged period will be followed prospectively for the occurrence of Giardia and or Amoebic infections.

These subjects will be divided into two groups:

I. Dacca expatriate residents: a group of >100 people from North-America, Europe or Australia who have lived in Dacca for more than one year.

II. Dacca expatriate newcomers: a group of > 100 people from North-America, Europe or Australia will be recruited during their first month in Dacca

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 routine examinations, 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 from, which will be suitable for coding.

The following laboratory tests will be done:

a) Chest-X-ray (if not done 1 yr)
b) PPD (if no BCG)
c) CBC, immunoglobulines, bloodgroup, serumantibodies against G. lamblia and E. histolytica
d) Urine-analysis
e) Stool for M.E. (2x) and culture (1x)
f) Saliva for measurement local immune response, in older children and adults.

Following this initial session each subject will keep a health diary in which illnesses are recorded. This will be recorded weekly.

Quarterly each subject will visit the 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 tests will be the same as the initial except the X-ray, PPD, and urine analysis will be annual.

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

Patients with diarrhea will have a standardized history, physical examination and the same blood, and stool and saliva tests as at admission and quarterly check up.

Patients with fever and or respiratory infections will have appropriate examination and treatment according to best clinical judgement.

As soon as amoebic or giardia cysts or trophozoites are found, during an episode of illness, on admission or at quarterly check-up, patients will enter the treatment study. Before entering the treatment study the medical history will be taken again and physical examination blood and stool and saliva examination will be performed.

Patients with amoebic cysts or trophozoites in their stool will have a proctoscopy and biopsy before entering the treatment study, however, this will be done only in patients of 16 years and older.

The following drugs will be used in the two treatment studies:

- Diloxanide Furoate (Furamide)
- Metronidazol (Flagyl)
- Tinidazol (Fasigyn)

Diloxanide furoate (Furamide^{R})

On search for new amebicides a number of anilides looked promising. One of them was selected for further investigation and ultimately, after extensive laboratory and clinical investigation it was marked in Britain (Diloxanide). Further investigations were carried out to find a derivate which would give better results. Finally they found out that the furoate ester did not only give better results in acute amebic dysentry, but also in the asymptomatic condition. This compound was given the name diloxanide furoate.

It is less soluble than its earlier parent compound and is more slowly absorbed from the bowel and excreted from the body, thus providing a higher concentration in the bowel wall and lumen for a longer period of time.

Studies carried out in various parts of the world with diloxanide furoate, in highly endemic areas and in returnees from endemic areas to England and France have claimed cure rates of more than 90%. [45-47] However only 40% cure rate was found for amebic dysentry in Durban, South Africa.[48] Other workers also consider diloxanide furoate inferior to other better absorbed drugs in acute amebic dysentry, where there is significant tissue invasion.[49] [50]

In '73 Wolfe[51] published his experiences with diloxanide furoate in the treatment of nondysenteric intestinal amebiasis. Excessive flatulence was a common, but the only significant side effect. The high effectiveness (83% cure rate) and the minimal toxicity indicated that this drug has numerous advantages over other primarily luminal acting amebicides.

Metronidozol (Flagyl^{R})

Introduced in 1959.

It is a nitroimidazole compound, used in the treatment of trichomoniasis, amebiasis and giardiasis.[52] It is effective at both intestinal and extraintestinal sites. It is well absorbed and seems to be more effective in symptomatic or invasive amebiasis than in the asymptomatic cyst-passer state.[53]

Usual side effects are nausea, headache and a metallic taste. Dizziness, vomiting, abdominal cramps and diarrhea are less common. Dark urine may occur from a metabolite of the drug. It may potentiate the anticoagulant effect of coumarin[54] and it should not be used with alcohol. Metronidazol has caused lung tumors in mice but not in hamsters and it is mutagenic to some bacteria.[52] These effects have not been seen in man. It should not be used in pregnancy. When used in amebiasis, metronidazole should always be followed by a lumenal-active drug[55].

Tinidazol (Fasigyn®)

This drug is chemically and structurally releated to metronidazol and also used for the treatment of trichomoniasis, amebiasis and giardiasis (see background). It is also rapidly and completely absorbed with peak serum levels one to two hours after a single 2 gm dose. The mean serum half-live of tinidazole is about 50% longer than the half-live of metronidazole.[34)]

For many reasons single dose therapy is preferred by the patient and by the community. The efficacity of tinidazole in one dose has been reported (background). Mild side effects related to the gastrointestinal tract have been reported, nausea, vomiting and diarrhea. As with metronidazol, Fasigyn may produce transient leucopenia.

