DD

R,B

Principal

Trainee Investigator (if any)

Application No. 85-009L

Supporting Agency (if Non-ICDDR,B)

Title of Study Cryptosporidiosis in Children in relation to nutritional Status & Clinical Status, Source of infection & Risk factors

Project status:
(✓) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

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

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

2. Does the study involve:
   (a) Physical risks to the subjects — Yes (No)
   (b) Social Risks — Yes (No)
   (c) Psychological risks to subjects — Yes (No)
   (d) Discomfort to subjects — (Yes) No Minor
   (e) Invasion of privacy — Yes (No)
   (f) Disclosure of information damaging to subject or others — Yes (No)

3. Does the study involve:
   (a) Use of records, (hospital, medical, death, birth or other) — Yes No
   (b) Use of fetal tissue or abortus — Yes (No)
   (c) Use of organs or body fluids — (Yes) No

4. Are subjects clearly informed about:
   (a) Nature and purposes of study — (Yes) No
   (b) Procedures to be followed including alternatives used — Yes (No)
   (c) Physical risks — Yes (No)
   (d) Sensitive questions — Yes (No)
   (e) Benefits to be derived — (Yes) No
   (f) Right to refuse to participate or to withdraw from study — (Yes) No
   (g) Confidential handling of data — (Yes) No
   (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — (Yes) No

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.

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

Nigar S. Shafiq
Principal Investigator

Trainee

SECTION I - RESEARCH PROTOCOL

1. Title : Cryptosporidiosis in children in relation to nutritional and immunological status sources of infection and transmission in family contacts.

2. Principal Investigator : Dr. Nigar S. Shahid

Co-Investigators Dr. A.S.M. Hamidur Rahman

Dr. D.A. Sack

Consultant : Prof. S.C. Sanyal

3. Starting Date : March 25, 1985

4. Completion Date : December 25, 1985

5. Total Direct Cost : U.S. $ 4602.30

6. Scientific Program Head :

This protocol has been approved by the Disease Transmission Working Group.

Signature of the Scientific Program Head _____

Date _____

7. Abstract Summary

A total of 20 Cryptosporidium cases, to non-Cryptosporidium controls will be enrolled into the study. Each child will have history and physical examination recorded along with nutritional and anergy assessment. The organism will be looked for in stools and nasopharengeal aspirates. All family members, animal and poultry birds of cases and controls will be followed on day 3,5,10,15 & 20th for excretion of the parasite.

SECTION II - RESEARCH PLAN

A. INTRODUCTION

This protocol is based on the findings of two pilot protocols of which one was a farmbased study on diary calves and their handlers and the other on the randomly selected 4% patients visiting ICDDR,B. In both the studies Cryptosporidium was detected and confirmed.

In the farm based study Cryptosporidium was detected in 14% of diarrhoeal and 1% of non-diarrhoeal cases as well as 8.5% of their handlers having diarrhoea but none from health individuals (25).

In the ICDDR,B hospital surveillance study Cryptosporidium was reported in 4% of patients during a one year period. Most of the cases occurred in children during the month of April to September.

1. Objectives

a. To determine if cryptosporidiosis in an index patient is associated with similar infection in other family members.

b. To determine if cryptosporidiosis infection is related to anergy and/or malnutrition

2. Background

Cryptosporidium, a protozoan (phylum, Apicomplexa; suborder, Eimeriorina) is responsible for an emerging zoonosis[1,2] that has inspired more than 100 reports since 1980. Many of those reports are case type, documenting the discovery of cryptosporidiosis in an additional species. This long list of species has been reviewed recently[3,4,5,6] and most of the information on cryptosporidiosis, summarized.

Cryptosporidiosis is widely recognized in veterinary medicine as a disease of calves[7,8] lambs[9], goats[10], pigs[11] and poultry birds[12,13,14]. Whereas the disease is thought to be primarily an enteric one in most species, in poultry birds the respiratory system has been a primary target as well as the conjunctional sac and the nasal passages of birds have also been infected[15].

