

ETHICAL REVIEW COMMITTEE, ICDDR,B.

222

Principal Investigator DR. A. R. SAMAD Trainee Investigator (if any) _____

Application No. 80-037(P) Supporting Agency (if Non-ICDDR,B) _____

Title of Study Identification, characterization Project status:
Description of Clinical Features of Yersinia () New Study
Anterocalitica Infection in Patients with Diarrhoea () 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 N.A

- Are subjects clearly informed about:
- (a) Nature and purposes of study Yes No N.A
 - (b) Procedures to be followed including alternatives used Yes No N.A
 - (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 N.A
 - (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 N.A

- 5. Will signed consent form be required:
 - (a) From subjects Yes No N.A
 - (b) From parent or guardian (if subjects are minors) Yes No N.A
- 6. Will precautions be taken to protect anonymity of subjects Yes No N.A
- 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)
 - N.A. Informed consent form for subjects
 - N.A. Informed consent form for parent or guardian
 - Procedure for maintaining confidentiality

- N.A. 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.

A. R. Samad

Principal Investigator

Trainee

80-037(P)

29 SEP REC'D

1. Name of Investigator : Dr. Aziz, R. Samadi & Dr.K. Watchsmuth
2. PRELIMINARY TITLE OF THE Protocol: Identification, characterization and description of clinical features of
3. Program Area : Yersinia Enterocolitica infection in patients with Diarrhoea.

PRELIMINARY
BUDGET REQUEST FORMAT
FY.- 81

(November-December 1980)

	<u>Existing^{1/} Resources Facilities</u>	<u>Additional^{11/} Requirement</u>	<u>Total</u>
1. Personnel Services	US \$ 1,840.00	—	
2. Travel	—	—	
3. Communication, Rent & Utilities	—	—	
4. Supplies & Materials	200.00	450.00	
5. Computer Services	—	—	
6. Printing & Reproduction	—	35.00	
7. Equipment	—	—	
8. Other Cost (Transportation of Things and Miscillaneous)	—	60.00	
	Total: US\$ 2040.00	545.00	
	Grand Total : US\$ = 2585.00		

1/ Facilities, equipment, personnel etc., which are already available within the Centre.

11/ Any new personnel, equipment, supplies, services etc., which must be financed.

SECTION I - LIMITED STUDY

1. Title: Identification, Characterization and description of clinical features of Yersinia Enterocolitica infection in patients with Diarrhoea.
2. Principle Investigator: Dr. Aziz R. Samadi
3. Co-Investigator: Dr. K. Watchsmuth & Md. Imdadul Huq
4. Starting Date: 5th November, 1980.
5. Completion Date: 30th December, 1980.
6. Total Direct Cost:
7. Scientific Program Head:

This protocol has been approved by the Working Group _____

A. Molla

* Signature of ^{the} Scientific Head: *K. M. S. Aziz*

8. Abstract Summary:

During the course of last decade Yersinia Enterocolitica has been isolated from man and animals and identified as an enteropathogen which causes diarrhoea that mimics diarrhoeas of ETEC, shigella and salmonella. This study is an attempt to identify and characterise Yersinia Enterocolitica as a probable enteropathogen by a new method of plasmids profile determination. Not only that, the technique which is used for characterization of Yersinia Enterocolitica also will be established as a new and efficient method for determination of pathogenicity of other microorganism existing in Bangladesh. The clinical feature of the disease also will be described.

* This signature implies that the Scientific Program Head takes responsibility for the planning, execution and budget for this particular protocol.

SECTION II - PLAN OF LIMITED STUDY

A. INTRODUCTION:

1. Objectives:

- a. Identification of *Yersinia Enterocolitica* in patients suffering from diarrhoea by using different well identified techniques.
- b. To introduce new method at ICDDR,B for characterization of plasmids of *Yersinia Enterocolitica* as well as other enteropathogens.
- c. To describe the clinical feature of *Yersinia Enterocolitica* infection in patients with diarrhoea.

