ETHICAL REVIEW COMMITTEE, ICDDR,B.

26

Principal Investigator J.N. WASSERHEIT Trainee Investigator (if any) —

Application No. 85-013P Supporting Agency (if Non-ICDDR,B) —

Title of Study Investigation of Prevalence & Etiologic Significance of ELO's in the Gastrointestinal Tract of Patients in Bangladesh

Project status:
(X) New Study (Pilot protocol)
( ) Continuation with change
No change (do not fill out rest of f

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 — NA Yes No
   (d) Sensitive questions — NA 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 — NA 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 t Committee:
   — Umbrella proposal - Initially submit overview (all other requirements be submitted with individual studies
   ✓ Protocol (Required)
   ✓ Abstract Summary (Required)
   — Statement given or read to subjects nature of study, risks, types (ions to be asked, and right to to participate or withdraw (Requ
   — Informed consent form for subject
   — Informed consent form for parent or guardian See Surveillance Protocol Comm
   ✓ 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 Ctte. 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.

Judith G. Wasserheit, MD
Principal Investigator

Trainee

85-013P
10/4/85

SECTION I - PILOT RESEARCH PROTOCOL

1. Title: Investigation of the Prevalence and Etiologic Significance of Campylobacter-like Organisms (CLO's) in the Gastrointestinal Tract of Patients in Bangladesh.

2. Principal Investigator: Judith N. Wasserheit, M.D.

   Co-Investigators: Jeffrey R. Harris, M.D.
   K. Zaman, M.B.B.S.
   M. Ansaruzzaman, M.A.
   Cynthia L. Fennell, M.T.
   Patricia A. Totten, Ph.D.

3. Starting Date: ~~January~~ May 15, 1985

4. Completion Date: ~~January~~ November 15, 1986

5. Total Direct Cost: $2926.00

6. Scientific Program Head: This protocol has been approved by the Disease Transmission Working Group.

   Signature of Scientific Program Head: Daniel R Lark
   Date: 7 March 1985

7. Abstract Summary: Over the past 15 months in the United States and Western Europe, a group of new organisms which resemble Campylobacters and which, therefore, have been called Campylobacter-like organisms (CLO's) have been identified. They are distinguished from traditional Campylobacters by their growth dynamics, microscopic and colonial morphology, biochemical and antibiotic sensitivity profiles, and DNA hybridization patterns. CLO's have been reported in patients being endoscoped for upper gastrointestinal symptoms such as dyspepsia or burping and may be associated with endoscopic or microscopic evidence of gastritis or ulcer disease. In the lower gastrointestinal tract, CLO's have been linked to a proctocolitis syndrome similar to that caused by C. jejuni. The histopathology at this site, too, is that of invasive disease.

Using minor modifications of previously employed Campylobacter culture techniques to examine the Matlab-Nayergaon diarrheal surveillance population, this study will help define the place of this new organism in diarrheal disease. Fundamental questions about the prevalence, seasonality, epidemiologic associations, clinical characteristics, and etiologic significance of CLO's remain to be answered. This study will address those questions.

8. Reviews: a) Ethical Review Committee: _____
   b) Research Review Committee: _____
   c) Director: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION:

1. Objectives: This pilot protocol will examine the prevalence, seasonality, epidemiologic associations, clinical characteristics, and etiologic significance of Campylobacter-like organisms (CLO's) in the Matlab diarrheal surveillance population. It will also investigate the patterns of other enteric organisms recovered with CLO's from these patients.

2. Background: Although gastric and fecal "spiral" bacteria have been noted intermittently for the past 80 years [1-5], it is only over the past 12 months that CLO's have been identified systematically at these sites in humans. They were first described in detail by Fennell, et al (6) from rectal swab specimens of homosexual men. Subsequently, several investigators have described CLO's in gastric biopsy specimens from patients undergoing endoscopy for gastritis, peptic ulcer disease, or other upper gastrointestinal complaints (7-11).

a) MICROBIOLOGIC CHARACTERISTICS - Microbiologically, CLO's resemble the currently recognized Campylobacter spp. in that they are microaerophilic, motile, oxidase- and catalase-positive, curved gram negative rods which cannot utilize glucose. CLO's differ from Campylobacter spp. in the following ways:

i) Growth characteristics -

CLO's grow more slowly than do Campylobacters. Plates must

therefore be held for 7 or 8 days before being considered negative (6).