Although believed not to occur with Fasigyn, related compounds when taken together with alcoholic beverages, have caused abdominal cramps, flushing and vomiting. Allergic skin reactions have been noticed. Abnormal neurological isgns (dizziness, vertigo, ataxia) have been reported. It should not be used in pregnancy.

Treatment Study Giardiasis

1. Objective: to compare the efficacy of Metronidazole with that of Tinidazole and placebo in clearing cysts of Giardia lamblia from the stools in comparable groups of patients, in a double blind clinical trial.

2. Patients are selected as described above; they have to give their informed consent to patticipate in the trial.

   Exclusion criteria

   - suspicion of a concomitant infectious disease
   - a history of intake of antibiotic or anti-giardia drugs within 10 days prior to entry to the trial
   - pregnancy or suspected pregnancy, lactating women

3. Treatment groups:

   I. patients receiving Flagyl

      adults : 1x 4 tablets à 600 mg

      children : 1x ml liquid formula metronidazole containing .. mg/ml dose 60 mg/kilo body weight

   II, patients receiving Fasigyn

      adult : 1x 4 tablets à 500 mg

      children : 1x ml liquid formula tinidazole containing mg/ml dose 50 mg/kilo body weight

   III. patients receiving Placebo

      adults : 1 x 4 tablets

      children : liquid formula containing ... mg/ml dose 50 mg/kilo body weight

TREATMENT STUDY AMEBIASIS

1. Objective: to compare the efficacity diloxanide furoate with that of metronidazol and placebo in clearing cysts of E. histolytica from the stools in comparable groups of amebic cyst passers, in a double blind clinical trial.

2. Patients are selected as described above, they have to give their informed consent to participate in the trial.

Exclusion criteria:

- Symptoms or signs suggestive of amebic dysentry or systemic amebiasis.
- Suspicion of a concomitant infectious disease.
- A history of intake of antibiotic or anti-ameebic drugs within 10 days prior to entry to the trial.
- Pregnancy or suspected pregnancy; lactating women.

3. Treatment Groups:

I. Patients receiving Furamide

Adults : 1 tablet à 500mg 3x per 24 hours for 10 days.
Children: 20mg/kg body weight daily in 3 divided doses for 10 days.

II. Patients receiving Flagyl

Adults : 1 tablet à 250mg 3x per 24 hours for 10 days.
Children: 20mg/kg body weight daily in 3 divided doses for 10 days.

III. Patients receiving Placebo

Adults : 1 tablet 3x per 24 hours for 10 days
Children: 20 mg/kg body weight daily in 3 divided doses for 10 days

DRUG SUPPLIES

Trial medication will be supplied by pfizer for the giardiasis treatment study and by Boots for the amebiasis treatment study.

Supplies will be packaged in packs labelled only with the patient's number and a code lot number. A sufficient amount of the drug for one treatment course will be packaged individually in a box for each patient number.

In the giardiasis treatment trial special packages will be made for the treatment of children. These packages will contain liquid preperations. The investigator will be supplied with an emergency list to provide him with the means of identifying in emergencies the individual trial medication allocated to each patient. A random assignment schedule will be provided.

All patients will get a trial number sequentially and started on treatment according to the pre-designed random assignment schedule.

Tablet counts will be made during and at the end of the period of treatment. Any other drugs given during the period of trial medication shall be recorded.

Unwanted effects:

All patients get a special form, on which they record daily whether or not any unwanted change has occurred in the state of health.

To follow the clinical course of the patient daily diary must be filled out on the subject of diarrhoea and other (related) complaints.

Drop-outs

- patients not complying with the trial protocol for reasons other than those included under "Treatment Failures".
- patients requiring concomitant treatment with anti-biotics and/or

other drugs which could interfere with any stage of E. histolytica.

Treatment Failures

- premature discontinuation of trial medication because of drug related unwanted effects.

