Attachment 1. Date Dec. 4 1980

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ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator ~~Dr. B. Glass~~ Mrs. S. R. Akhtar Trainee Investigator (if any) ______

Application No. 80-046(P) Supporting Agency (if Non-ICDDR,B) ______

Title of Study Characterization of isolates of Campylobacter jejuni in Patients, Asymptomatic Carrier & animals

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

(PILOT)

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)

   Laboratory based study

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)

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

   NA

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) Pilot
   - ✓ 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
   - NA ___ 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.

S. Rukaiya Akhtar
Principal Investigator

______
Trainee

80-046(P)
Rec'd.
4.12.80

SECTION 1 - RESEARCH PROTOCOL

1. Title: Characterization of Isolates of Campylobacter jejuni from Patients, Asymptomatic Carriers and Animals

2. Principal Investigator: Mrs. S.Q. Akhtar

   Co-Investigator: Dr. R. Glass

3. Starting Date: 1.1.1981

4. Completion Date: 31.1.81

5. Total Direct Cost: $2,900

6. Availability of Funds:

7. Scientific Program Head:

This protocol has been approved by the Disease Transmission Working Group

Signature of Scientific Program Head: [signature]

Date: 4/12/1980

8. While Campylobacter jejuni is commonly isolated from patients, healthy individuals, and animals in Bangladesh, it is usually not associated with illness. We plan to characterize the isolates of Campylobacter jejuni from patients, asymptomatic carriers, and animals to determine whether new biochemical marker or toxin tests can be used to discriminate between pathogenic and non-pathogenic strains. Ten isolates of Campylobacter from diarrhea patients with no other enteric pathogens, asymptomatic carriers, and animals will be grown on plates and in broth. The growth response of these isolates will be compared for differences due to variation in carbon source of the medium. We would sonicate isolates and test the whole cell, sonicate, and filtrate of the sonicate in the rabbit ileal loop, CHO cell, infant mouse modules. fatty acid

composition of the lipopolysaccharide membrane will be examined as well.

9. REVIEW:

(a) Ethical Review Committee_____

(b) Research Review Committee_____

(c) Director_____

(d) BMRC_____

(e) Controller/Administrator_____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective: While Campylobacter jejuni is commonly isolated from patients, healthly individuals, and animals in Bangladesh, it is usually not associated with illness. We plan to study ten isolates from each of these groups using a variety of physiochemical test (e.g. toxin test, biochemical test) to identify one which could help to discriminate pathogenic from non-pathogenic strains.

2. Background: Campylobacter fetus ssp. jejuni is a newly recognized in common human enteric pathogen in the developed world. It has avoided identification for so long because culture techniques were not available to select Campylobacter from other enteric overgrowth. Because Campylobacter usually has been isolated from individuals who sought medical attention because of diarrhoea, the clinical spectrum of Campylobacter enteritis has been defined from studies of these hospital and clinic patients. In the developing countries, Campylobacter has been isolated from patients with diarrhoea but its role as a pathogen has not been well established. Bakkenheuser for example in South Africa and de Mol in Zaire showed the Campylobacter was frequently isolated from children who had no symptoms whatsoever. These findings suggests that some aspects of the epidemiology and pathogeneeity of Campylobacter infections may not be so straightforward and may differ between the developed and the developing countries.

In Bangladesh, we have found that Campylobacter infections are quite different from a similar infections in the developed world. Campylobacter is a common isolate in patients with diarrhoea and in healthy controls. We have found no distinct picture of disease in patients presenting with diarrhoea to the hospital and no clear history of diarrhoeal illness in healthly control children are cultured positive. We are left with an agent in search of a disease. There is some evidence that both human and animal strains of Campylobacter exists and that animal strains may not be pathogenic to man. Several systems using serologic markers, phage typing, and biochemical tests are being tried to distinguish subgroups of Campylobacter and to identify the human and animal strains and those which may be pathogenic for man. Todate none of these have been completely discriminating.

3. Rationale: This study is an attempt to identify of physiologic test that can discriminate between Campylobacter isolated from animals, asymptomatic carriers, and patients with diarrhoea who have no other enteric pathogens.

