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ETHICAL REVIEW COMMITTEE, ICDDR,B.

Asma Islam, A. Hall,

Principal Investigator A. Tomkins

Trainee Investigator (if any)

Application No. 84-005(P)

Supporting Agency (if Non-ICDDR,B) Wellcome Trust

of Study Development and evaluation of immunological techniques for the detection of Giardia lamblia in a pilot study of childhood diarrhoea in rural Bangladesh.

Project status:

- (X) New Study
- ( ) Continuation with change
- ( ) No change (do not fill out rest of form)

Give 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
- d) Physical risks to the subjects Yes  Yes  No
- e) Social Risks Yes  Yes  No
- f) Psychological risks to subjects Yes  Yes  No
- g) Discomfort to subjects Yes  Yes  No
- h) Invasion of privacy Yes  Yes  No
- i) Disclosure of information damaging to subject or others Yes  Yes  No
- j) Use of records, (hospital, medical, death, birth or other)  Yes  No
- k) Use of fetal tissue or abortus Yes  Yes  No
- l) Use of organs or body fluids  Yes  No
- m) Subjects clearly informed about:
  - n) Nature and purposes of study  Yes  No
  - o) Procedures to be followed including alternatives used  Yes  No
  - p) Physical risks  Yes  No
  - q) Sensitive questions  Yes  No
  - r) Benefits to be derived  Yes  No
  - s) Right to refuse to participate or to withdraw from study  Yes  No
  - t) Confidential handling of data  Yes  No
  - u) 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:

- NA Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
- Protocol (Required)
- Abstract Summary (Required)
- Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
- Informed consent form for subjects
- Informed consent form for parent or guardian
- Procedure for maintaining confidentiality

NA Questionnaire or interview schedule \*

\* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:

1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee: for review.

REF  
QX 70. JB2  
H174d  
1984

SECTION 1 - RESEARCH PROTOCOL :

(1) Title: Development and evaluation of new immunological techniques for the detection of Giardia lamblia in a pilot study of childhood diarrhoea in Bangladesh.

(2) Principal Investigators: Andrew Hall, Asma Islam

Co-Investigators: Andrew Tomkins, Mujibur Rahaman

(3) Starting date: 1st February 1984.

(4) Completion date: 31st July 1984.

(5) Total direct cost: \$ 3000

(6) Scientific Programme Head:

This protocol has been approved by the

Pathogenesis-Therapy  
Working Group.

Signature of Scientific Programme Head: D Butler

Date: 1-19-84

(7) Abstract summary:

It is proposed to develop and evaluate immunological methods for the diagnosis of Giardia lamblia in faeces which would be suitable for testing large numbers of samples in investigations of diarrhoea among young children in developing countries. Countercurrent immunoelectrophoresis and enzyme-linked immunosorbent assay for the detection of Giardia in faeces will be developed at the London School of Hygiene and Tropical Medicine. Their sensitivity, specificity and predictive value in comparison with light microscopy will be evaluated in children attending the International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh, and in a pilot study of a rural area in which an evaluation of the impact of an improved water supply on diarrhoeal disease morbidity, transmission of enteropathogens and nutritional status is planned.

## SECTION II - RESEARCH PLAN

### A. INTRODUCTION

#### 1. Objective

To develop immunological methods for the detection of Giardia lamblia in faeces. To evaluate these methods in a pilot study of infected children in Bangladesh, both in a hospital and in a rural community, in order to be able to undertake future investigations of the impact of Giardia on the growth and nutritional status of young children.

#### 2. Background

Diarrhoea continues to be a major cause of morbidity and death among young children in developing countries and frequently precipitates malnutrition (1). Although recent research has focused on viral and bacterial enteropathogens (2), diarrhoea associated with parasitic infections, and in particular with Giardia lamblia, has received little attention, especially in terms of its effects on nutritional status and growth. The highest prevalence of infection with Giardia usually occurs between 2 - 5 years of age, after which partial immunity may develop and infections are often asymptomatic (3). Symptomatic infections result in a considerable diversity of clinical features, none of which are pathognomonic. In our previous studies we have examined the mechanisms of malabsorption in acute and chronic diarrhoea in adults (4). Clinical studies of children with giardiasis have shown similar findings but there is little data on the significance of Giardia lamblia among children within a community where the infection is endemic. However, an association between diarrhoea, growth faltering and parasitic infections has been described by Mata in Guatemalan children (5) and a limited study of the chemotherapy of Giardia lamblia indicated that treatment may have some effect on nutritional status (6).

