

## ETHICAL REVIEW COMMITTEE, ICDDR,B.

84

Principal Investigator Dr. ASM H. Rahman Trainee Investigator (if any) NIL

Application No. 84-097(P)

Supporting Agency (if Non-ICDDR,B)

Title of Study Experimental Cryptosporidiosis Study in Baby Chicks.

Project status:

(A PILOT PROTOCOL)

(✓) New Study

( ) Continuation with change

( ) No change (do not fill out rest of form)

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

## 1. Source of Population:

(a) Ill subjects Yes (No)

(b) Non-ill subjects Yes (No)

(c) Minors or persons under guardianship Yes No

## 2. Does the study involve:

(a) Physical risks to the subjects Yes (No)

(b) Social Risks Yes (No)

(c) Psychological risks to subjects Yes (No)

(d) Discomfort to subjects Yes (No)

(e) Invasion of privacy Yes (No)

(f) Disclosure of information damaging to subject or others Yes (No)

## 3. Does the study involve:

(a) Use of records, (hospital, medical, death, birth or other) Yes (No)

(b) Use of fetal tissue or abortus Yes (No)

(c) Use of organs or body fluids Yes (No)

## 4. Are subjects clearly informed about:

(a) Nature and purposes of study Yes No

(b) Procedures to be followed including alternatives used Yes No

(c) Physical risks Yes No

(d) Sensitive questions Yes No

(e) Benefits to be derived Yes No

(f) Right to refuse to participate or to withdraw from study Yes No

(g) Confidential handling of data Yes No

(h) Compensation &amp;/or treatment where there are risks or privacy is involved in any particular procedure Yes No

NA

## 5. Will signed consent form be required:

(a) From subjects Yes (No)

(b) From parent or guardian (if subjects are minors) Yes No

## 6. Will precautions be taken to protect anonymity of subjects Yes No

## 7. Check documents being submitted herewith to Committee:

Umbrella proposal - Initially submit a 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.

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

Principal Investigator

Trainee

84-047(p)  
29/11/84

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SECTION I - RESEARCH PROTOCOL

1. TITLE : Experimental Cryptosporidiosis  
in baby chicks.
2. PRINCIPAL INVESTIGATOR : Dr. A.S.M. Hamidur Rahman  
CO-INVESTIGATORS : Drs. K.A. Al-Mahmud, Nigar S.  
Shahid & Mr. K.M.N. Islam  
CONSULTANT : Thomas C. Butler  
Prof. S.C. Sanyal
3. STARTING DATE : December 15, 1984
4. COMPLETION DATE : May 15, 1985
5. TOTAL DIRECT COST : US \$4,300/-
6. SCIENTIFIC PROGRAM HEAD : I. Ciznar

This protocol has been approved by the HDWG.

Signature of the Scientific Program Head

*Irene Ciznar*  
24/11/84

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02 JUL 2002

7. ABSTRACT SUMMARY:

The natural history of cryptosporidiosis will be studied on baby chick model focusing on the prepatent/incubation period, the developmental stages of the parasite on the epithelium of gastrointestinal and respiratory tracts, the pathophysiology of the disease including the morbid anatomy. Of 150, 1-day-old baby chicks, one-third will receive peroral inoculum from naturally infected calf cryptosporidiosis cases, another third will receive inoculum from human sources and the rest, as a control, will be given only the phosphate buffer saline. The knowledge gathered by this study will help in the treatment and control of the cryptosporidiosis in both animals and human.

8. REVIEWS:

- a. Research involving human subjects \_\_\_\_\_
- b. Research Review Committee \_\_\_\_\_
- c. Director \_\_\_\_\_
- d. BMRC: \_\_\_\_\_
- e. Controller/Administrator \_\_\_\_\_

## SECTION II - RESEARCH PLAN

### A. INTRODUCTION :

#### 1. Objective

Since nothing is clearly known regarding the nature of the initial infection, the prepatent period (i.e. oocysts to oocysts in the faeces of the infected animal) etc. of the infection - the study will let us know the following:

- i) the exact time of appearance of the cryptosporidial oocyst in the gut mucosa following the inoculation (the incubation period).
- ii) the time/period required to complete the life cycle of the protozoan in days.
- iii) the sequential developmental stages of the protozoan on the host epithelium (merozoites/trophozoite, schizonts, macro-gametocysts, microgametocyst etc.).
- iv) the extent of damages done in the organs/systems due to crypto-infection in relation to those in the normal healthy controls.

#### 2. Background

Cryptosporidium sp. is a member of the family Cryptosporidiidae, suborder Eimeriorina, but it differs from most other enteric coccidia in that the organisms are smaller (diameter, 3 to 4  $\mu$ m) and only invade the microvillous border of the gut enterocytes.

