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ICDDR,B LIBRARY
DHAKA 1212

STUDY

Attachment 1.

Date 4/1/82

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. Thomas C. Butler Trainee Investigator (if any) _____

Application No. ✓ 82-016(P) Supporting Agency (if Non-ICDDR,B) _____

Title of Study Pilot Survey for Spirochetal and Chlamydial Agent in Diarrheal Patients 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).

- 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

- Will signed consent form be required:
 - (a) From subjects Yes No
 - (b) From parent or guardian (if subjects are minors) Yes No
- Will precautions be taken to protect anonymity of subjects Yes No
- 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:

- 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.
- Examples of the type of specific questions to be asked in the sensitive areas.
- An indication as to when the questionnaire will be presented to the Cttee. for review.

15 JUN 1982

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

Thomas Butler
Principal Investigator

Trainee

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QW 152.JB2
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1982

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DHAKA 1212

82-016PP
2/4/82

SECTION I - RESEARCH PROTOCOL

1. Title: Pilot Survey for Spirochetal and Chlamydial Agents in Diarrheal Patients
2. Principal Investigator: Dr. Thomas C. Butler
3. Co-Investigator: Dr. Barbara Stoll, Dr. Michael Bennish, Dr. Richard McGlaughlin, Dr. Peter Speelman, Dr. M. Imdadul Huq
4. Starting Date: April 1982
5. Completion Date: July 1982
6. Total Direct Cost:
7. Scientific Program Head: Dr. Thomas C. Butler

This protocol has been approved by the Pathogenesis and Therapy Working Group.

Signature of Scientific Program Head:

Thomas C. Butler

Date:

March 31, 1982

8. Abstract Summary:

Two hundred diarrheal patients undergoing the ICDDR,B surveillance procedure and 100 patients entered into the colitis study will be screened for the presence of intestinal spirochetes and chlamydia. The spirochetes will be detected by phase contrast microscopy and silver staining of colonic biopsies. Chlamydial infection will be screened by submitting paired sera for serological testing.

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15 JUN 1998

9. 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: The aim of this pilot study is to determine whether some cases of diarrhea in Bangladesh, which presently occur for unknown cause, are caused by spirochetal or chlamydial agents. If this pilot study detects evidence for either of these occurring, a full study protocol will be written.
2. Background: At the ICDDR,B the ongoing surveillance study detects diarrheal pathogens in about 70% of cases and the colitis study likewise establishes the cause of dysentery in about 2/3 of cases. Thus, about 30% of patients in both of these studies cannot be diagnosed. They are thus termed "unknown etiology" or "non-shigella dysentery."

The spirochetes are an important group of bacteria that are capable of causing many diverse diseases of man and animals. The three genera of pathogenic spirochetes are Borrelia, Leptospira, and Treponema. Spirochetes are classified as bacteria because they share the typical features of other bacteria. For example, they are single-celled and have an outer membrane or cell wall. They are procaryotes, that is, the nuclear material is not organized into chromosomes or bounded by a nuclear membrane, and multiplication

occurs by binary fission. The spirochetes contain both DNA and RNA and are capable of cellular metabolism and protein synthesis. Furthermore, they are susceptible to the action of antibiotics. On the other hand, they differ from other bacteria by their unique helical structure, spirillary motility, and growth requirements. They range in length from about 5 to 40 microns. Their motility appears to be a rotational corkscrew-like motion but actually consists of complex patterns of contrarotation of opposite ends, helical waves, and flexing motions. The organelles of motility are flagella, which vary in number from 1 to 20 in the pathogenic spirochetes and are unique by their location between the outer membrane and inner cytoplasmic membrane. In general, the spirochetes are fastidious in their growth and require long-chain fatty acids. They will not form colonies on agar medium, but the Leptospirae and Borreliae can be cultivated in media supplemented with bovine serum albumin and rabbit serum. Certain Treponemes are even more fastidious because of extreme sensitivity to oxygen toxicity outside of mammalian tissues and have defied all attempts at in vitro cultivation.

Spirochetes are ubiquitous in nature. In addition to the pathogenic genera, there are free-living spirochetes that inhabit bodies of water, marshes, and sewage. Animals, such as the mollusks, have their digestive tracts colonized by spirochetes. Spirochetes colonize the oral cavities and intestinal tracts of man and other animals, in which they constitute part of the normal endogenous bacterial flora.

Only rarely are these spirochetes associated with disease and the pathogenic roles of these endogenous spirochetes in initiating certain diseases are unclear. One such disease is periodontitis, in which the oral treponemes on the subgingival plaque proliferate to much greater than usual numbers when there is inflammation, tissue destruction, and bone loss. Acute necrotizing ulcerative gingivitis, or trench mouth, is another sort of oral inflammation in which oral spirochetes appear to play a pathogenic role. Another example of oral inflammation is Vincent's angina, a form of tonsillitis in which Treponema vincentii spirochetes exist in large numbers. In the swine intestine Treponema hyodysenteriae is capable of invading the epithelial cells and producing dysentery.

