Revised

ETHICAL REVIEW COMMITTEE, ICDDR,B.

7 March 1984

8/3/84

ICDDR,B Library

Principal Investigator Dr. M. Bennish

Trainee Investigator (if any)

Application No. 84-009

Supporting Agency (if Non-ICDDR,B) 26

Title of Study EVALUATION OF CHLAMYDIA TRACHOMATIS AS A POSSIBLE DIARRHEAL PATHOGEN.

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)
   (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 N/A
   (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.

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

Michael Bennish M.D.
Principal Investigator

Trainee

SECTION 1 - RESEARCH PROTOCOL

1. TITLE: EVALUATION OF CHLAMYDIA TRACHOMATIS AS A POSSIBLE DIARRHEAL PATHOGEN.

2. PRINCIPAL INVESTIGATOR: Dr. M. Bennish

   CONSULTANTS: Dr. Thomas Butler

   Dr. Julius Schachter, University of California, San Francisco.

3. STARTING DATE: 1 July 1984

4. COMPLETION DATE: 30 June 1985

5. TOTAL INCREMENTAL COST: US $ 39,489.00

6. SCIENTIFIC PROGRAMME: This protocol has been approved by the Pathogenesis & Therapy Working Group.

Signature of the Associate Director, PTWG T Butler

Date: 7-3-84

7. ABSTRACT SUMMARY:

Chlamydia trachomatis is an intracellular bacterial organism that is a known cases of a number of diseases in man. Although it has been isolated from the gastrointestinal tract, it's role as a possible enteric pathogen has not been systematically evaluated. A previous serosurvey of 96 diarrheal patients at ICDDR,B found a 24% prevalence of antichlamydial antibodies. As the next step in evaluating it's role in the gastrointestinal tract, we will establish facilities for the isolation of chlamydia and attempt to isolate the organism from surveillance patients, non-diarrheal control patients, and patients with pneumonia by culturing rectal swabs, stool, conjunctivae, and naso-pharyngeal aspirates.

8., REVIEWS:

(a) Ethical Review Committee: _____

(b) Research Review Committee: _____

(c) Director: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION:

1. Objectives:

(a) To determine the prevalence of rectal and stool sheding of C. trachomatis among 300 consecutively samples surveillance patients.

(b) Based on the initial culture results, to obtain rectal swab and stool cultures from 200 surveillance patients at high risk (as defined by results of the initial 300 cultures) of C. trachomatis infection, and to compare the infection rate in these patients with that of 200 age and neighborhood matched non-diarrheal control patients.

(c) To determine if C. trachomatis is a cause of pneumonia among infants in Bangladesh.

2. Background:

Chlamydia are bacteria that are unusual in that they are obligate intracellular parasites (1). Within the genus Chlamydia are two seperate species, C. psittaci, which primarily infects avians and other animals, and is only an occasional pathogen in humans, and C. trachomatis, for which man is the primary host (1). C. trachomatis is further divided into 15 serotypes which differ significantly in their biological activity.

Three of these serotypes are lymphogranuloma venereum strains, which tend to be more invasive than the 12 oculogenital strains which in vivo grow only in epitheleal cells (1).

C. trachomatis infection of humans has been associated with a number of different disease manifestations. Inclusion conjunctivitis and trachoma were the first diseases that were attributed to C. trachomatis infection. Identification of the characteristic intracytoplasmic chlamydial inclusion bodies in scrapings of conjunctival epithelium from patients with these two diseases occured in the first decade of this century (1).

With the advent in the late 1950's and early 1960's of yolk-sac and tissue culture methods for isolating the organism, the number of diseases associated with C. trachomatis infection has rapidly expanded. In addition to trachoma and inclusion conjunctivitis, syndromes currently associated with C. trachomatis infection include urethritis, cervecitis, salpingitis, infant pneumonia, and proctitis. (1).

A major unanswered question in defining the clinical spectrum of chlamydial diseases is the role of C. trachomatis in body sites from which it has been cultured but where it's clinical role has not yet been systematically evaluated. Among these sites the possible association of enteric disease with chlamydial infection of the gastrointestinal tract has been the source of much speculation (1-6).