2. Background Information:

Although *Yersinia Enterocolitica* was first isolated from human in United States In 1963 (1), its bacteriological characteristics and clinical features in human and animal have become better known over last decade when *Yersinia Enterocolitica* infections in human have been reported with increasing frequency from different parts of the world (2). While the number of the countries at present reporting infection due to *Yersinia Enterocolitica* is considerable, the actual incidence of the cases and distribution of the different types of strains vary greatly. Although *Yersinia Enterocolitica* has been isolated most often in the cooler climates, it also has been reported that the microorganism was found in tropical areas such as Algeria, South Africa and Zaire (3).

As the source of infection is concerned, *Yersinia Enterocolitica* has been traced to a wide variety of environmental sources. The organism has been recovered from wild and domestic animals (4), raw milk (5) and water supplies (6). The theory that animals are important reservoirs of infection is reinforced by recent work with experimentally infected mice, demonstrating fecal excretion of pathogenic strains of *Yersinia Enterocolitica* up to four months after the initial infections (7). The possibility of fecal oral spread from animal to man or man to man seems likely. Person to person transmission has been strongly suggested by the description of a hospital outbreak in Finland, where staff members became ill after caring for a young patient with gastroenteritis (8).

Studies of the pathogenesis of acute gastroenteritis by *Yersinia Enterocolitica* have now revealed that both invasive and toxigenic mechanisms are involved. Some strains of *Yersinia Enterocolitica* have been shown (i) to produce a heat stable enterotoxin similar to that of enterotoxigenic *E. coli* (ETEC) and (ii) to invade host tissue in a variety of animal models (9).

The clinical aspects of *Yersinia Enterocolitica* was comprehensively reviewed by Kohl (10) who suggested that *Yersinia Enterocolitica* infection in children under the age of five years virtually is indistinguishable from gastroenteritis caused by other invasive enteric pathogens including salmonella, shigella, ETEC and entamoeba histolytica. Polymorphonuclear-neutrophils have been found in stool specimens and may be helpful in narrowing differential diagnosis. Fever, vomiting and/or abdominal pain occur in 60-80 per cent of the patients. Blood in stool occurs in less than 10% of the patients studied. Some patients have profuse watery diarrhoea caused by toxin-producing bacteria. The duration of the disease is generally for two to three weeks but, less frequently, chronic diarrhoea lasting for several months. In children over five years of age abdominal pain is a common presenting complaint and the symptom complex may mimic that of acute appendicitis. Findings at surgery generally include mesentric lymph adenitis or terminal ileitis. Cutaneous manifestations of *Yersinia Enterocolitica* that occur infrequently in children include erythema nodosum, erythematous maculopapular rash and erythematous multiform-like eruption. Arthritis also can occur in adults. Septecemia also has been reported in malnourished and healthy persons.

In adults in addition to fever, diarrhoea and abdominal pain nonsuppurative polyarthrititis or arthralgia occur much more frequently.

In Bangladesh Dr. R. Black unsuccessfully attempted to isolate *Yersinia Enterocolitica*. His failure to isolate *Yersinia Enterocolitica* might have been related to criteria for selection of the cases for study (11).

Recently Dr. K. Watchsmuth of CDC, U.S.A. has shown interest and suggested to determine the incidence of *Yersinia Enterocolitica* infection in Dacca in relatively cooler autumn months (12). If the occurrence of *Yersinia Enterocolitica* is rare or nonexistent in Dacca, it would be possible to use this type of plasmid marker approach to pathogenicity of the organisms known to occur in Dacca.

3. Rationale:

This pilot project concerning isolation, identification and characterization of *Yersinia Enterocolitica* will allow us to study a new pathogen causing diarrhoea in man and wide variety of animal. This also will help us in establishing the agarose gel electrophoretis method for the characterization of the plasmids in *Yersinia Enterocolitica* as well as in different enteric pathogens which will indicate its probable pathogenicity and toxicity. This may lead to a full pledged protocol to characterise the plasmids in the resistant enteric bacteria isolated from people suffering from diarrhoea.