Recent reports of intestinal spirochetes found in patients with rectal symptoms and diarrhea suggest that spirochetes may be pathogenic (1-3).

The chlamydiae are small intracellular organisms that are barely visible by light microscopy. Thus, they are larger than viruses but resemble viruses by being obligate intracellular organisms. They are smaller than bacteria but resemble bacteria by being susceptible to the action of antibiotics. Chlamydiae are the recognized cause of ornithosis, trachoma, lymphogranuloma venereum, and non-gonococcal urethritis. In male homosexuals, chlamydial infection causes proctitis and the organism can be cultured from the stool. In birds, this is a common intestinal inhabitant. In children with trachoma in Egypt, the organism could be cultured from stool as well as from the eye. Chlamydia can cause neonatal pneumonia and diarrhea,

B. SPECIFIC AIMS:

1. By phase contrast microscopy and histopathology of colonic biopsy, to look for evidence of intestinal spirochetosis in diarrheal patients at the ICDDR,B.
2. By serological screening, to look for evidence of chlamydial infection as a cause of diarrhea.

C. METHODS OF PROCEDURE:

1. Patient selection. The 4% systematic sample of patients for surveillance will be used. Also patients admitted to the hospital under the colitis study will be used.
2. Stool exam. A freshly passed stool or an aliquot obtained by rectal catheter will be observed under phase contrast microscopy for spirochetes.
3. Histopathology. For colitis patients undergoing colonoscopy and biopsy, the blocks of tissues will be cut to prepare a slide for staining with Dieterle's silver stain.
4. Serology. Blood will be obtained by syringe from adults and by finger stick from children for chlamydial serology. A follow-up visit at home will be scheduled 2 weeks later to obtain a convalescent specimen. The sera will be frozen and sent to Dr. Schachter's laboratory in San Francisco for serological testing.
5. Informed consent. Prior to obtaining blood, written informed consent will be requested of all patients.

D. SIGNIFICANCE AND RATIONALS:

In about 30% of diarrheal cases at the ICDDR,B, diagnostic efforts fail to reveal a cause. If either intestinal spirochetes or chlamydia can be detected in some of these patients, we will have found new causative agents. Such new information on causes of diarrhea will be valuable to us in seeking better therapies and control measures for diarrheal disease in tropical countries.

REFERENCES

1. Lee, F.D., Krazewski, A., Gordon, J. et al. Intestinal spirochetosis, Gut 12:126-133, 1971
2. Crucioli, V., Busuttill, A. Human intestinal spirochetosis. Scand. J. Gastro. 16 (Suppl 70) 77-79, 1981
3. Douglas, J.G., Crucioli, V. Spirochetosis: a remediable cause of diarrhea and rectal bleeding. Brit Med. J. 283:1362, 1981

Abstract Summary for Ethical Review Committee

1. This pilot survey intends to examine stool and serum of patients with diarrhea for evidence of infection with new pathogenic agents. The populations of patients to be studied will be from two existing studies at the ICDDR,B which have already been approved by the Ethical Review Committee "Surveillance of Urban Diarrhea" and "Colitis in patients with Campylobacter, V. parahemolyticus, and Shigella infections." The choice of these patients was made because they are already being examined and interviewed; this information can be shared in the present study. The only addition to the previous protocols will be obtaining acute and convalescent blood specimens from patients in the surveillance study. Blood is already being obtained in the colitis study and an aliquot of this blood will be used in the present study.
2. There is minimal risk in this study. Obtaining 5cc of blood on two occasions will cause minimal discomfort only.
3. The procedure to minimize risk will be to draw blood in a professional and skillful manner so as to reduce discomfort.
4. Confidentiality will be safeguarded by using the surveillance numbers and hospital numbers rather than names of patients in our record-keeping.
5. Signed informed consent will be obtained using the same form as in the two previously approved protocols. For the surveillance patients, the addition to the informed consent will be the venepuncture to obtain blood during the first hospital visit and two weeks later when the patient will be asked to return for a convalescent specimen.
6. No new interview will be added.
7. The potential benefits are the new information on the causes of diarrheal diseases in Bangladesh. If new agents of diarrhea are discovered, it should be possible to improve treatment and plan further work that will lead to prevention.
8. The study will not require records or bodily tissues other than serum.

SECTION III - BUDGET

A. DETAILED BUDGET1. Personnel Services

<u>Name</u>	<u>Position</u>	<u>% of effort</u>	<u>Project Required</u>
Dr. T.C. Butler	Principal Investigator	-	-
Dr. M.I. Huq	Co-Investigator	-	-
Dr. B.J. Stoll	"	-	-
Dr. P. Speelman	"	-	-
Dr. R. McGlaughlin	Guest Scientist	10%	-
Dr. M. Bennish	Guest Scientist	50%	-

2. Supplies and Materials:

A. Syringes and needles	: \$600	\$1,200
B. Test tubes	: \$600	150

3. Equipment : Nil4. Patient hospitalization : Nil5. Outpatient care : Nil

Total	:	<u>\$1,350</u>
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