A number of lines of evidence suggest the Chlamydia might be an enteric pathogen in humans. It is a known cause of proctitis among certain populations (7). Chlamydia are diarrheal pathogens in a number of animal species (8). Rectal shedding of Chlamydia trachomatis is a frequent occurence in children who are colonized with C. trachomatis at other sites (2-4). Beem and colleagues at the University of Chicago found that of 115 infants 1 week to 6 months old with either conjunctival or nasopharyngeal Chlamydia infections, 43% also had rectal cultures positive for Chlamydia (2). Schachter et. al. found that in prospectively studied infants born to Chlamydia infected mothers, four of the 12 infants who subsequently developed Chlamydia infection had rectal cultures that were positive (4).

Although chlamydial infections of infants are presumed to be aquired vertically, a number of cross-sectional studies of children in non-trachoma endemic areas have found a steady increment in the prevalence of antichlamydial antibodies in pre-adolescent children (9-10). The transmission patterns and clinical correlates underlying this acquisition of antibody have not been evaluated. A fecal oral mode of transmission would be one possible explanation for acquisition of antibody in an age group where vertical and venereal transmission are unlikely to play a major role.

In an initial attempt to assess the possible association of chlamydial infection and diarrheal illness, from April to June 1982 we evaluated the antichlamydial antibody status of 93 ICDDR,B patients.

Antichlamydial antibodies were detected in twenty four (26%) of these patients (11). Three patients had elevated IgM class antibodies, and two of seven patients from whom convalescent sera were obtained had a fourfold change in titer (11).

We also attempted to isolate the organism from rectal swabs obtained from 87 of the 93 patients. Twenty four cultures were not evaluable because of fungal or bacterial overgrowth of the cell sheet. From the remaining 63 cultures we had one Chlamydia isolate, which was C. trachomatis serotype D (12). Because of the numerous freeze-than cycles which the specimens underwent prior to culturing, the isolation rate was presumably lower than it would have been had conditions been optimal.

Three has been frequent speculation on the possible role of C. trachomatis as an enteric pathogen. In an attempt to better define the role of Chlamydia in the gastrointestinal tract we hope to establish facilities here at ICDDR,B for isolation of the organism, and to begin sampling patinets for the presence of Chlamydia in stool or rectal swabs.

B. SPECIFIC AIMS: See objectives.

C. METHODS AND PROCEDURES:

(1) Isolation method:

A McCoy cell line system adopted to microtiter plates as described by Yoder et. al. will be used for isolation of the organism (13). This meicromethod has been found to be equal in sensitivity and less costly than the older vial-cover slip system. Cells will be treated with cyclohexamide to enhance sensitivity (14). Staining of inclusions will be done with Jones-iodine solution. To reduce contamination of the cell sheet, both rectal swab specimens and stool samples will be diluted prior to innoculation.

(2) Specimen collection:

Rectal and conjunctival culture will be obtained with a calcium alginate swab. Nasopharynegeal cultures will be obtained by aspiration of the posterior nasopharynx using a 10.c.c. syringe and size five French or eight French feeding tube.

(3) Patient selection:

(i) Initial evaluation of the culture system:

In order to evaluate the tissue culture system we will process 200 stool and 200 rectal swab specimens during the four weeks that the technical collaborators are at ICDDR,B.

Samples will be obtained from surveillance patients and other treatment centre or hospitalized patients who are having rectal swabs or stool samples obtained.

Technical problems that might require modifications during this time could include optimal dilution of specimen and the choice of antibiotics in the overlay required to minimize bacterial and fungal contamination of the culture system.

(ii) Surveillance patients:

Rectal swab, stool sample if available, and conjunctival swabs will be obtained from 300 consecutive surveillance patients for purposes of culture. Specimens will be put into transport media and innoculated the day they are obtained, or held at $4^{\circ}C$ until they can be innoculated. Stool specimens will be centrifuged with sterile glass beads and the supernatant will be extracted for innoculation.

(iii) Case control study:

Following analysis of the culture results of the first 300 sampled patients, 200 surveillance patients at high risk of Chlamydia infection as defined by the initial study will be sampled. They will be compared with 200 age and neighborhood matched non-diarrheal control patients.