B. SPECIFIC AIMS:

1. Isolation, identification and characterization of *Yersinia Enterocolitica* from patients admitted to the hospital with diarrhoea.
2. Introduction of a new efficient method for evaluation of pathogenicity and toxigenicity of *Yersinia Enterocolitica* and other enteric pathogens found in Bangladesh by characterizing plasmid in those organisms.
3. To use the same method for evaluation of antibiotic resistant strains of other microorganism.
4. To describe the clinical feature in different age group of patients in Dacca.

C. METHODS AND MATERIALS:

About two hundred patients of different age admitted in the hospital or treatment centre will form the subject of this study. The criteria for selection of the cases are based on: (1) patients suffering from diarrhoea, fever and abdominal pain. (2) Duration of diarrhoea should not exceed one week prior to admission. (3) The patients should not have received any antibiotics before admission. Medical history and physical examination will be performed on admission (Annex 1). The first stool specimen will be collected for laboratory examination.

Stool will be plated on to MacConkey agar and be enriched in broth in cold at 4°C. The plates will be incubated at 26°C (RT) as well as 37°C. The cold enriched broth will be subcultured after 48 hours and 7 days. On to MacConkey agar. Stool will also be plated onto TTCA, SS as well as enriched into Bile Peptone (tellurite). Stool from children under 5 will be looked for Rotavirus.

Plasmid DNA isolation from the bacteria under study will be done by SDS lysis using modified Hirt procedure. Plasmid DNA isolation can also be made by using Triton lysis procedure of Hershfield. The isolated plasmid DNA can be passed through vertical slab gel agarose electrophoresis system and after proper straining can be viewed with U.V. transillumination. A photographic image of the strained gel is more superior as it can actually detect small amounts of DNA not seen by direct viewing with U.V. transillumination.

D. SIGNIFICANCE:

While *Yersinia Enterocolitica* is a known pathogen in developed countries, its role in Bangladesh is not known. This limited study will enable us to identify its existence in Dacca and to characterize its pathogenicity by plasmid profile determination. The same technique of agrose gel electrophoresis which is used for characterization of *Yersinia Enterocolitica* also can be used as an established method in ICDDR,B for evaluation of pathogenicity of other existing enteropathogens as well as for study of antibiotic resistant microorganism.

FACILITIES:

1. Office Space: No additional space.
2. Laboratory Space: Some freezer space for storage of specimens and working space for two persons are required (already available).
3. Hospital Resources: No additional hospital resources are required.
4. Animal Resources: Nil.
5. Logistic support: Personnel and a lab technician assigned to work with the study. These are included in the budget.
6. Major Items of Equipment: Included in the budget.

REFERENCES

1. Gilbert, R. Interesting cases and unusual specimens. Annual Report of the Division of Laboratories and Research, Albany, P57, 1933.
2. Botton, E.J. *Yersinia enterocolitidis*: a panoramic view of a charismatic microorganism. CRC Crit. Rev. Microbiol. 5:211-241, 1977.
3. WHO Chronicle, 30: 494-496, 1976.
4. Hubert, W.T., *Yersiniosis* in mammals and birds in United States: Case Report Review. Am S. Trop. Med. Hyg., 21:458, 1972.
5. Schiemann, D.A. and Toma, S. Isolation of *Yersinia enterocolitica* from raw milk. Appl. Environ. Microbiol 35: 54, 1978.
6. Highsmilk, A.K., Feeley, J.C. Skalty, P., et al. Isolation of *Yersinia enterocolitica* from well water and growth in distilled water. Appl. Environ. Microbiol 34:745, 1977.
7. Ricciardi, I.D., Pearson, A.D., Suckle, W.G. et al. Longerm fecal excretion and resistance induced in mice infected with *Yersinia enterocolitica*. Infect. Immun., 21:342, 1978.
8. Tolvanen, P., Alkonen, L., Toivane, A., et al. Hospital outbreak of *Yersinia enterocolitica* infection. Lancet, 1: 801, 1973.
9. Gemski, P., Lazerve, J.R. and Casey, T. Plasmid Associated with pathogenicity and calcium dependency of *Yersinia enterocolitica*. Infect. Immun. 27: 682-685, 1980.
10. Khol, S. *Yersinia enterocolitica* infection in children. Ped. Clin. Nor. Am. Vol 26 No.2: 433-443, 1979.
11. Greenough W.B. Personal Communication, 1980.
12. Watchsmuth, K. Proposal for short-term Research Project. CDC. 1980.