Cryptosporidium sp. infections were described first in tamed wild mice (1,2) and subsequently in C57 mice (3). Infection associated with diarrhoea have also been reported in a variety of animals and in humans (4 - 13).

Studies in guineapigs (14-15) suggested that Cryptosporidium sp. is species specific, but recently, subclinical infections were recorded in 1-day-old specific-pathogen-free (SPF) suckling mice and rats inoculated with faeces containing Cryptosporidium sp. taken from calves, lambs and humans. Asymptomatic infections were reported to occur in mice (16), rabbits (17-18), cats (19) and several-week-old piglets (20-21).

The present study aims at observing the fate of the Cryptosporidium inoculated to the 1-day-old baby chicks and observing the infection pattern and the pathogenic role of the protozoan in the baby chick so that the chick may produce diarrhoea and/or gastroenteritis.

#### B. SPECIFIC AIMS:

1. To test whether the baby chick acts as a satisfactory model for cryptosporidiosis & if found suitable, the following observations will also be done:
  - a. to observe the actual time of re-appearance of the oocysts in the baby chick gut/faeces after per oral inoculation.

- b. to observe the developmental stages of the protozoan (life cycle) in the gastrointestinal tract of the baby chick.
- c. to measure the extent of damage done in the host in reference to the non-inoculated controls.

### C. METHODS:

#### Source:

Cryptosporidium oocysts will be obtained from the stools of the patients coming to ICDDR,B hospital (Dr. Nigar's study) or from the faeces of the naturally infected calves in Savar Dairy Farm. The faeces containing oocysts will be mixed with an equal volume of 2.5 per cent potassium dichromate solution ( $K_2Cr_2O_7$ ) and stored at 4°C for more than six months prior to use.

#### Purification:

Oocysts will be purified and concentrated by flotation in Sheather's Sugar Solution (granulated sugar - 45g; distilled water - 355 ml and liquified phenol - 6.6 ml) washing three times by centrifugation in phosphate-buffered-saline (PBS), PH 7.2 , and incubating in PBS containing 0.1 per cent (weight to volume) glucose, penicillin (5000-IU/ml), streptomycin (5 mg/ml) and amphotericin - B (20  $\mu$ /ml.) for 3 hours at 37°C to kill microbial contaminants (22).

Study Design:

One hundred and fifty 1-day-old baby chicks (Starbro-strain) collected from Savar Poultry Complex will be used for the experiment. The chicks will be grouped into three batches of 50 each. Two batches will get inoculum, one from ICDDR,B crypto positive patients (Group A) and the other from Crypto-positive calves from Savar Dairy Farm (Group B) and the remaining group will receive only Phosphate Buffered Saline (Group C).

Food will be given after autoclaving. Boiled and then cooled water will be provided ad libitum to prevent any unknown natural crypto infection in the defined experimental groups of chicks.

Stool will be examined daily by standard rapid-acid-fast method for crypto-oocysts (23). When a positive case will be detected, the chick will be sacrificed painlessly following inhalation of a sub-lethal dose of ether. Autopsy will be done and touch-smears will be examined from jejunum, ileum and large gut for the parasite. The entire gastrointestinal and respiratory tracts will be preserved in formal-saline for histopathological studies. Once the smears are found positive from any segments/sections, the relevant portion will be subjected to detailed histopathological studies.

D. RATIONALE:

The exact nature regarding the initial acquisition of the cryptosporidium and the subsequent production of the disease is not well known in both animals and humans. The present study will hopefully enhance our knowledge for better understanding of the protozoan's life cycle.

E. FACILITIES REQUIRED:

The facilities of ARB, Microbiology and the Histopathology laboratory will be required.

F. COLLABORATIVE ARRANGEMENTS:

No collaborating arrangement would be needed except partial discussion with Prof. S.C. Sanyal.

## PILOT PROTOCOL

### Experimental Cryptosporidiosis in baby chicks.

#### Abstract Summary:

One hundred and fifty, 1-day-old baby chicks will be used to conduct the Experimental Cryptosporidiosis Study. A batch of 50 chicks will get inoculum from naturally infected calf cryptosporidiosis cases and another from human sources. Similar 50 will get only phosphate buffer saline which will act as control. The propagation of the developmental stages along with the damages done in the G.I tract will be recorded. The knowledge gathered regarding the pathogenesis of the protozoan in experimental animal will help taking effective treatment and control measures both in animals and humans suffering from cryptosporidiosis.