Microaerophilic incubation at 35-37 C. rather than at 25 C. or at 42 C. is optimal for CLO growth (6).

ii) Microscopic and colonial morphology -

CLO's are more delicate in appearance than is C. jejuni. They have variously been reported to be slender spiral organisms 1.5-5.0um in length and 0.3-0.7um in diameter (4, 6-11) with a periodicity of 0.9-1.2 um (7,11). Although the rectal isolate characterized by Fennell, et al (6) had a single polar flagellum like traditional Campylobacters, the gastric isolates of Rollason, et al (7) had no flagellum, those of Phillips et al (11) had bipolar flagella, and those of Marshall and Warren (9) and Langenberg, et al (10) had as many as five sheathed polar flagella.

Colonies of CLO's look like miniature C. jejuni colonies. They grow from pinpoint transluscent colonies at 48 hours into small, greyish-white, nonhemolytic, flat, spreading, "wet" colonies at 72 hours (6).

iii) Biochemical and antibiotic sensitivity profile -

In contrast to C. jejuni, but like most C. fetus subspp. fetus, CLO's do not hydrolyze hippurate (6). Like other Campylobacters, the majority of Fennell et al's strains reduce nitrate (6), however three of their strains and all of Marshall and Warren's isolates (9) were reductase negative. CLO's cannot be distinguished from classical Campylobacters on the basis of H_2S production either in triple-sugar iron agar or in agar with lead acetate strips because both are negative in the former case and at least trace positive in the latter (6).

One of the most important differences between CLO's and C. jejuni is the sensitivity of the former to cephalothin (MIC's in the 4 to 64ug/ml range for most strains) (6). Cephalothin must, therefore, be deleted from the selective growth medium for recovery of CLO's. Like C. jejuni, CLO's are inhibited by 30ug naladixic acid discs (6).

iv) DNA homology -

Hybridization did not occur between the CLO strains of Fennell, et al (6) and any of their reference Campylobacter spp. Strains of each of the three types of CLO's described by them did, however, exhibit homology with itself and with other strains of the same type.

b) CLINICAL MANIFESTATIONS - Both upper and lower gastrointestinal tract syndromes have been linked preliminarily with CLO infection:

i) Upper gastrointestinal tract -

Several authors have reported culture and/or silver stain evidence of CLO's in the stomachs of patients with ill-defined complaints such as dyspepsia or burping (8-10) or with endoscopic evidence of peptic ulcer disease or gastritis (7-11, 13-14). These studies have, however, demonstrated no eradication of bacteria following ulcer healing. CLO's have also been identified in 8 (50%) of 16 symptomatic patients with normal endoscopic examinations (9) and in 6 (25%) of 25 asymptomatic patients (10). In both studies, however, histologic evidence of gastritis was subsequently documented in almost all culture-positive patients (see below: histopathologic associations).

ii) Lower gastrointestinal tract -

Among homosexual patients studied by Quinn, et al (12), the presenting lower gastrointestinal tract symptoms of infection with CLO's were similar to those caused by infection with C. jejuni. They constituted a proctocolitis-like syndrome of bloody diarrhea, abdominal cramps, tenesmus, and hematochezia with or without anal discharge. In two of the 13 symptomatic patients from whom CLO's were identified as the sole pathogen, fever was also noted. CLO bacteremia resembling that seen in patients with C. fetus subspp. fetus infection was documented in two other patients from Texas (15). In Quinn's series (12), 6 (19%) of the 32 homosexual patients with CLO infection and 2 (17%) of the 12 homosexual patients with C. jejuni infection denied gastrointestinal symptoms. Neither CLO's nor other Campylobacters were recovered from asymptomatic heterosexuals. Symptomatic heterosexual patients were not evaluated in that study.