A WHO working group recently recommended that "studies should be carried out in developing countries in children below 5 years of age to determine the ratio of symptomatic to asymptomatic cases and any effect that giardiasis has on growth and development" (7).

Our studies in rural Nigeria (8) and peri-urban Gambia (unpublished data) have demonstrated that close, reasonably accurate surveillance of individuals for the incidence of diarrhoea is possible in developing countries. However in order to undertake studies of Giardia lamblia it will be necessary to improve methods of diagnosing infections which currently often rely on the time consuming, microscopical examination of faeces. Problems in microscopical techniques arise because cysts are intermittently excreted in faeces in very variable numbers and may be obscured by faecal detritus, while trophozoites on the other hand are generally only found in fresh, diarrhoeal faeces (9).

The purpose of the proposed investigations is to develop and

evaluate promising immunological techniques for the diagnosis of Giardia lamblia in order to be able to undertake future studies of its role in diarrhoeal disease and malnutrition in children.

Immunological techniques have been used for the serological diagnosis of G.lamblia infections by immunofluorescent techniques (10) and enzyme-linked immunosorbent assay (ELISA;11). However neither technique was sufficiently specific as a small proportion (about 12%) of uninfected control subjects in one study (11) also had circulating antibodies, probably as a result of previous infection.

More recently a single study has used countercurrent immunoelectrophoresis (CIE) to detect Giardia lamblia in faeces (12). Initial results indicate that this technique is sensitive and specific: it detected all infections (100%) diagnosed by the microscopical examination of either faeces (which detected 94%), duodenal fluid or duodenal biopsy specimens.

Besides detecting serum antibodies, the ELISA technique has been used to distinguish between different in vitro cultures of trophozoites (13) but has not so far been used to detect Giardia lamblia in faeces. It is proposed to develop an ELISA method, to detect Giardia lamblia in faecal specimens. Both immunological techniques would be developed and evaluated with the aim of using them in future long term studies of the role of Giardia lamblia in diarrhoeal disease, growth and the nutritional status of young children in rural Bangladesh, where Giardia is known to be endemic (14,15).

## B. SPECIFIC AIMS

The objectives of this study are:

1. To develop immunological methods for the diagnosis of Giardia lamblia.
2. To determine the sensitivity, specificity and predictive value of the immunological techniques in relation to microscopical methods.
3. Undertake a pilot study of a rural community where a long term study of the impact of Giardia lamblia infections on the growth and nutritional status of young children could be undertaken.

## C. METHODS

(1). Cysts of Giardia lamblia obtained from patients at the Hospital for Tropical Diseases, London will be extracted from faeces and concentrated by centrifugation in ether, then further purified by centrifugation in a gradient of a Ficoll and Urografin mixture (11). After filtration through an 8 $\mu$  nucleopore membrane they will be washed and suspended in sterile aqueous solution containing penicillin G and gentamycin, checked for sterility by bacterial culture under aerobic and anaerobic conditions, counted in a haemocytometer and then stored at -20 $^{\circ}$ C prior to inoculation into rabbits (16).

Cultures of trophozoites will be obtained from Dr. D. Warhurst of the Hospital for Tropical Diseases (HTD) and subcultured in modified Diamonds medium filter sterilised as described by

Visvesvara (17), but containing 10% inactivated horse serum instead of bovine serum, and 40 µg/ml gentamycin sulphate (18). After checking for contamination, trophozoites will be washed and resuspended in Hanks balanced salt solution, then counted and stored at - 70° C.

Antisera to cysts and trophozoites will be produced by immunisation of New Zealand white rabbits by injection of parasite preparations with Freund's adjuvant, and collected by venesection.