#### 1. Population:

The study will require animals only (Baby Chick, Starbro Strain). The Biman Poultry Farm at Ganakbari, Savar will supply as special request.

#### 2. Potential risk:

The baby chicks will be maintained in appropriate humane nature while conducting the research.

#### 3. Procedure for minimizing potential risks:

Pains to the animals will be minimized by using anaesthetics.

#### 4. Safe guarding confidentiality:

Not applicable.

5. Informal consent:

Does not arise.

6. Interview Informations:

The research will not require any interview.

7. Potential benefits to the subject:

The findings of study will let the researcher know more precisely about the acquisition, propagation and damage to the individual animal host in terms of cryptosporidial infection.

8. Medical Records:

No use of records, organs, tissues, or body fluids from humans will be needed.

REFERENCE

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3. Hampton, J.C., and B. Rosario. 1966. The attachment of protozoan parasites to intestinal epithelial cells of the mouse. *J. Parasitol.* 52:939-948.
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SECTION III - DETAILED BUDGET

. Personnel Services:

| Name                | Designation     | % Effort | Salary   |        |
|---------------------|-----------------|----------|----------|--------|
|                     |                 |          | Taka     | Dollar |
| Dr. A.S.M.H. Rahman | Prin. Investgr. | 20       | 15,500/- | -      |
| Dr. K.A. Al-Mahmud  | Co- Investgr.   | 5        | 7,500/-  | -      |
| Dr. Nigar S. Shahid | -do-            | 5        | 5,000/-  | -      |
| Mr. K.M.N. Islam    | -do-            | 20       | 12,500/- | -      |
| Mr. Md. Shafi Ullah | Tech. Officer   | 20       | 8,500/-  | -      |
| Mrs. S. Pashi       | R.O             | 20       | 8,000/-  | -      |
|                     |                 |          | 57,000/- |        |

Supplies and Materials:

| Item                                      | Amount | Unit cost (Taka) | Project Requirement |        |
|-------------------------------------------|--------|------------------|---------------------|--------|
|                                           |        |                  | Taka                | Dollar |
| Baby Chick (day-old)                      | 200    | 10               | 2,000/-             | -      |
| Anaesthetise (ether)                      |        |                  |                     | 200/-  |
| Surgical Instruments (complete full sets) |        |                  |                     | 200/-  |
| Stains (Jenner's, Giemsa, Acid fast)      |        |                  |                     | 200/-  |
| Syringe, Gloves, Needle etc.              |        |                  |                     | 200/-  |
| Bacteriological media                     |        |                  |                     | 200/-  |
| Paraffin                                  | 5 lbs  |                  |                     | 200/-  |
| Reagents & Chemicals                      |        |                  |                     | 200/-  |
| Electrolytes                              | 100    | 12               | 1,200/-             | -      |
| Histological specimens                    | 100    | 80               | 8,000/-             |        |

| Item                                                                                                                                                 | Amount | Unit cost<br>(Taka) | Project Requirement |          |
|------------------------------------------------------------------------------------------------------------------------------------------------------|--------|---------------------|---------------------|----------|
|                                                                                                                                                      |        |                     | Taka                | Dollar   |
| Liq. Phenol, slide, cover<br>glass, glass pencil                                                                                                     |        |                     |                     | 400/-    |
| Imersion Oil, Lens paper,<br>micropipette etc.                                                                                                       |        |                     | 2,500/-             |          |
| Equipment                                                                                                                                            | None   |                     | Nil                 | -        |
| Patients                                                                                                                                             | None   |                     | Nil                 | -        |
| Outpatient                                                                                                                                           | None   |                     | Nil                 | -        |
| ICDDR,B Transport<br>Centre to Biman Poultry Farm<br>and back to collect baby chick<br>60 miles once in a month for 6<br>months @ Tk. 12.50 per mile |        |                     | 6,500/-             | -        |
| Travel & Transportation<br>of person                                                                                                                 |        |                     |                     | 1,500/-  |
| Transportation of things                                                                                                                             |        |                     | Nil                 | -        |
| Rent, Communication &<br>Utilities                                                                                                                   |        |                     | Nil                 | -        |
| Painting and Reproduction                                                                                                                            |        |                     | 2,500/-             | -        |
| Other contractual services<br>(Pathology Consultant)                                                                                                 |        |                     | 9,500/-             | -        |
| Construction, Rennovation                                                                                                                            |        |                     | -                   | -        |
| Total Taka =                                                                                                                                         |        |                     | =25,000/-           | =3,300/- |
| Project requirement: US \$ 4,300/-                                                                                                                   |        |                     |                     |          |
| (1 US \$ = Taka 25.00)                                                                                                                               |        |                     |                     |          |