- patients still passing cysts and/or trophozoites in their stool after 2, 3 or 4 weeks will be considered as treatment failures. If stool specimens are positive after 4 weeks, a reinfection cannot be excluded, however, this seems very unlikely. Parasitological failures after treatment of giardiasis have been described after 8 weeks of follow-up[34] in a country where no endemic giardiasis occurs. However, most of the failures have been detected in two to four weeks. In a recent study of Jokipii only 2 out of 22 failures were detected after more than 4 weeks.

Patients with positive stools following treatment with one of the trial medications will be retreated with Fasigyn (Giardiasis) or Furamide (Amebiasis). Again they will have a follow-up.

- To identify cases and clusters of cases, a family study will be done. The index patient will answer questions regarding a symptom in other members of the family and household servants. Also a member of the family will be designated as "recorder" for the family. The study nurse will visit the family, talk to the "recorder" and explain the family study to him/her. Stool specimens (4x) for microscopic examination will be requested from all members of the family and servants and these people will be under surveillance for symptoms of a infection for 28 days after the treatment of the index patient. The nurse will talk to the recorder every week to inquire about symptoms.

If any of these persons proves to have a Giardia or Amoebic infection, he shall be asked to enter the study and therapy-trial.

Examinations during - and/or post - treatment

1. One week after the start of treatment the study nurse will visit the patient to collect 2 control stool specimens, the diary and the unwanted effects form and to give new forms. On this occasion she

will collect the first stool specimens of family members and servants.

II. Two weeks after the start of treatment the patient has to come back to the traveller's clinic to bring two control stool specimens and again stools specimens from family - members and servants. On this occasion a second blood - sample will be drawn for measurement of serum-antibodies and the patient has to give a second saliva - sample.

III. Three weeks after treatment = one week after treatment, see I.

IV. Four weeks after starting treatment the patient has to come back to T.C. to bring the last stool specimens and to give blood and saliva.

V. 3, 6, and 12 months after treatment blood will be drawn and saliva be collected. Should reinfection occur, the same procedure starts again.

N.B.: Those patients, not enrolled in this study, who come to the T.C. with complaints of diarrhoeal disease and prove to have Giardia lamblia or Entamoeba histolytica cysts or trophozoites in their stools will be asked to enroll the study or at least the treatment study.

Data Analysis

The data will be recorded on speial data sheet for entering onto computer discs.

Laboratory Studies

Bacteriology:

All stool specimens will be tested for Salmonellae, Shigellae, Vibrio, E. coli, (LT/ST) Campylobacter Yersinia and Rotavirus antigen.

Haematology:

Blood will be drawn from the finger for C.B.C., immunoglobulines and bloodgroup.

Parasitology:

Morphology identification of Giardia lamblia and Entamoeba histolytica will be performed after direct and (if negative) formal-ether concentration. Samples will be examined unstained and after iodine-staining.

Immunology:

- cultivation of E. histolytica for isoenzyme characterization will be performed on a modified Dobell's medium.
- Detection of parasite antigens in the stools will be performed with (in principle) the method used for Rotavirus detection.
- For detection of serumantibodies against Giardia lamblia and/or Entamoeba histolytica ELISA performed with polyvalent anti-IgG conjugates will be used, if using class-specific conjugates.
- Detection of total and specific secretory IgA in the saliva will be determined according to Sohl-Åkerlund et al. For specific SIgA antibodies ELISA and/or IF with anti-Sc-conjugates will be used.

INDIRECT IMMUNOFLUORESCENCE (IF)

I. Serum dilutions

Dilute the patient sera in phosphate-buffered saline, pH 7.4 (PBS). The dilution of 1/10 is recommended to be the first serum dilution. From that serial dilution can be prepared. Two-step dilution is recommended for human routine serology.

II. Chessboard titration of the conjugate

(Huldt, G., Ljungström, I. and Aust-Kettis, A. (1975) Ann. N.Y. Sci., 254, 304-314).

For each batch of fluorescein-conjugated anti-human antibody (conjugate) endpoint titrations of antisera against serial dilutions of conjugates, has to be done. The working dilution should be one next to the plateau endpoint.

III. Counter staining

In order to avoid nonspecific staining the slides are immersed in Evan's Blue, dilution 1/500 in PBS, pH 7.4 (see sect. V:12).

IV. Controls

Positive serum with known titer.

Negative serum (sera)

Buffer, for testing the specificity of the second layer and/or the third layer in the staining procedure.

