

Library

ETHICAL REVIEW COMMITTEE, ICDDR, B.

Principal Investigator Dr. ASMH Rahman Trainee Investigator (if any) _____

Application No. 82-0587P

Supporting Agency (if Non-ICDDR, B) _____

Title of Study Studies on Cryptosporidium

Project status:

a possible etiologic agent of diarrhoea in
slaves and their attendants in Bangladesh.

- 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 overview (all other requirements will be submitted with individual studies)
- Protocol (Required)
- Abstract Summary (Required)
- Statement given or read to subjects or 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.

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

ASMH Rahman

Principal Investigator

02 JAN 1973

SECTION I - RESEARCH PROTOCOL

82-058(P)
29/12/82

1. Title : Studies on Cryptosporidium
as a possible aetiologic agent of
diarrhoea in calves and their
attendants in Bangladesh.
2. Principal Investigator: Dr. A.S.M. Hamidur Rahman

Co-Investigators : Dr. K.A. Al-Mahmud
Dr. S.C. Sanyal
To be named (Savar Dairy Farm)
- Consultant : Dr. Bruce C. Anderson
3. Starting Date : January, 1983
4. Completion Date : June, 1983
5. Total Direct Cost : \$3,000/-
6. Scientific Program Head:

This Protocol has been approved by Disease Transmission
Working Group.

Hamidi

Signature of Scientific Programme Head

Date _____

7. Abstract Summary:

Faecal specimens of five hundred of diarrhoeic ^{and non-diarrhoeic} calves aged 1-4 weeks from the Savar Dairy Farm including their extension program and another five hundred from diarrhoeal and non-diarrhoeal attendants of the farm and the extension project will be included in the study. These stool specimens will be screened for the presence of Cryptosporidium oocysts by phase-contrast microscopy.

8. Reviews:

- a) Research Involving human subjects: _____
- b) Research Review Committee: _____
- c) Director: _____
- d) B M R C: _____
- e) Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION:

1. Objective: Cryptosporidiosis is recognised basically as a veterinary problem. However, during recent years there have been several reports implicating Cryptosporidium in the aetiology of diarrhoea of normal and immunocompromised humans. The relationship between man and animals in the developing countries especially in Bangladesh is very close. Mostly, they share the same premises for their living leading to close association. Therefore, the possibility of cross infection between man and animal is quite high.

In the present study we aim at recognising the existence of Cryptosporidium as a possible cause of diarrhoea amongst domestic animals especially in calves as well as in man attending them in Bangladesh.

2. Background: There is no known published report of Cryptosporidiosis in Bangladesh but calf morbidity and mortality is quite high in the dairy farms showing non specific watery diarrhoea. Cryptosporidiosis, an infection of the intestinal mucosa by a protozoan of the enteric Coccidia group

2. Background: (Contd.)

has been recognised in animals since the beginning of this century¹. It has been identified in a wide range of vertebrates, having been reported in reptiles (snakes)², avians (turkeys and chickens)³ and mammals (rabbits, mice, guineapigs, dogs, calves, sheep and monkeys)²⁻³. The infection causes enterocolitis in turkeys, guinea pigs and calves and gastritis in snakes. The disease was recognised as a significant cause of diarrhoea in calves in a well managed, closed herd of approximately 300 lactating Holstein cows. The morbidity in 1-2 week old calves were almost 100 per cent although mortality was low. Clinically mild to moderate depression, hair standing on and raised temperature in the range of 103 to 104°F (39.4 to 40°C) were first noted 6 to 10 days postnatally. Twelve to 24 hours later yellow watery diarrhoea followed. Rectal temperature gradually came down to about 101°F (38.3°C) during the next five to seven days. During this period, the faeces gradually thickened to a tenacious, pasty, mucoid consistency. Results of faecal floatation examination revealed the presence of Cryptosporidium oocysts usually on the second or third day after the onset of diarrhoea. Shedding of oocysts usually ceased within three to six days or when the faeces

2. Background (Contd.):

became thick and pasty. The optimal day for shedding the oocysts, in the group of calves studied, was 12th day of life.⁴

Cryptosporidiosis was first noted as a human infection in 1976⁵ when the protozoan was detected in the watery diarrhoeal stool specimen of an immunosuppressed patient and since then there has been a growing awareness of this parasitic protozoan as a potential human pathogen.