Most veterinary reports have been from cases in calves. Upto 30% of the calf necropsies from diagnostic laboratories in the USA and Canada have revealed crypto, usually mixed with other enteric pathogens both viral and bacterial[16]. One prospective study of the dairy calf population in the state of Idaho, USA, documented that cryptosporidial infection was present in upto 66.7% of the dairy herds[17]. In some of these herds, other known enteric pathogens have not been found. These included entero-toxigenic E. coli, Salmonella sp. Campylobacter sp. Rotavirus and Corenavirus.

In man infection with Cryptosporidium have been considered rare and the result of opportunistic infection with a pathogen outside its normal host range. Of 12 published cases of cryptosporidiosis[18], ten occurred in patients who were immunologically compromized[2]. In more recent communication from CDC, 21 cases of cryptosporidiosis, were reported in patients with the recently recognized acquire immunodeficiency syndrome. AIDS victims have served to bring cryptosporidiosis to international prominance as a high priority disease. The course of infection is protracted in these people and often fatal due to extensive fluid loss[2]. There is no known successful treatment[20] although recently spiromycin or clindamycin-quinine combinations

seemed to cure some, but not all AIDS victims with cryptosporidiosis. Another groups at risk is chemotherapy patients.

Cryptosporidium readily infects immunocompetant individuals and the outcome of infection with children may be severe diarrhoea with moderate to severe dehydration although this is easily corrected by oral rehydration therapy. Infection could be serious in children with severe PEM owing to their altered immune function in analogy with observations in immunodeficient individuals.

The life cycle of this organism is similar to the life cycle of other coccidia having both asexual and sexual phase. Its reproductive potential is enhanced by the formation of autoinfective thin walled oocysts which reinitiates the life cycle in the infected individual. In the presence of a defective immune system, this feature accounts for prolonged diarrhoea[3]. These details have been elucidated in chicken embryo[18] and tissue culture studies.

Prospective studies in human populations have shown that Cryptosporidium is present in Costa Rican[19], British[20] and Liberian children[21]. There appeared to be a beneficial effect of children being breast fed and an increased risk to children in slums[19].

The pathogenetic mechanisms of cryptosporidial diarrhoea have been suggested by light[22] and electron microscopic studies[23]. The organism does not cause overt necrosis of enterocytes but does apparently hasten the turnover rate of villus epithelial cells. There is villous atrophy, crypt hyperplasia,

fusion of villi, reduction of absorptive surface, loss of microvilli and inflammatory cells infiltrates into the lamina propria. Ultrastructurally there is disruption of the microvillar border, the terminal web and the microvillar rootlets. Reduction in enteric digestive enzymes has been documented in the guinea pig.

Cryptosporidium diarrhoea may evolve into chronic diarrhoea and may induce malnutrition in young children as has been shown to be the case in other specific diarrhoeas. The most popular and widely used technique for diagnosis of Cryptosporidium today is the acid fact technique[31]. The method is rapid and sensitive and the diagnostician has the choice of many acid fast methods some of the most rapid methods work best but false positives are known to occur. It is suggested that repeat sampling and/or concurrent use of floatation or giemsa techniques be used in the few questionable specimens. Oocyst shedding takes place for several to many days; there are plenty of positive specimens available in an infected patient..

ICDDR,B researchers have been committed to investigating cryptosporidiosis in Bangladesh for a couple of years. In one study at the Savar Dairy Farm, Cryptosporidium was detected in calves with diarrhoea and their attendants. Additionally, the organism was found in some family members of these infected attendants (manuscript by Rahman ASMH, Sanyal SC, Al-Mahmud KA, Sobhan A, and Hossain KS)[25]. Further more in another study by Rahman ASMH and Sanyal, SC the pattern and shedding Cryptosporidium oocysts in calves was observed and this resembled the life cycle of the protozoan in experimental animals as shown in Current[3].

For the past year 4% of the diarrhoeal cases from the ICDDR,B hospital surveillance project were randomly selected for screening for cryptosporidial oocysts. Using the standard giemsa technique, a number of suspect cases were identified and most of these have been verified by use of the acid fast stain. These 69 positive cases are the basis of an initial publication by Dr. Nigar Shahid and co-workers[26]. The positive cases have been verified by Dr. Bruce C. Anderson, who has been working on cryptosporidiosis at the University of Idaho, USA since 1978.