A CIE method will be established using 1% agarose gel in Tris-barbital/sodium barbital buffer on slides with antisera and faecal homogenate placed in opposing wells (12).

An ELISA method using antisera prepared as above and diluted with carbonate buffer will be developed according to the method of Yolken *et al* (19). Diluted antisera will be placed in the wells of microtitre plates and incubated at 4° C prior to washing with P.B.S.-tween. Faecal homogenates and foetal calf serum diluted in P.B.S.-tween will be added to each well. A colour change will be measured quantitatively in a colorimeter using the conventional alkaline phosphatase/p-nitrophenyl phosphate interaction.

This work will be performed in collaboration with Dr. J. Ackers of the Dept. of Medical Protozoology, L.S.H. & T.M. and Mr. A. Moody and Dr. D. Warhurst at the HTD. We would like to liaise with Dr. M. Farthing of St. Bartholemews Hospital at several stages of this project and preliminary discussions indicate that he is willing to collaborate.

(2) Faecal samples from children with diarrhoea attending the out patient department of the International Centre for Research in Diarrhoeal Diseases, Bangladesh (ICDDR,B) will be examined for *Giardia lamblia* by microscopical techniques after formol-ether sedimentation (20) and will be tested using the CIE and ELISA methods. Sensitivity, specificity and predictive values for each method will be calculated and analysed using McNemar's test (21).

In order to determine if differences (of 10%) in the values for these indices of diagnostic reliability are significant, we estimate that at least 180 samples will require examination by each method.

In addition the critical number of cysts necessary to produce a positive diagnosis by each technique will be assessed by serially diluting a known concentration of cysts in faeces, estimated in a haemocytometer.

In view of the need to keep fresh faecal samples collected in the field in cold boxes for several hours before they can be tested in the laboratory, a comparison of diagnostic methods will be undertaken using sequential tests of aliquots of 25 faecal specimens maintained at different temperatures for 6, 12, 24, 36, 48 and 72 hours after collection. Statistical analysis will be performed using Fischers Exact test (21).

In order to assess the value of the immunological methods in diagnosing intermittent excretion of cysts, a cohort of asymptomatic children in Nandipara, a peri-urban village of Dhaka where *Giardia* is endemic, will be studied. Twenty children who are excreting cysts will have faecal samples collected and examined on alternate days for 6 - 8 weeks.

(3) Finally, provided the CIE and ELISA techniques have proved satisfactory in stages 1 and 2, we shall undertake a pilot study in which faecal samples from 200 - 300 children aged 0 - 5 years near the village of Mirjapur about 40 miles from Dhaka will be examined for Giardia lamblia and other intestinal parasites. This village has been selected by ICDDR,B for an evaluation of changes in diarrhoeal disease morbidity, transmission of pathogens and nutritional status in response to improved water supplies. The children we propose to study will not be involved in the ICDDR,B investigation. The sampling and analysis of data for this study will be decided after a site visit and discussion with epidemiologists at ICDDR,B and the Tropical Epidemiology Unit, LSH&TM.

#### Data analysis

The analysis of results will be performed at ICDDR,B using an Epson HX 10 microcomputer and at LSH&TM using several computer packages.

In order to determine if differences (of 10%) in the values of the index of diagnostic reliability are significant it is estimated that at least 180 faecal samples will require examination by each method. The sensitivity, specificity and predictive value of each diagnostic test will be tested for statistical significance using McNemar's test.

A statistical analysis of the effect of temperature on the diagnostic reliability of each technique will be performed using Fischers Exact Test.

#### D. Significance

The techniques currently used to diagnose Giardia lamblia are either unreliable or physically invasive. The proposed study would develop immunological techniques for detecting Giardia in faeces which would be both non-invasive and more reliable. These techniques would be used as diagnostic tools in proposed epidemiological investigations and in intervention studies of the impact of Giardia on the growth and nutritional status of young children in rural Bangladesh.

Parasitological and nutritional expertise would be provided by Dr. Hall to ICDDR,B during his stay in Dhaka.