(iv) Infants with pneumonia:

Hospitalized infants less than six months of age who have diarrhea, and signs and symptoms of the Chlamydia pneumonia syndrome, such as paroxysmal cough, lack of fever, bilateral rales, eosinophilia, and interstirial infiltrates on x-ray, will be evaluated to see if Chlamydia trachomatis infection is present. Conjunctival, naso-pharyngeal, rectal swab, and stool cultures will be obtained from such patients.

(4) Data analysis:

Chlamydia culture negative and Chlamydia culture positive patients from the initial unselected sample of surveillance patients will be grouped, and differences in the frequency of clinical and laboratory findings searched for. If differences are found their significance will be evaluated by the X^2 or Fisher exact tests. Based on this analysis, a group of 200 surveillance patients at high risk of infection will be sampled, and compared with a control population of 200 patients. The significance of differences in these groups will again be initially evaluated using the Chi-square or Fishers exact test.

D. SIGNIFICANCE:

Chlamydia trachomatis is an intracellular bacterial pathogen that causes subacute and chronic infection at a number of body sites. C. trachomatis is known to infect the gastrointestinal tract, but the consequences of such infection have not been systematically evaluated. In a substantial percentage of ICDDR,B patients with acute diarrhea and in a majority of patients with chronic diarrhea no pathogen can be identified. A previous serologic study of diarrhoea patients at ICDDR,B found a 26% prevalence of antichlamydial antibodies. As the next step in the evaluation of C. trachomatis as a possible diarrheal pathogen we will attempt to isolate the organism from stool and rectal swab specimens from diarrhea patients and non-diarrhea age and neighborhood matched controls patients.

E. FACILITIES REQUIRED:

Available laboratory and office facilites will initially be used for this project. With completion of the new pathology facilities on the ground floor of the IPH building, the laboratory will be moved to this facility.

F. COLLABORATIVE ARRANGEMENTS:

This study will done in collaboration with Dr. Julius Schachter, Professor of Epidemiology, The University of California, San Francisco. His senior laboratory technician will spend four weeks in Dhaka to help with the establishment of the isolation procedure, and his laboratory will provide stock chlamydia to use as a positive control.

BIBLIOGRAPHY:

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4. Schachter J, Grossman M, Holt J, Sweet R, Spector S. Infection with Chlamydia trachomatis: Involvement of Multiple anatomic sites in neonates. J Infect Dis 139 : 232-234, 1974.

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6. Alexander ER, Harrison HR. Chlamydial infections In: Evans As, Feldman HA, eds. Bacterial infections of Humans: Epidemiology and control, New York : Plenum 1982.

7. Quinn TJ et. al.: Chlamydia trachomatis proctitis. NEJM 305 : 195-200, 1981.

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9. Black SB, Grossman M, Cles L, Schachter J. Serologic evidence of chlamydial infection in children. J Peds 98 : 65-67, 1981.

10. San Joaquin UH, Rettig PJ, Newton SY, Marks MI. Prevalence of Chlamydial antibodies in children AM J Dis Child 136 : 425-427, 1982.

11. Butler TC, Bennish M, Schachter J, Stoll B. Serologic evidence for Chlamydial infection in patients with diarrhea. In press: Trans Roy Soc Trop Med Hyg.

12. Bennish M. Unpublished data.

13. Yoder BL, Stamm WE, Koester CM, Alexander ER. Microtest procedure for isolation of Chlamydia trachomatis. J Clin Microbiol 13 : 1036-1039, 1981.

14. Schachter J, Dawson CR, Hoshiwara I, Daghfous T, Banks J. The use of cyclohexamide-treated cells for isolating trachoma agents under field conditions. Bull WHO 56 : 629-632, 1978.