V. Staining procedure:

: 1 Make a list protocol

: 2 Place the number of slides to be used in room temperature and allow to dry before the staining procedure.

: 3 Mark the slides according to the test protocol.

: 4 Place the slides horizontally in a moist chamber.

: 5 Pipette the serum dilutions onto the slides, according to the test protocol, so that the antigen spots are completely covered.

: 6 Incubate the slides, in the moist chamber, for 30 min at room temparature

: 7 a. Wash the slides one at a time by dipping the slides into PBS.

b. Remove the excess of PBS by touching the edge of the slide against filter paper.

c. Place the slides together in a washing basin containing PBS and let stand for 5 min. Repeat twice with fresh PBS in the basin sect.V:7b,c.

: 8 Remove all buffer from the slides with filter paper and place the slides in the moist chamber (see sect. V:4). DO NOT ALLOW THE SECTIONS TO DRY!

: 9 Pipette the conjugate, suitably diluted in PBS (see sect. II), onto the sections, so that the sections are completely covered.

:10 Incubate as before - sect. V:6

:11 Wash as before - sect. V:7

:12 Place the slides in a basin containing Evan's Blue, diluted 1/500 in PBS.

:13 Incubate for 13 min at room temperature.

:14 Wash as before - sect. V:7.

:15 Remove all buffer on both sides of the slide with filter paper. DO NOT ALLOW THE SECTIONS TO DRY during the procedure.

:16 Add one drop of glycerine buffer (1 part PBS, pH 7.8 - 7.9 to 9 parts of glycerin) to each dot on the slide and place a cover glass on the top.

:17 Examine the sections under a fluorescence microscope equipped for FITC-fluorescence..

Comments:

Class-specific conjugates can be used instead of anti-Ig. Sera from various species can be examined. In that case a conjugated anti-species gamma globulin has to be used. If a conjugated anti-sepcies gamma globulin is not available a third layer can be used, e.g.,mouse-antibody, goat-anti-mouse-antibody and anti-goat-antibody-FITC.

ELISA = Enzyme Linked ImmunoSorbent Assay

Reference: Engwall, E. and Perlmann, P. (1972) J. Immunol. 109, 129-135.

1. Coat microtiterplate with 0.2 ml/well of the antigen dissolved in coating buffer to a suitable concentration.
   Incubate at room temperature over night.
2. Wash the plate three times in washing solution.
3. Dilute the testserum to suitable concentrations in the incubationbuffer.
   Incubate the plate with 0.2 ml/well of the serum dilution for 1 hour at 37°C.
4. Wash the plate three times in washing solution.
5. Incubate with 0.2 ml/well of the enzyme-labelled anti-immunoglobulin diluted in incubation buffer for 2 hours at 37°C.
6. Wash the plate three times in washing solution.
7. Measure the enzyme activity bound to the plate by adding 0.2 ml/well of the substrate.
   The reaction is stopped by adding 0.05 ml/well of 1 M NaOH.
   The reaction product is measured in a spectrophotometer at 449 nm.

Substrate

Reference: Ruitenberg, J. et al, (1976) J. Immunol. Methods, 10, 67.

A: 5-amino-2-hydroxy bensoic acid, pH 6.0 (40 mg/50 ml distilled water)

B: 0.05 per cent H_2O_2 9 parts of A and 1 part of B.

SOLUTIONS

Coating buffer 0.05 M carbonate pH 9.6

1.59 g $Na_2 CO_3$

2.93 g $Na HCO_3$

0.2 g $Na N_3$

Solve in 1 liter distilled H_2O.

Incubation buffer

900 ml PBS

0.45 ml Tween 20

Washing solution 5 liter

45 g NaCl

2.5 ml Tween 20

Solve in 5 liter distilled H_2O.

PBS (SBL) pH 7.4

8 g NaCl

0.2 g $KH_2 PO_4$

2.89 g $Na_2 HPO_4 12H_2O$

0.2 g KCl

Solve in 1 liter distilled H_2O.

D. SIGNIFICANCE

This study will provide valuable information on the magnitude of the problem of infection with Giardia lamblia and Entamoeba histolytica among patients consulting our travellers clinic.

It will also tell us more about the clinical features, the epidemiology, the efficacy of treatment and the value of different kinds of treatment. We will get information about the immunological response of the patient, and the value of antibody assay (local and systemic antibody responses in giardiasis and amoebiasis.