3. Rationale :

No work has been done on Cryptosporidiosis in this region. Since the organism was found in 4% of patients coming to ICDDR,B we would like to define the circumstances in which the infection occurs in Bangladesh.

B. SPECIFIC AIMS

1. To identify symptomatic/asymptomatic Cryptosporidium infection among family members in relation to cases with and without cryptosporidiosis.
2. To determine the duration of excretion of Cryptosporidium in man.
3. To identify a possible reservoir of Cryptosporidium infection in animals.
4. To determine if Cryptosporidium infection is related to anergy and/or malnutrition.

C. MATERIALS AND METHODS

Patients and Controls

Index cases will be selected from among those diarrhoea patients included in the 4% surveillance system who have Cryptosporidium detected in their stool sample. Index cases will be limited to children 0-5 years who live within a motorable distance to ICDDR,B. We will exclude children who have a concurrent infection with V. cholerae, Salmonella, Shigella, ameba and giardia, C.jejuni who have taken antibiotics during the previous 7 days.

Control cases will be children with non diarrhoeal mild illness being treated at Dhaka Medical College Outpatient Department. Controls will be age (± 3m) and sex matched and will be concurrent (± 2 weeks). cases will be stratified into ≤3 years & >3 years of age.

Clinical Work up

The history of the patient will be taken in the special emphasis on nutritional factors, previous history of measles, other childhood infections as well as a history of the current diarrhoeal episode. The physical examination will include signs of dehydration, systemic illness, nutritional status (height, weight, arm circumference). Laboratory evaluation will include : blood for Hct, WBC, differential count and plasma specific gravity. Immunological studies will include an anergy screening (i.e. skin test with intermediate PPD, candida and trichophyton).

A chest X-ray will be taken to look for evidence of pneumonia and to examine the thymus.

a. Laboratory Methods

All stool samples will be screened initially by the modified Acid fast method as described by Bailey and Scott. A smear will be made on a glass slide with sediment of centrifuged stool. The slide will be stained with carbol faschin, heated revised and decolorized with 5% H_2SO_4 solution and counter-stained with methyline blue. For the purpose of confirmation of a suspected positive sample stool will be stained with Giemsa stain to look for morphology.

b. Nasopharangeal Aspirate

Nasopharangeal aspirate will be obtained from children with cough by inserting a polyvinyl tube in the nasopharyx, producing a cough reflex on the child and sucking the aspirate. The aspirate will be washed in normal saline, centrifuged and the sediment smeared and stained by modified acid fast method and confirmed by Giemsa staining.

Family Followup

Each index case and control will be visited by a team, consisting of a male and female Health Assistant. Visits will occur on day 3,5,10,17,20 and every 10 days until all family members are negative for Cryptosporidium.

On the first visit the following information will be obtained.:

1. Causes of family members with sex and age
2. Diarrhoea in family members during last 6 days.
3. Characterization of any diarrhoea (watery diarrhoea or dysentery)
4. Census of any household animals including birds

5. Diarrhoea occurring in animals. A stool specimen will then be obtained from each family member and animal. (See Family Followup Form). Additionally the height, weight and arm circumference will be obtained.

On subsequent visits the same clinical information and stool specimens will be obtained.

The stool specimens will be examined for Cryptosporidium only.

If any family members are found to have infection with Cryptosporidium, they will be requested to visit ICDDR,B to have the following tests; Hct, WBC, differential count, plasma specific gravity, anergy screen and chest X-ray.

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ABSTRACT SUMMARY

1. The study population will be children 0-5 years of age because the organism was found mostly in children.

2. No potential risk.

3. NA

4. All data will be handled confidentially and in coded format.

5. Signed consent will be obtained from guardian.

6. Interview and sample collection at the house will take about an hour.

7. The index children and their families will be provided with full medical coverage during the time family work up is being done.

8. Blood 2cc will be required from the index children.

CONSENT FORM

Your child has been infected with Cryptosporidium which is one of the newer agents for diarrhoea. Not much is known about its epidemiology, and course of infection in children. If you let your child enter our study you will be helping us to answer some very vital and preliminary questions on this infection.