SECTION III - BUDGET

A. DETAILED BUDGET:

1. Personnel services:

Name	Designation	% effort	Salary Taka	Salary Dollar
Dr. Michael Bennish	Int. Research Associate	20%		7,400
To be named	Senior Research Officer	100%	40,000	

2. Equipment 16,484

Revco ult 1885 B	4,620
Lab-line -20°C Freezer 3552-10	1,270
Lab-line 4°C refrigerator 812	1,010
Laminar flow hood-Labconoco 110000	930
Incubator-Isotemp 11-633-231 D	467
International centrifuge-model K	4,120
Centrifuge head #276	366
Vortex genie	143
37° water bath	480
Measuring cylanders - 500, 100, 50 ml - 2 each	53
Microtiter carriers -Eco microtiter	250
Microscope AO XH 110B6F	2,725

			US Dollar
3. Supplies and Materials			7,005
Glassware:			3,093
Microtiter plates - 96 well-Falcon 3040	# 250		1,564
Pasteur pipetes	1000		420
Disposable calibrated pipettes:			
1 ml	# 500		94
5 ml	500		153
10 ml	500		176
Pipete Bulbs - safety type	3		55
Screw cap bottles:			
500 ml	12		40
100 ml	12		25
Screw cap tubes - 16 X 150 mm	1000		250
75 CM^2 Falcon tissue culture flasks	200		213
15 ml screw cap centrifuge tubes	500		103
Reagents:			2,662
		Gibco cat #	
Fatal calf serum - 500 ml	12	200-6140	1,476
L. Glutamine - 50 ml	12	810-1051	155
Eagles MEM with earlts salt - 500 ml	12	330-1380	411
$NaHco_3$ - 100 ml	12	895-1810	102
Glucose 50 ml	12	820-5023	146
Trypsin edta - lypholized - 50 ml	12	820-5023	260
Iodine - 300 ml	4		112

Antibiotics:	Gibco #		US $ 500
Gentamicin 50 mg/ml	600-5750	6	
Vancomycin		6	
Nystatin	600-5325	6	
Moxalactam		6	
Cyclohexamide		6	

Miscellanecous — US $ 750

Labels

Lab markers

Calcium Alginate swabs

Tape

Glass beads

Forceps - medium pointed - 3

		Taka	Dollar
4.	Patient Hospitalization 100 days at 250 Taka/day	25,000	1,000
5.	Outpatient care	-	Nil
6.	ICDDR,B transport		500
7.	Travel and transportation of persons		
	Trip by Dr. Schachters Technician, to Dhaka and 30 days Guest house stay		2,500
8.	Transportation of things		2,500
9.	Rent, Communication and Utilities		Nil
10.	Printing and Reproduction		500
11.	Other contractual services		Nil
12.	Construction, Renovation, Alteration		Nil

Total Budget: US $ = 39,489

Conversion rate US $ = 25 Taka

B. BUDGET SUMMARY:

	US $
1. Personnel services	9,000
2. Supplies and Materials	7,005
3. Equipment	16,484
4. Patient hospitalization	1,000
5. Outpatient care	Nil
6. ICDDR,B Transport	500
7. Travel and Transportation of persons	2,500
9. Rent, Communications	Nil
10. Printing and Reporduction	500
11. Other contractual services	Nil
12. Construction, Renovation, Alterations	Nil
TOTAL	39,489

ABSTRACT SUMMARY

The purpose of this study is to determine if a particular bacteria, Chlamydia trachomatis, is associated with diarrheal illness.

1. Requirements for study population:

Three study populations will be used; (a) Surveillance patients (b) Community control patients (c) Hospitalized children with pneumonia. All three patient groups will include children, as they are likely to be at highest risk of such infection. The surveillance patients will be included as part of the study as they are a systematic sample of ICDDR,B patients on which much baseline information is already being obtained. Age and community matched non-diarrheal control patients will be necessary in order to assess if infection with Chlamydia is associated with diarrhea. Infants less than six month of age with pneumonia will be studied because they are known to be at high risk of Chlamydial infection.

2. Assessment of risk:

There is no physical, psychological, social or legal risks involved in the procedures proposed in the protocol.

3. Minimizing risk:

Not applicable (no risk involved)

4. Confidentiality:

All information collected on surveillance and control patients will be used for research purposes only.

The identity of individual patients will not be apparent in any publications generated by these studies.