SECTION III - BUDGET

1. <u>Personnel</u>	Effort	Annual salary	<u>Project Requirement</u>	
			<u>Taka</u>	<u>Dollar</u>
Dr. Samadi	P.I. 20%	\$ 36000		US \$ 1200.00
Dr. Watchsmuth	P.I. 100%			
Mr. I. Huq	C.I. 20%	Tk. 140,280	Tk.4676.00	
Lab Technician	100%	Tk. 29,655	Tk.4940.00	
2. A. <u>Supplies and Materials</u>				
	No. of culture samples 200 (Plating & Enrichment)		Tk.3000.00	
B. <u>Chemicals</u>				
				US \$ 450.00
3. A. <u>Equipment:</u>				
	a) Vertical get slab tank with spares			
	b) Micropipettes			
4. <u>Patient Hospitalization:</u> None				
5. <u>Out Patient Care:</u> None				
6. <u>CRL Transport:</u>				
	100 miles of inland travel		Tk. 900.00	
7. <u>Travel & Transportation:</u>				
8. <u>Transportation of Things:</u>				
9. <u>Rent Communication & Utilities:</u>				
	<u>Guest House accommodation</u> for the P.I. 2 months			

10. Printing & Reproduction: Tk. 500.00

11. Other Contractual Services: None

12. Construction, Renovation, Alteration: None

Total	Tk. 14016 (US \$935)	US\$ 1650
Grand Total		US\$ 2585

SECTION IV

ABSTRACT SUMMARY

During the course of last decade *Yersinia Enterocolitica* has been isolated from man and animals and identified as an enteropathogen which causes diarrhoea that mimics diarrhoeas of ETEC, shigella and salmonella. This study is an attempt to identify and characterise *Yersinia Enterocolitica* as a probable enteropathogen by a new method of plasmids profile determination. Not only that, the technique which is used for characterization of *Yersinia Enterocolitica* also will be established as a new and efficient method for determination of pathogenicity of other microorganism existing in Bangladesh. The clinical feature of the disease also will be described.

1. Population:

About two hundred stool samples will be collected from patients with diarrhoea.

2. Potential risks:

There is no risk at all to this study since no extra interview or medical procedures are performed.

3. Procedures for Minimizing Risks:

There are no risks, so this section is not relevant.

4. Safeguarding Confidentiality:

Confidentiality of the data collected will be insured. Patient names will not be used in analysis of data. In stool specimens only hospital number with serial number of study will be used to identify the patient.

5. Informed Consent:

A signed consent will not be used since no interview or medical procedure except routine medical history is taken, routine physical examination is performed and stool specimen is examined.

6. Interview Information:

There is no need for interview. Although the study require use of the hospital records, a clinical study form has been prepared as a guide line (Annex 1) in order to insure that complete medical entry and physical examination are performed.

7. Potential Benefits to the Subjects

Individual patients with diarrhoea will benefit in that the cause of their illness will be found. The health authority will be made aware of probable existence of pathogenic yersinia enterocolitica in order suitable steps to be taken to prevent the disease in the community.

CLINICAL FORM

FO YERSINIA ENTEROCULITICA INFECTION

LIMITED STUDY

Hospital Number.....

Study Number.....

A. Personal History:

Age

Sex

Address

B. Medical History:

Duration in days (prior to admission)

Fever

Diarrhoea

Vomiting

Abdominal Pain

Antibiotic Received

Frequency of diarrhoea in past 24 hours

Frequency of vomiting in past 24 hours

C. Physical Examination:

Pulse

Respiration

Blood Pressure.....

Temp.....

Mucous Membrane

Skin Turgor

Fontanel(in infants)

Eyes

CNS :

Irritable.....

Turpur

Semicomalne

Chest:

Abdomen :.....

Skin Rash (type) :

Arthralgia

Polyarthritis

Schock

Specimen of Stool: Date & time collected

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