#### E. FACILITIES REQUIRED

1. No new office space is required.
2. Personnel:
  - Fieldworker assistance for 4 months
  - Occasional laboratory assistance for 6 months
3. Laboratory space in Clinical Pathology and Immunology labs.
4. Occasional use of colorimeter for ELISA tests.
5. Hospital support - faeces from outpatients at ICDDR,B.
6. Transport to and from field area.

## 7. COLLABORATIVE ARRANGEMENTS

This study would involve collaboration between Drs. Hall and Tomkins of the London School of Hygiene and Tropical Medicine and Drs. Islam and Rahaman of ICDDR,B. All expenses will be paid for by a grant from the Wellcome Trust and administered by LSH&TM. Some technical assistance will be provided by ICDDR,B.

## REFERENCES

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SECTION III - PERSONNEL

Personnel Services

<u>name</u>	<u>Position</u>	<u>% of effort</u>
Dr Andrew Hall	Principal Investigator	100%
Dr Asma Islam	Coinvestigator	25%
Dr Andrew Tomkins	Coinvestigator	10%
Dr Mujibar Rahaman	Coinvestigator	10%



### ABSTRACT SUMMARY

Diarrhoea during infancy is a major cause of morbidity, mortality and growth faltering in Bangladesh. Although bacterial and viral pathogens have been isolated the role of parasitic infections appears to have received little attention. Giardia lamblia is a protozoan parasite which inhabits the upper intestine and is known to cause acute and protracted diarrhoea with malabsorption. A major problem in studying the epidemiology and impact of Giardia infections is that of reliable diagnosis : the parasites are only intermittently excreted in faeces and in very variable numbers. It is proposed to develop and evaluate the use of two immunological techniques for the detection of Giardia in faeces: counter current immunoelectrophoresis (C.I.E.) and enzyme linked immunosorbent assay (E.L.I.S.A.). These methods which detect the antigen of Giardia will be compared with conventional diagnostic techniques using light microscopy in studies of patients attending the I.C.D.D.R-B out patient clinic at Dhaka and subjects in a rural community.

CONSENT FORM

DEVELOPMENT AND EVALUATION OF NEW IMMUNOLOGICAL TECHNIQUES  
FOR THE DETECTION OF GIARDIA LAMBLIA IN A PILOT STUDY OF  
CHILDHOOD DIARRHOEA IN RURAL BANGLADESH.

(Statement to be read to the patient or guardian when  
consent is obtained).

Diarrhoea is a very common illness during childhood. The  
I.C.D.D.R,B is testing methods to detect one cause of  
diarrhoea. We would like to collect some faeces from your  
child.

If we find that your child is infested with worms then  
we will give treatment.

If you agree to participate for your child to participate  
then please sign your name or give your thumb impression.

\_\_\_\_\_  
Signature of Investigator

Date:

\_\_\_\_\_  
Signature or left thumb  
print of parent/guardian

## সন্মতি পত্র

৷ জিয়ারডিয়ার উপর গবেষণা ৷  
"সন্মতি নেওয়ার পূর্বে রোগী বা অভিভাবককে পড়িয়া শুনাইতে হইব"

শিশু বয়সে ডায়রিয়া একটি খুব সাধারণ রোগ । আই, সি, ডি, ডি, আর, বি ডায়রিয়ার একটি কারণ নির্ণয়ের জন্য বিভিন্ন পরীক্ষা নিরীক্ষা চালিয়ে আসছে । আমরা আপনার সন্মানের কিছু (পায়খানা) মল সংগ্রহ করার ইচ্ছা রাখি । পরীক্ষার পর যদি আমরা দেখতে পাই যে আপনার সন্মান বিভিন্ন কৃমিতে ভূগছে, তাহলে আমরা তার চিকিৎসা করব ।

যদি আপনি আপনার সন্মানকে এই পরীক্ষা নিরীক্ষায় অংশ গ্রহণ করতে চান তবে এই সন্মতি পত্রে স্বাক্ষর করুন বা টিপ সহি দিন ।

স্বাক্ষর, প্রধান গবেষক,

তারিখ-

স্বাক্ষর / টিপ সহি  
(পিতা-মাতা/অভিভাবক)