5. (a) Surveillance patients having only a rectal swab or stool sample obtained are already having informed consent obtained for these procedures, and no additional informed consent will be obtained. If a conjunctival culture is to be obtained, informed consent will be obtained for this sample. Informed consent will be obtained from all non-diarrheal control patients, or their parent or guardian. Informed consent will not be obtained from patients suspected of having pneumonia due to Chlamydia. No informed consent will be obtained as the information obtained will be used in the clinical management of the patient.

(b) Information on culture results will not be made available to surveillance patients. As is true for most of the surveillance data, such information is used for study purposes only and will not immediately affect the care of such patients. Culture results on infants with pneumonia will be available to the parents of patients, and the information will be used to guide therapy.

(c) None of the proposed procedures involves potential risk to the patient.

6. Non-diarrheal community control patients will be interviewed for the purposes of this study. Interviews will take place at their residence. The interview will consist of questions similar to those asked of the surveillance patients to whom the controls will be compared. The interview should not take longer then five to ten minutes.

7. This study will not directly benefit either surveillance or community control patients. Infants with pneumonia will benefit as culture results will potentially influence their care. Society will benefit as the study might identify a potentially treatable cause of diarrhea. As no risk to patients is involved, the potential benefits will out weigh the risks.

8. Information collected on the surveillance patients will include that collected on the surveillance record sheet. Hospitalized patients with pneumonia will have information abstracted from the hospital record.

Consent form

We are trying to find out what germs are responsible for causing diarrhea in Bangladesh. As part of this work we need to examine the stool of patients who have not had diarrhea within the last two weeks. We would like to obtain a stool sample from you, as well as a rectal swab specimen. A rectal swab means putting a small cotton tipped wooden stick in the anus for a few seconds. This is not painful and no harm can come from it.

We would also like to ask you a few questions concerning the last time you had diarrhea, and also about your living circumstances.

If you agree to do this, please sign or put your thumb print below.

Informed consent!

"After listening the above information and all my questions having been answered, I accept to be included in this study. I understand that if I decide to withdraw from it, I shall not be refused adequate treatment nor be penalised in any way".

_____

Signature or thumb print

Date: _____

সম্মতি পত্র

আমরা বাংলাদেশে উদরাময় রোগ সৃষ্টিকারী জীবাণুসমূহ সনাক্ত করার চেষ্টা করিতেছি। এই কর্মসূচীর অংশ হিসাবে গত দুই সপ্তাহের মধ্যে উদরাময় রোগাক্রান্ত হয় নাই, এইরূপ রোগীদের মল পরীক্ষা করিতে হইবে। আমরা আপনার নিকট হইতে মলের নমুনা এবং উহার সংগে মলাশয়ের নমুনা (সোয়াব) গ্রহণ করিতে চাই। মলাশয়ের নমুনা (সোয়াব) অগ্রভাগ তুলায় আবৃত একটি ছোট কাঠির সাহায্যে মলদ্বার দিয়া নেওয়া হইবে। ইহা যন্ত্রণাদায়ক বা ক্ষতিকারক নয়।

ইহা ব্যাতীত আপনি শেষ কখন উদরাময় রোগাক্রান্ত ছিলেন, এবং আপনার জীবনযাত্রার অবস্থা সম্পর্কেও আপনাকে কিছু প্রশ্ন রাখিতে চাই।

আপনি যদি সম্মত হন, তবে নীচে আপনার স্বাক্ষর বা বৃদ্ধাংগুলির ছাপ প্রদান করুন।

সজ্ঞান সম্মতি :

"উপরোক্ত তথ্যসমূহ শুনিয়া এবং আমার উপস্থাপিত সকল প্রশ্নের উত্তর পাইয়া, আমি এই পর্যবেক্ষণে অংশ গ্রহণ করিতে সম্মত হইলাম। ইহাও জ্ঞাত আছি যে, যদি আমি পরবর্তিতে ইহা করিতে অস্বীকার করি, আমাকে উপযুক্ত চিকিৎসা হইতে বঞ্চিত করা হইবে না বা আমাকে কোনরূপ শাস্তি প্রদান করা হইবে না।"

স্বাক্ষর/বৃদ্ধাংগুলের ছাপ

তারিখ----------