At sigmoidoscopy, focal and diffuse mucosal friability and ulceration were seen in symptomatic patients (12). These lesions extended beyond 15 cm. in 3 of 4 patients in whom only CLO infection was detected.

c) HISTOPATHOLOGIC AND CYTOPATHOLOGIC ASSOCIATIONS - Both in the upper and lower gastrointestinal tract studies to date are more consistent with an invasive than with a toxin-mediated pathophysiology:

i) Upper gastrointestinal tract -

A strong association between the presence of CLO's and the presence of histologic evidence of gastritis has been reported in each of the five studies in which this relationship has been examined (7-10, 14). The

association persists among asymptomatic patients (10) and among patients without concomittant peptic ulcer disease (8,9). Although endoscopic evidence of peptic ulcer disease was associated with the presence of CLO's in 5 of 7 studies (8-11, 13), in two of these studies histopathologic examination revealed concomittant microscopic gastritis or gastric metaplasia (11, 13). Two additional studies (7, 14) argue against an association between ulcers and CLO's.

ii) Lower gastrointestinal tract -

Rectal biopsies from patients infected with CLO's and with C. jejuni both showed polymorphonuclear leukocyte (WBC) infiltration of the lamina propria with or without crypt abscess formation (12). Gram stains of rectal swabs from symptomatic patients with CLO infection had an average of 8.2 + 3.6 WBC per 1000X field while those from asymptomatic patients had and average of 0.9 + 0.5 WBC per 1000X field (12).

d) EPIDEMIOLOGIC PATTERNS - To date, no population-based studies of the point prevalence, incidence, or seasonality of upper or lower gastrointestinal CLO infections have been published. The reservoirs of CLO infection also have not been established. Campylobacters are commonly encountered both as commensals and as pathogens in domestic pets and in farm animals (16). Similarly, CLO's have been recovered from cats, dogs, seagulls, hogs, monkeys, and ocelots (4). The human mouth is also a reservoir for commensal Campylobacters (9), but CLO's have not been studied in this mileau.

The mode of spread of CLO infection is not yet understood. The

fecal-oral route proposed in homosexual men (12) may well not explain transmission in heterosexual patients.

3. Rationale: In light of the fact that a bacterial, viral, or parasitic pathogen could be identified in only 56% of Matlab patients with diarrhea (17) and 66% of Dhaka station patients with diarrhea (18), ongoing research into new gastrointestinal pathogens is mandatory. CLO's are now taking their place as new gastrointestinal pathogens in developed countries and, due to their probable animal reservoir, are likely candidates for disease in Bangladesh.

Prior studies of C. jejuni in both rural and urban Bangladesh (19, 20) found frequent infection both in diarrhea patients and in healthy controls which decreased with increasing age. These studies, however, employed culture conditions which would have precluded recovery of CLO's. By minor modifications of previously employed Campylobacter culture techniques, we will be able to assess the role of CLO's in gastrointestinal disease in Bangladesh.

B. SPECIFIC AIMS:

1. To examine the age-specific prevalence, and seasonality of CLO's in Matlab field station.

2. To establish the clinical manifestations and etiologic significance of CLO's in a third world setting and to compare them to those in a developed country.

3. To evaluate the patterns of co-infecting organisms detected with CLO's in the Matlab population.

C. MATERIALS AND METHODS:

Rectal swabs from patients in the Matlab surveillance study will be examined for CLO's in the following manner:

1. Culture techniques: Rectal swabs will be inoculated on to a selective Campylobacter agar consisting of brucella agar base (Difco Laboratories, Detroit; 42 gm with 1000ml distilled water) which has been autoclaved for 15 minutes at 121 C. and 15 lbs pressure, cooled in a water bath to 50 C., and enriched with 100ml of sterile, defibrinated sheep blood and two vials of Oxoid Campylobacter antibiotic supplement (order no. SR69). This supplement contains vancomycin (10 ug/ml), polymyxin B (2.5 IU/ml), and trimethoprim (5.0 ug/ml). If fungal overgrowth becomes a problem, amphotericin B (2.0 ug/ml) will also be added to the medium. Subculture agar will be made using the same recipe, but deleting SR69 antibiotic supplement. If absolutely necessary, swabs may be held in modified Cary-Blair transport medium at 4 C. for up to 24 hours before inoculation of plates.

Within one hour of inoculation of selective isolation medium, plates will be incubated microaerophilically in BBL GasPak jars with the catalyst removed. A maximum of 8 plates/GasPak 100 jar or 24 plates/GasPak 150 jar will be set to optimize the microaerophilic environment. One GasPak H + CO envelope with 10 ml of water will be placed in each 100 jar (or 3 envelopes

in each 150 jar). If GasPak envelopes are unavailable, incubation may be attempted using CampyPak envelopes, but the former are preferable. Jars must be incubated at 35-37 C. and should be examined every 48 hours for 7 to 8 days.