E. FACILITIES REQUIRED

1. The office and clinic space is already provided.
2. Laboratory space is already provided.
3. Hospital resources: hopefully none will be needed.
4. No animal resources.
5. Logistic support: the study nurse will have to visit each house several times: one vehicle three hours, 5x a week.
6. Major items of equipment: none
7. Specialized requirements: some spare parts for procto-sigmoidoscopies, biopsy forceps, light handle, transformer etc.
   Several drugs and placebo.

F. COLLABORATIVE ARRANGEMENTS:

This study will be a collaborative study between

- ICDDR,B (Dr. P. Speelman) and

- The National Bacteriological Laboratory
  S-10521 Stockholm, Sweden
  Dr. I. Ljungström, Dr. G. Huldt and Dr. M.S. Grundy

Authors will include - but not necessarily be limited to -

Drs. Speelman, Ljungström, Huldt and Grundy

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Abstract Summary

1. Two hundred expatriates will participate in this study, including infants and children, because they are a major group at risk. It is not possible to perform this study among Bangladeshi patients because of the very high background pathology.
2. Risks of the proposed are minimal. A fingerprick bloodspecimen will be taken quarterly and 2 and 4 weeks after starting treatment for infection with G. lamblia or E. histolytica. In the treatment trials only well established drugs are used with a known low risk of toxicity. The question of whether patients in an area of high endemicity should be treated is still open. So the use of a placebo in this treatment trial can be justified and cannot harm any patient. Patients with positive stools following treatment will be retreated with an active drug. Protoscopy and biopsy - if indicated - in adult amebic cyst passers is without any substantial risk.
3. All patients will have complete histories taken and physical examination. Patients who are lactating, pregnant or who are allergic to one of the trial-drugs will not be included in the treatment study. Proctoscopy in amoebic cyst passers will be performed in adult patients only.
4. All clinical records will be maintained in a locked file in the clinical 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.

../2

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 prevention of diarrhoeal disease.
8. The planned research will use the clinic records (which are being collected as research records) and will use blood, saliva and stool, and in some cases a rectal mucosal biopsy.

SECTION III - BUDGET

A. DETAILED BUDGET

1. Personnel Services

	Position	% of effort	Taka	Dollar
Dr. P. Speelman	Investigator	50%	-	12,500
Dr. G. Huldt	Investigator	-	-	-
Dr. I. Ljungström	Investigator	-	-	-
To be named	Co-Investigator	20%	8,000	-
To be named	Study nurse	100%	-	12,000
Mrs. N. Boone	Study nurse	50%	-	6,000
Daniel Ascension	Secretary	75%	17,000	-
To be named	Techn.Clin.Path.	100%	20,000	-
To be named	Techn.Immunology	100%	20,000	-
To be named	Techn.Bacteriology	50%	10,000	-
To be named	Driver	50%	8,000	-
		Sub total :	83,000	30,500

2. Supplies and Materials

a.	Clinical supplies - vaccines, clinical medications		1,000
	- Other clinic supplies : drapes syringes, needles, cups, plastic etc.		1,000
	- 1 microscope (light)		1,000
	- 1 refrigerator		500
b.	Lab. supplies - glassware, media, reagents		5,000
c.	Lab. tests - CBC ± 1500 x 7.30	10,950	
	- stool ME ± 3000 x 4.75	14,250	
	- stool culture ± 1000 x 15.00	15,000	
	- chest X-rays ± 120 x 25	3,000	
	Sub total :	43,200	8,500

		Taka	Dollar
3.	Equipment		
	Proctosigmoidoscope 11 mm x 25	-	500
	Biopsy forceps		
	Replacement lamps		
	Light handle		
	Transformer		
	Sub total :	0	500
4.	Patient hospitalization	-	-
5.	Outpatient care		
	This will be done through special clinic	-	-
6.	ICDDR,B transport		
	Mileage - Dacca 5000 miles estimated (x 30/mile)	15,000	-
	Sub total :	15,000	0
7.	Travel and Transportation of persons		
	- international travel to present paper at international meeting	-	2,500
	- international travel for one person of Swedish group to set up new technique	-	2,500
	Sub total :	0	5,000
8.	Transportation of things		
	- import supplies and equipment	-	2,500
	- transport of specimens	-	1,000
	Sub total :	0	3,500
9.	Rent, communications and utilities		
	Postage	-	100
	Telephone (study nurse)	2,000	-
	Cables	-	100
	Sub total :	2,000	200