B. SPECIFIC AIMS

To discriminate differences in isolates with Campylobacter from patients animals, and asymptomatic carriers on the basis of 1) toxin test (rabbit ileal loop CHO cell assay, infant mouse assay) 2) growth response 3) simple biochemical assays 4) analysis of lipopolysaccharides.

C. METHODS AND PROCEDURES

Ten isolates each from asymptomatic carriers, patients with diarrhoea and no other pathogens, and animals will be studied intensively using a variety of standard microbiological procedures. These will include 1. toxin testing - (rabbit ileal loop CHO cell assay, infant mouse assay) of agar grown colonies, sonicates, filtrate of sonicates, supernatent and filtrate of broth culture. 2. growth responsive isolates 3. simple biochemical tests - each sub two H_2S production, salt toerance, temperature variation, glycine fermentation and 4. qualitative and quantitative analysis of lipopolysaccharide.

Problems can be expected in getting a heavy broth culture of Campylobacter since the organism is microaerophilic. Analysis will proceed by camparing the three groups for significant differences. Test that appear discriminating will be applied to fresh isolates from the Campylobacter protocol.

D. SIGNIFICANCE

Characterization of new tests to discriminate between strains of Campylobacter by toxigenic of physiologic chracteristic will be helpful to distinguish pathogenic and non-pathogenic strains. It would also aid clinical and epidemiologic investigation of this organism.

E. FACILITIES REQUIRED

1. Office Space : None

2. Laboratory Space : None

3. Hospital resources : None

4. Animal resources: 150 Guinea Pigs.

5. Logistic support: None

6. Major item of equipment:

   a. Gas liquid chromathography

   b. Chromatography kit

   c. Spectrophotometer

All equipments are available in the laboratory.

F. COLLABORATIVE ARRANGEMENTS

None

REFERENCES

1. Bokkenheuser VD., Richardson NJ., Bryner JH et al. Detection of enteric Campylobacter in children. J Clin Microbiol 1979, 9:227-232.

2. De Mol, P., Bosmans E. Campylobacter enteritis in Central Africa Lancet 1978, 1:604

3. Sereny, B. 1955, Experimental Shigella kerato-conjunctivitis. A preliminary report. Acta Microbiol Acad Sci Hung 2:293-296

4. Donta, S., King, M. Induction of steriodogenesis in tissue culture by cholera enterotoxin. Nature (New Biol) 243:246-247, 1974.

5. Guerrant RL., Brunton LL., Schnaitman TC., Rabhun LI., Gilman, AG. cyclone adenosine monophosphate and alteration of chinese hamster ovary cell morphology; a rapid sensitive in vitro assay for the enterotoxin of V. cholera and E. coli. Infect Immun 10:320-327, 1974.

6. Dean, AG., Ching YC., Williams RG., Harden LB: Test for E. coli enterotoxin using infant mice: application in a study of diarrhoea in children in Honolulu. J Infect Dis 125:407-411.

7. Westphal, O., Jann, K. In R.L. Whistler (ed) methods in carbohydrate chemistry vol 5 Academic press. New York 1965, p 83-85

SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

Name	Position	%Time Used	Annual Salary	Project Requirements Taka	Dollar
S.Q. Akhtar	Assistant Scientists	10%	56,016	5600	
Dr. R. Glass	Scientist				
Mr. Biren Pal		10%	15,600	1560	

2. SUPPLIES AND MATERIALS

Media, reagents and chemicals		2000
ST/LT/CHO	5000	
toxin preparation & others		

3. EQUIPMENT - None

4. PATIENTS HOSPITALIZATION - None

5. OUTPATIENT CARE - None

6. ICDDR,B TRANSPORT - None

7. TRAVEL AND TRANSPORTATION OF PERSONS - None

8. TRANSPORTATION OF THINGS - None

9. RENT, COMMUNICATION AND UTILITIES - None

10. PRINTING AND REPRODUCTION - None

11. OTHER CONTRACTUAL SERVICES - None

12. CONSTRUCTION, RENOVATION, ALTERATIONS- None

13. ANIMAL REQUIREMENT - 7 rabbits 1000

	13160	2000

Grand Total: 13160
30000

Tk. 43160