Your child and your whole family will be under complete medical coverage throughout the period of the study. We shall take 0.5 cc blood from your child to perform some routine blood examinations. A chest X-ray will also be performed to see whether your child has a normal immune status we shall test for some common antigens which can be tested all at one time.

Your family will be visited on day 3,5,10,15,20 and every ten days till the infection is present in any of your family members. You will be required to provide us with stool of all your family members on these days. We shall also be taking faecal samples from all domestic animals and poultry birds in your household.

You are at liberty to withdraw the study anytime you wish after have joined it.

If you decide to join the study please sign on the paper.

Signature _____

LT impression of guardian _____

সম্মতি পত্র

আপনার সম্মতির জন্য জানানো যাইতেছে যে আপনার সন্তান ক্রিপ্টোস্পোরিডিয়াম নামক জীবাণু দ্বারা সংক্রমিত হইয়া পেটের পীড়ায় (ডায়রিয়া) ভুগিতেছে। এই জীবাণু সম্পর্কে জ্ঞান সীমিত। এই পরিপ্রেক্ষিতে আপনার সন্তানকে আমাদের পরীক্ষায় অন্তর্ভুক্ত করিয়া এই জীবাণুকে জানিবার জন্য সহায়তা করিতে পারেন।

এই পরীক্ষা চলাকালীন সময় আপনার সন্তান এবং পরিবারের অন্যান্য সদস্যদের চিকিৎসার ব্যয় বহন করা হবে। আমরা সকল নিয়মিত পরীক্ষার জন্য আপনার সন্তানের মাত্র ০.৫ মিলি রক্ত নেব। বুকের একটি এক্সরে তোলা হইবে। এটা যাচাই করা হইবে যে আপনার সন্তানের মধ্যে কোন প্রতিরোধের ক্ষমতা কতটুকু উপস্থিত। আপনার পরিবারকে ৩, ৫, ১০, ১৫, ২০ এবং প্রত্যেক ১০ দিন অন্তর যেখানে উল্লেখিত দিন পর্যন্ত আপনার পরিবারে রোগটির প্রাদুর্ভাব থাকবে। পরিবারের সকল সদস্য/সদস্যাদের মল পরীক্ষার জন্য নেওয়া হইবে। আপনার গৃহপালিত পশু-পক্ষীর মল ও পরীক্ষার জন্য নেওয়া হইবে। এই পরীক্ষা চলাকালীন যে কোন সময় আপনি মর্জি মাফিক বর্জন করিতে পারিবেন।

আপনি যদি এই পরীক্ষায় আমাদের সহযোগিতা করিতে চাহেন তবে অনুগ্রহ করিয়া স্বাক্ষর - বা টিপসই দিন। ধন্যবাদ।

স্বাক্ষর / টিপসই

তারিখ

SECTION III - BUDGET

A DETAILED BUDGET

Personnel Services	% Time	Annual Salary $	Project Requirement $
Dr. Nigar Shahid	10	5,630.00	562.00
Dr. ASMH Rahman	15	3,850.00	578.00
Mr. N.Rahman	5	2,688.00	135.00
Mr. Asadullah	100	1,690.00	1,690.00
H.A. Male & 1 animal attendant	100	1,690.00	3,380.00
H.A. Female	100	1,690.00	1,690.00
			8,035.00

Supplies & Materials			
Slides & Coverslips, immersion oil lens paper			500.00
Staining material AFB & Gemsa			100.00
Antigenic material			600.00
Medicine for family study			400.00
		Sub-Total:	1600.00

Bacterial cultures of stool			
Salm/Shig.V.chol, Jejuni	Tk. 128/- x 16		205.00
Rotavirus assay	Tk. 45/- x 16		72.00
E.coli (LT & ST)	Tk. 50/- x 16		80.00
Blood films (CBC)	Tk.3.20 x 16		5.12
X-Ray	Tk.25/- x 16		40.00
		Sub-Total:	402.12

ICDDR,B Transport	2,000.00

B BUDGET SUMMARY

Supply & Material	$ 1,600.00
Lab. investigation	402.00
ICDDR,B Transport	2,000.00
	$ 4,002.00
15 °/. increment cost	600.30
Grand Total: US	$ 4,602.30