			Taka	Dollar
10.	Printing and Xerox			
	Printing forms		7,500	
	Publication costs			1,000
	Xerox		10,000	
		Sub total	17,500	1,000
11.	Other contractual services		-	-
	Consultant fees		-	-
	Patient payments		-	-
	Other Payments		-	-
12.	Construction, renovation, alterations		-	-
		Total :	160,700	49,200

B. Budget Summary

		Taka	Dollars
1.	Personnel	85,000	30,500
2.	Supplies	43,200	8,500
3.	Equipment	-	500
4.	Hospitalization	-	-
5.	Outpatients	-	-
6.	ICDDR,B transport	15,000	-
7.	Travel persons	-	5,000
8.	Transportation things	-	3,500
9.	Rent/Communication	2,000	200
10.	Printing/Reproduction	17,500	1,000
11.	Contractual service	-	-
12.	Construction	-	-
	Total :	160,700	49,200
	Total US $:	10,480	49,200

Grand Total :US $ 59,245

(Conversion rate US $1.00 = Tk.16.00)

PERMISSION FORM

Amoebiasis and Giardiasis

The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) (formerly Cholera Research Laboratory) is carrying out research into the cause, prevention and treatment of diarrhoeal disease. As you may know diarrhoeal diseases are quite prevalent in Bangladesh and are a major health problem. Expatriates who live in Bangladesh also frequently develop diarrhoeal 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 ICDDR,B under the supervision of Dr. P. Speelman is planning to carry out a study of amoebiasis and giardiasis among expatriates living in Dacca. The main reason for doing this study is to find out if patients get complaints when they acquire this infection, if they make antibodies to the causative organism, if they infect other people in the household and to study the results of different treatment schedules in amoebiasis and giardiasis.

We invite you to participate in this study of amoebic and giardia-infections 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 (if available), 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. Speelman. Also you will be able to see your clinical and research records should you desire.

3. You would be expected to come to the Clinic every three months for certain routine tests and examinations. These quarterly examinations would consist of obtaining samples of blood, stool and saliva.

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.

5. If you become illwe want to know about your illness early in 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. If you acquire an amoebic infection, we shall perform a proctoscopy and biopsy before you enter the treatment study. Proctoscopy means an examination of the last few inches of your bowel. This examination including the biopsy, is without substantial risk and will not cause pain.

7. For most illnesses you will be treated with standard medical treatment (that is no investigational treatment). However, if you get an infection with amoebas or Giardia you will be included in a treatment study and you will receive:

- in case of giardiasis:	or Flagyl (metronidazol)
	or Fasigyn (tinidazol)
	or Placebo (sugar pill)
- incase of amoebiasis:	or Flagyl (metronidazol)
	or Furamide (Diloxanide furoate)
	or Placebo (sugar pill)

This treatment study is double blind, that means that neither the doctor nor the patient knows which treatment you get. Flagyl, a drug already known for 20 years, is effective in giardiasis and more or less in amoebiasis. Fasigyn is a rather new drug, chemically and structurally related to Flagyl; probably it ismore effective in giardiasis and has less side-effects.

Reported side-effects of Flagyl are: nausea, headache, metallic taste, dizziness, abdominal cramps, dark urine and adverse reactions when taken together with alcohol. The side-effects of Fasigyn are the same but probably less frequent and serious. Furamide is a safe drug. The only reported side-effect is flatulence.

When the treatment is not effective and you still have complaints, you will be retreated with Fasigyn in case of giardiasis and with Furamide in case of amoebiasis. You will get a special form on which you can record daily whether you have experienced any side-effect of the medication.

8. We need a detailed record of your illness; therefore, during illness you will be expected to complete a record of your symptoms.

9. One and three weeks after start of treatment the nurse will come to your house to collect two stoop specimens from you. On this occasion she will also ask for stool specimen from your family-members and servants. This is to identify clusters of cases of giardiasis or amoebiasis. Two and four weeks after start of treatment you have to come back to the travellers clinic to bring two stool specimens from you and one specimen of every family-member and servant. On these occasions we will take blood samples from your finger and we will ask saliva-specimen.