2. Identification tests: If typical colonial morphology and gram stain characteristics are observed (see above, Background), the following identification procedures will be performed:

a) OXIDASE & CATALASE TESTING - by standard methods (6). These tests should both be positive if CLO's are present.

b) HIPPURATE HYDROLYSIS - using ninhydrin (21). CLO's will be negative in this test.

c) NITRATE REDUCTION - using brain-heart infusion broth with 0.2% KNO_3 and 0.3% agar by standard methods (22). Although Fennell's type 1 CLO's did reduce nitrate, types 2 and 3 did not (6).

d) MOTILITY - in trypticase soy broth (or other broth medium) under darkfield microscopy. The slide will be examined for characteristic spiraling and back-and-forth darting motion (6). If activity persists following addition of cholera antiserum, distilled water will be added and the test will be considered positive if the motion is extinguished. The predictive value of a darkfield examination performed in this manner in conjunction with stool microscopy which is positive for leukocytes and

erythrocytes has been demonstrated to be approximately 90% for C. jejuni (5).

e) SENSITIVITY AND TEMPERATURE TESTING - Colonies found to be consistent with CLO's by the above criteria will be subcultured to 5 plates and incubated as follows:

i) Plate no. 1 - at 37 C. after placing a 30 ug naladixic acid (NA) disc and a 30 ug cefalozin (CZ) disc.

ii) Plate no. 2 - at 37 C. after placing a 5 ug rifampin (RA) disc.

iii) Plate nos. 3-5 - at 25 C., 37 C., and 42 C., respectively (without discs).

Antibiotic susceptibility patterns will be interpreted as follows:

i) NA sensitive - 'C' types 1 & 2.

ii) NA intermediate or sensitive & CZ resistant - C. jejuni, C. coli, NA resistant and CLO type 3 NA sensitive.

iii) NA resistant & CZ sensitive - C. fetus subssp fetus, C. fetus subsp venerealis, C. faecalis.

Temperature susceptibility patterns will be enterpreted as follows:

i) Growth at 25 C. & 37 C. - C. fetus subspp fetus & C. fetus subsp venerealis.

ii) Growth at 37 C. & 42 C. - C. jejuni, C. coli, C. faecalis, and CLO type 3 (light growth only).

iii) Growth at 37 C. only - CLO types 1 & 2.

3. Microscopy: Stool character, pH, and cell counts (WBC, RBC, and macrophage) will be recorded as per the surveillance study protocol. Using a

0.8% carbol fuschin counterstain instead of safranin, gram stains of suspicious colonies will be examined and saved.

4. Confirmatory procedures: Isolates consistent with CLO's by the above criteria will be lyophilized and sent for confirmatory studies to Cynthia Fennell, M.T. at the University of Washington in Seattle, Washington. Stool specimens will also be spotted on nylon membrane filters and sent to Patricia Totten, Ph.D. at the University of Washington for DNA probe analysis. In addition, Dr. Totten will receive nitrocellulose filters on which suspected CLO's have been spotted (as 10 aliquots of a 3 McFarland suspension of the organism in 50% inactivated horse serum and 50% trypticase soy broth). Using her rapid Taxonomic Spot Blot Test (23) the samples will be compared with the currently recognized CLO types.

5. Epidemiologic considerations: If CLO's are recovered from the Matlab diarrheal surveillance population, the surveillance questionaire, the standard laboratory examinations and the CLO studies discussed above will be obtained from sex- and age-matched (<18 mo.: ±1 yr. for >18 mo. & <5 yr.: ±2 yr. for >5 yr. & < 20 yr.; ±5 yr. for >20 yr.) controls who have not had diarrhea within the past 14 days. These controls will be selected from the first eligible house in the next bari as specified in the DSS census.

D. SIGNIFICANCE:

This study will be the first in Bangladesh to examine the role of CLO's, a newly recognized gastrointestinal pathogen in other parts of the world. Because of the vast numbers of patients with diarrheal diseases seen at

ICDDR-B, if CLO's are found in Bangladesh, this study will help the international medical community define the spectrum of disease caused by this organism.

E. FACILITIES REQUIRED:

No new facilities will be required.

F. COLLABORATIVE ARRANGEMENTS:

Ms. Cynthia Fennell and Dr. Patricia Totten of the University of Washington in Seattle, Washington will collaborate on this project and will perform confirmatory studies on the CLO isolates. This work will be done in Seattle.