A case of human cryptosporidiosis in an animal handler was reported by Auburn University in Alabama in 1981. About 3 weeks before the onset of the symptoms, the patient, a normal healthy 25 - year old male, free of immune deficiencies, had started a survey of calves for Cryptosporidium sp.⁶ Clinical features of his illness included nausea and low-grade fever, moderate abdominal cramps, anorexia, 5-10 watery and frothy stools a day, followed by constipation. Fourteen days after the onset, the patient improved and was eating a full diet.

Since the detection of the protozoal pathogen in the animal handler concerned with the calf cryptosporidiosis study, stool examinations were done for 16 other animal handlers at the University who had

2. Background (contd.):

contacts with animals involving three separate, unrelated outbreaks of calf cryptosporidiosis. From those 16 persons, 11 additional cases of human infections were identified. All involved previously healthy individuals; no abnormality was noted in their levels of serum globulins at the time of infections, and no deficiency in cell mediated immune response was detected by lymphocyte blastogenesis technique. Symptoms occurred within 1-2 weeks after the individual had first contact with the infected calves. Four of these 11 patients had clinical symptoms similar to those described earlier; another 4 had diarrhoea and moderate abdominal cramps; 1 had fever, constipation, and abdominal cramps, and 2 were asymptomatic. Before this outbreak in Alabama, not more than a dozen cases of human cryptosporidiosis had been reported in literature. Of these, 5 involved patients who had prolonged illness and were shown to be immunologically deficient,⁽⁷⁻¹¹⁾ 2 other patients were undergoing immunosuppressive chemotherapy,^(12, 5) and 4 were otherwise apparently healthy individuals^(6, 13-15). Thus Cryptosporidiosis occurs, not only in immunologically compromised persons but also apparently healthy individuals.

B. SPECIFIC AIMS:

To detect Cryptosporidium oocysts in stool specimens or diarrhoeic calves and their attendants by phase-contrast microscopy after faecal floatation technique.

C. METHODS OF PROCEDURES:

1. Sample Selection: (Calves/attendants)

Faecal specimens of calves, (both diarrhoeal and non-diarrhoeal) during the first 1-4 weeks of lives will be collected in clean glass bottles daily and preserved at 4°C in the Savar Dairy Farm. The Specimens will be brought to the Centre for microscopy once in a week. A total of 500 samples will be examined. Another 500 specimens of diarrhoeal and non-diarrhoeal attendants of the farm as well as the extension program will also be collected for examination. The specimens from the latter group will be collected from their houses by the Veterinary Extension Officer. In both the cases the non-diarrhoeal group will act as control.

In seventies cases of human cryptosporidiosis used to be diagnosed only after histologic examination of the intestine or rectal biopsies. Recent experience in studies of clinical cases and experimental infections clearly demonstrates that human Cryptosporidiosis can be diagnosed and monitored by

1. Sample Selection (Contd.)

detection of oocysts in the faeces. It has been found that coverslip floatation using Sheather's sugar solution followed by examination with phase-contrast microscopy is the best technique for detection of Cryptosporidium oocysts in the faeces of humans, calves, and rodents⁶. Anderson also recommended faecal floatation to be the best technique for demonstrating Cryptosporidium oocysts in calf faeces⁴. The oocysts survive in stool specimen for weeks together at 4°C and for several days at ambient temperature¹⁶. Floatation tests for Cryptosporidium oocysts will be done with approximately 5g of faeces, suspended in 15-20 ml of water, filtered through 4 layers gauze and centrifuged for 10 minutes at 500x g. The supernatant will be decanted and the sediment will be resuspended in a Levitation solution of specific gravity 1.27 (granulated sugar, 454 g; distilled water, 355 ml; and liquified phenol, 6.7 ml). Centrifugation will be repeated at 500 x g for 10 minutes. One loopful (7 mm diameter) of fluid from the meniscus will be taken on a glass slide, coverglassed and examined at 400 x and 1000 x.