10. Your medical records will be kept confidential.

11. 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

12. If you agree one or more of your children (less than 13 years old) to participate in the study, please sign your name and the name of your child(ren) here.

_____ Your name

_____ Child

_____ Child

_____ Child

_____ Child

_____ Date

Amoebiasis - Giardiasis - Study

Admission Form (History)

Date :

Name :

Patient No.:

Sex : Birth date :

How long in Bangladesh now :

Expected stay in Bangladesh :

Nationality : Marital status : Children :

Countries lived in during last 2 years (>3 months) :

Country	Diarrhoea
_____	_____
_____	_____
_____	_____

Past medical history:

Previous hospitalizations :

Significant acute illness :

Significant chronic illness :

Hereditary diseases :

Operations :

Drugs :

Present medical history:

Do you have any complaint now?

Are you allergic to any

Are you alle

Do y

If y and in wha

Did you regain weigh

Amoebiasis - Giardiasis - Study
Immunization form

Date : _____ Name : _____ Patient No._____

Vaccine	Last Received	Next Due	Date Given
Small Pox			
Typhoid			
Mumps			
Rubella			
Yellow Fever			

Vaccine	Last Received	Next Due	Date Given	Next Due	Date Given	Next Due	Date Given
Polio							
Cholera							
DKTP							
DPT							
DT							
Gamma Globulin							
B.C.G.							

Amoebiasis - Giardiasis - Study

Admission Form (phys. exam. and laboratory)

Date :

Name :

Patient No.:

Weight :

Length :

Temperature :

Pulserate :

Blood-pressure :

Routine Screening; unusual findings :

Laboratory : Specimen obtained?

Blood : CBC

Immunoglobulines

Serum-antibodies

Blood group

Urine : Complete analysis

Stool : ME_1

ME_2

Culture

Rotavirus

Saliva :

X-ray-chest :

P P D :

Weekly Health Surveillance Form

Amoebiasis - Giardiasis - Study

Name : _____

Patient No.: _____

Date : _____

	If yes, give date
Were you ill :	
Did you have any abdominal discomfort :	
Did you have any nausea or did you vomit :	
Did you have more than 3 stools / day :	
Did you have loose or watery stools :	
Did you have any "cold" symptoms :	
Did you have any fever (>100° / >38°) :	
Did you have loss of appetite :	
Did you have any other symptom :	

1 = yes

2 = no

3 = no answer

Amoebiasis - Giardiasis - Study

Quarterly follow-up form No.___

- Date :

  Name :

  Patient No. :

  Weight :

  Length :

  Temperature :

  Pulserate :

  Blood-pressure

  Routine Screening : unusual findings :

- Weekly health-records completed : Yes __ No __

  All illnesses recorded : Yes __ No __

  If not, describe -

  Episode 1 :

  Episode 2 :

- Laboratory : specimen obtained?

  Blood : CBC

  Immunoglobulines

  Serum-antibodies

  Stool : ME_1

  ME_2

  Culture

  Rotavirus

  Saliva :

Amoebiasis - Giardiasis - Study

To be used when :

- stool positive for Giardia or Amoeba
- patient has diarrhoeal illness
- during treatment

Date :

Name :

Patient No. :

Treatment No. :

- Which complaints do you have :

  - no complaints
  - diarrhea
  - nausea / vomiting
  - abdominal cramps
  - abdominal distention
  - loss of appetite
  - flatulence
  - fever
  - general weakness

  - other :

- What about your stool :

  - normal
  - more than 3x / day
  - only in the morning
  - foul-smelling
  - mucus
  - blood

- What about the consistency :

  - normal
  - like water
  - loose
  - semiformed

- Did you take any drug : Yes ___ No ___

  If yes, name -

Amoebiasis - Giardiasis - Study
Treatment Study

Nurse Report

Date :

Name :

Patient No. :

Amoebiasis / Giardiasis :

Address - patient :

Telephone - residence :

Telephone - office :

Recorder - family study :

Start treatment :

Treatment No. :

Pregnant / lactating :

Follow - up	I week Home	II week T C	III week Home	IV week T C
2 stool specimen patient				
1 stool specimen household/servants				
blood patient				
saliva patient				
unwanted-side-effect effects				
complaints - form				
family - form				