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ABSTRACT SUMMARY

This study is designed to examine the role of a new pathogen, Campylobacter-like organisms (CLO's), in gastrointestinal disease in Bangladesh. In doing so, it will also further define the epidemiologic, clinical, and microbiologic spectrum of disease produced by this bacterium.

1. Subject population - the Matlab-Nayergaon surveillance population, a systematic sampling of DSS patients presenting for treatment of diarrhea. This group will include children because they constitute the majority of patients presenting with diarrhea. In these cases, verbal consent will be obtained from parents or guardians as per the surveillance protocol.

2. Potential risks - none. The study will require only additional microbiologic laboratory work on specimens which are already being collected for the surveillance protocol.

3. Methods for protecting against or minimizing potential risks - not applicable. No risks are involved.

4. Methods for safeguarding confidentiality - as in the surveillance protocol, confidentiality will be maintained by assigning each patient a unique number by which he/she will be identified in the data forms.

5. a)Waiver of signed consent - as in the surveillance protocol, verbal rather than signed, written consent will be obtained because no risks to the patient are involved. Instead, participation will provide the clinician with additional information which might aid in patient care.

b)Withholding of information - no information will be withheld from the patient.

c)Compensation and/or treatment for risks - not applicable. No risks are involved.

6. Interview procedures - each patient (or parent/guardian) will be interviewed for about 5 minutes after urgent patient-care decisions have been made.

7. Potential benefits - for individual patients, the availability of additional laboratory data may result in beneficial theraputic decisions. Longterm, should CLO's prove to play a significant role in enteric disease in Bangladesh, this study may allow us to reduce the number of cases of diarrhea in which no etiologic agent is identified. By characterizing a new organism, it may provide a scientific approach to a broader spectrum of disease. It may also be the first of several studies to link upper and lower gastrointestinal symptoms of infectious etiology.

8. Required specimens - the same stool and rectal swab specimens required by the surveillance study will be used in this study.

SECTION III: BUDGET

A. DETAILED BUDGET:

1. Personnel services:

Name	Position	% Time	Salary/yr Taka	Dollar
J.N. Wasserheit	Principle Investigator	2	---	620.00
J.R. Harris	Co-Investigator	NA	(Covered by vaccine trial surveillance study)	
K. Zaman	Co-Investigator	5	6410	---
M. Ansaruzzaman	Co-Investigator	5	3600	---
C.L. Fennell	Co-Investigator	NA	(U. of Washington funded)	
P.A. Totten	Co-Investigator	NA	(U. of Washington funded)	
(Subtotal			10010	620.00)

2. Supplies and materials:

Item	Unit cost-$	Number	Total cost-$
Skirrow's antibiotic supplement (SR69)	30.25	7	211.75
Antibiotic susceptibility testing discs			
30ug cephalothin	20.85	2	41.70
30ug naladixic acid	20.85	2	41.70
5ug rifampin	24.55	2	49.10
Triketohydrindene crystal hydrate	16.15	1	16.15
Hippuric acid sodium	38.20	1	38.20
Oxidase test reagent	20.70	10	207.00
(Subtotal			605.60)

3. Equipment: None ---

4. Patient hospitalization: None ---

5. Outpatient care: None ---

6. ICDDR-B Transport: None ---

7. Travel and transportation of persons: 1200.00
One round-trip air ticket between Seattle, Washington and Dhaka for Dr. Wasserheit to work with Ms. Fennell and Dr. Totten on collaborative parts of study

8. Transportation of things: 100.00

Transport of lyophilized specimens, nylon membrane filters, and nitrocelllulose filters from Dhaka to Seattle

9. Rent, communications, and utilities: None ---

10. Information services: None ---

11. Printing and reproduction: None ---

12. Other contractual services: None ---

13. Construction, renovation, alterations: None ---

B. BUDGET SUMMARY:

		Dollars/yr*
1.	Personnel services	1020.40
2.	Supplies and materials	605.60
3.	Equipment	---
4.	Patient hospitalization	---
5.	Outpatient care	---
6.	ICDDR-B transport	---
7.	Travel and transport of persons	1200.00
8.	Transport of things	100.00
9.	Rent, communications, and utilities	---
10.	Information services	---
11.	Printing and reproduction	---
12.	Other contractual services	---
13.	Construction, renovation, alterations	---
	Total	2926.00

* Conversion from taka to dollars is at the rate of 25 taka to 1 dollar.

C. The consent forms used in the surveillance protocol will also be used in this investigation.