When observed with phase-contrast microscopy, the spherical to sub spherical oocysts measured 4-5 μ m;

1. Sample Selection (contd.)

highly refractile, and containing 1-6 prominent dark granules and numerous fine dark granules are seen⁶.

RATIONALE:

In our dairy farms a large number of calves die due to diarrhoea of unknown aetiology. However, during recent years Cryptosporidium species has ~~be~~ been found to be responsible for many cases of calf diarrhoea in different countries. This study has the possibility of elucidating the role of Cryptosporidium in the aetiology of calf-diarrhoea in Bangladesh which might be of help in formulating preventive measures for the great loss in our Livestock.

Further, this protozoan has been implicated during recent years in diarrhoeagenesis of animal attendants indicating the zoonotic nature of the disease. Detection of this protozoan in farm attendants and other diarrhoeal calf attendants may confirm this hypothesis.

REFERENCES

1. Tyzzer GC. A sporozoan found in the peptic glands of the common mouse. Proc Soc Exp Biol Med 1907; 540: 12-3.
2. Brownstein DG, Strandberg JD, Montoli RJ, Bush M, Eortner J: Cryptosporidium in snakes with hypertrophic gastritis. Vet Pathol 1977; 14: 606.
3. Levine ND: Protozoan Parasites of Domestic Animals and of Man. Minneapolis, Burgess Publishing Co. 1961 PP 245-246.
4. Anderson BC. Pattern of shedding cryptosporidial oocysts in Idaho calves. Am. J. V.M.A. 1981; 178: 982-984 (14)
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5. Meisel JL, Perera Dr. Meligro C, Rubin CE. Over-whelming watery diarrhoea associated with Cryptosporidium in an immunosuppressed patient Gastroenterology 1976; 70: 1156-60.
6. Reese NC. Current WL, Ernst JV, Bailey WS. Cryptosporidiosis of man and calf: a case report and results of experimental infections in mice and rats. Am J Trop Med Hyg 1982; 31: 226-9.
7. Lasser KH, Lwein KJ, Rynning FW. Cryptosporidial enteritis in a patient with congenital hypogammaglobulinemia. Hum Pathol 1979; 10: 234-340.
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10. Sloper KS, Dourmaskin RR, Bird RB, Slavin C, Wester ADB. Chronic malabsorption due to cryptosporidiosis in a child with immunoglobulin deficiency. *Gut* 1982; 23: 80-2.
11. Bird RG, Smith MD, Cryptosporidiosis in man: parasite life cycle and fine structural pathology. *J Pathol* 1980; 132: 217-33.
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13. Nime FA, Burek JD, Page DL, Holscher MA, Yardley JH. Acute enterocolitis in a human being infected with protozoan Cryptosporidium: *Gastroenterology* 1976; 70: 592-8.
14. Tzipori S, Angus KW, Gray EW, Campbell I, Vomiting and diarrhoea associated with Cryptosporidium infection. *N Engl J Med* 1980; 303: 818.

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15. Anderson BC, Donndelinger T, Wilkins RM, Smith J.
Cryptosporidiosis in a veterinary student. J Am Vet
Med Assoc 1982; 30: 408-9.

16. Pohlenz J, Moon HW, Cheville NF, et al: Cryptospori-
diosis as a probable factor in neonatal diarrhoea of
calves. J Am Vet Med Assoc 172; 452-457, 1978.

*msi.

PILOT PROTOCOL

Studies on Cryptosporidium as a possible aetiologic agent of diarrhoea in calves and their attendants in Bangladesh.