Performed

Blood-test 3 months after treatment : Date : _____

" 6 " " " : Date : _____

" 12 " " " : Date : _____

Amoebiasis - Giardiasis - Study
Treatment Study

Side-effects of drugs

Date :

Name :

Patient No.:

Side-effects / Day	1	2	3	4	5	6	7
Nausea							
Vomiting							
Headache							
Dizziness							
Metallic taste							
Diarrhea							
Constipation							
Fever							
Skin rash							
Loss of appetite							
Pruritus ana							
Abdominal pain							
Dark-urine							
Flatulence							
Others							

Amoebiasis - Giardiasis - Study
Family-form

Date: _____ Name : _____ Patient No. _____ Treatment No. _____

Date start treatment : _____

Name other family members and house staff

No.	Name	Age	Sex	Position
1.				
2.				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				

Symptoms before and after start treatment index patient

2 wk before	1 wk before	1 wk after	2 wk after	3 wk after

0 Healthy
1 Diarrhoea (3x/day)
2 Nausea or vomiting
3 Abdominal cramp
4 Abdominal distention
5 Loss of appetite
6 Flatulence
7 Fever
8 General weakness
9 Other

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH-TRAVELLERS DISEASES IN DACC

HEMATOLOGY FLOW SHEET FORM M

Name_____ Study No._____ (51 - 54)

Card No. 1-2	Date 3-8	Hct 9-10	WBC 11-16	P.atlets 17-22	PMN 23-24	Bands 15-26	Lymph 27-28	Mono 29-30	Eos 31-32	Bas 33-34	Juv 35-36	Nuc 37-38	RBC 39-40	Retic 41-42	Malaria 43	Filar 44	Sed. Rate 45-46	Sp. Gr. 47-50	(78-80
M 1																			212
M 2																			212
M 3																			212
M 4																			212
M 5																			212
M 6																			212
M 7																			212
M 8																			212
M 9																			212
M 10																			212
M 11																			212
M 12																			212

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH-TRAVELLERS DISEASES IN DACCA

STOOL MICROSCOPY FLOW SHEET FORM N

Name_____ Study No._____

(39 - 42)

Card No.	Date	Colour	Consis	PH	Blood	Mucous	Guaiac	Fecal Leaks	Pus Cells	RBC	Macro Phage	N. Fat	Other	Ameba Cysts	Ameba Trophs	Giar Cyst	Giar Trophs	Tricho	HW	Asc	Str	Trich	Pin Worm	
1-2	3-8	9	10	11-12	13	14	15	16-18	19-21	22-24	25-26	27	28	29	30*	31	32	33	34	35	36	37	38	(78-80)
N 1																								212
N 2																								212
N 3																								212
N 4																								212
N 5																								212
N 6																								212
N 7																								212
N 8																								212
N 9																								212
N 10																								212
N 11																								212
N 12																								212

* 0 = Neg
1 = Without rbc
2 = With rbc

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URINE FLOW SHEET FORM O

Name _____ Study No. _____ (34 - 37)

Card No. 1-2	Date 3-8	Colour 9	Trans 10	Sp. Gr. 11	PH 12-13	Alb 14.	Sugar 15	Acetone 16	Bile Salt 17	Bile Pig 18	Others 19	Pus 20-22	RBC 23-25	Ep. Cells 26-28	Cast				Culture Obtained (Y/N) 33	(78-80)
															Gram 29	WBC 30	RBC 31	Other 32		
O 1																				212
O 2																				212
O 3																				212
O 4																				212
O 5																				212
O 6																				212
O 7																				212
O 8																				212
O 9																				212
O 10																				212
O 11																				212
O 12																				212

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH-TRAVELLERS DISEASES IN DACCA

MICROBIOLOGY FLOW SHEET FORM P

Name _____ Study No. _____ (31 - 34)

Card No. 1-2	Date 3-8	Source of Spec 9-10	Quant Count 11-17	Sal 18	Shig 19	Vibrio 20	E. coli 21	Kleb 22	Aeromonas 23	Other 24	Other 25	Other 26	Pathogenic Test LT 27	Pathogenic Test ST 28	Pathogenic Test Invasive 29	Rota 30	(78-80)
P 1																	212
P 2																	212
P 3																	212
P 4																	212
P 5																	212
P 6																	212
P 7																	212
P 8																	212
P 9																	212
P 10																	212
P 11																	212
P 12																	212