Abstract Summary:

Cryptosporidiosis in the past, was recognised as a veterinary problem. During recent years there have been several reports implicating Cryptosporidium in the aetiology of diarrhoea both in healthy and immunodeficient humans. There is no known published report of Cryptosporidiosis in man and/or animals in Bangladesh. However report of calf morbidity and mortality in the dairy farms, showing non-specific watery diarrhoea are available.

In the present pilot study we aim at recognising the existence of Cryptosporidium as a possible cause of diarrhoea amongst domestic animals especially in calves as well as in man attending them.

1. Population:

Faecal specimens of five hundred diarrhoeic calves from the Savar Dairy Farm and another five hundred from diarrhoeal and non-diarrhoeal attendants will be included in the study.

2. Potential risk:

There is no significant risk to the study.

Rectal faecal specimens will be collected from the diarrhoeic calves with minimum handling by the

technician. Animal attendants will be provided with clean specimen container for collection of their stools.

3. Procedure for minimizing potential risks:

Since there is no risk this section is not applicable.

4. Safeguarding confidentiality:

Confidentiality of human subjects will be ensured by coding them with numbers.

5. Informal Consent:

Consent will be taken from the animal attendants letting them know the prospect of the study.

6. Interview informations:

The research will not require any interview.

7. Potential benefits to the subject:

The study has the possibility of being beneficial to the farm by making proper diagnosis of the diarrhoea cases especially those of parasitic aetiology which will lead to proper treatment. Further, it has the potentiality of saving many calves from death due to Cryptosporidiosis and curing man suffering from this disease and thus will accrue great benefit to the society.

8. Medical Records:

The research will not require any records except those of calves maintained in the farm.

SECTION III - BUDGET

A. DETAILED BUDGET

PERSONNEL SERVICES:

<u>Name</u>	<u>Position</u>	<u>% or No. of day</u>	<u>Annual Salary (TAKA)</u>	<u>Project Requirement (TAKA)</u>
r. A.S.E.H. Rahman	Principal Investigator	30%	48,000/-	7,200/-
r. K.A. Al-Mahmud	Co-Investigator	5%	90,000/-	2,250/-
r. S.C. Sanyal	CO-Investigator	5%	--	25,000/-
to be named (Savar Farm)	Co-Investigator	5%	--	--
to be named (Savar Farm, Extension)	Co-Investigator	5%	--	--
Technician	New Position	100%	18,000/-	9,000/-
r. B.C. Anderson	Consultant	-	--	--

SUPPLIES AND MATERIALS:

<u>Item</u>	<u>Amount</u>	<u>Unit Cost</u>	<u>Project Requirements</u>
Specimen Bottle (glass)	500/-	5	2,500/-
Elastic spoon	1,000/-	1	1,000/-
Slide & Cover slips	500/-	-	500/-
Glass Ware	--	-	500/-
Glass Pencil, marker, dropper	--	-	500/-
Granulated sugar	20 lbs.	-	500/-
Liquid Phenol	6 Bottle 500 ml	-	500/-
Anthelmintic Drug	--	-	3,750/-

EQUIPMENTS

Nil

PATIENT HOSPITALIZATION

Nil

INPATIENT CARE

Nil

A. DETAILED BUDGET (Contd.)

<u>ICDDR,B TRANSPORT</u>	<u>TAKA</u>
ICDDR,B to Savar Farm and back 50 miles/week for 26 weeks, Tk. 4.5/mile	5,800/-
TRAVEL & TRANSPORTATION OF PERSONS	Nil
TRANSPORTATION OF THINGS	Nil
RENT, COMMUNICATION & UTILITIES	Nil
PRINTING AND REPRODUCTION	1,000/-
OTHER CONTRACTUAL SERVICES	Nil
CONSTRUCTION, RENNOVATION	Nil

TOTAL TK. 60,000/-

US \$2600/-