Attachment 1.

Date 22.10.81

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Prof. S. C. Sanyal

Trainee Investigator (if any)

Application No. 81-096

Supporting Agency (if Non-ICDDR,B)

Title of Study Studies on the Pathogenic Mechanisms of Campylobacter fetus ssp. jejuni isolated in Bangladesh and their role in the aetiology of Diarrhoea

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 NA
   (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 NA
   (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
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.

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

Principal Investigator

Trainee

SECTION I - RESEARCH PROTOCOL

1. Title : Studies on the Pathogenic Mechanisms of Campylobacter fetus ssp. jejuni isolated in Bangladesh and their role in the aetiology of Diarrhoea.

2. Principal Investigator : Prof. Suhas C. Sanyal, M.D., Ph.D.

3. Starting Date : November 1, 1981

4. Completion Date : October 31, 1982

5. Total Direct Cost : U.S.$ 57,000.00

6. Availability of Fund :

7. Scientific Program Head :

The protocol has been approved by the Disease Transmission Working Group.

Signature of the Scientific Program Head : _____

Date : 29/10/81

8. Abstract Summary:

Campylobacter fetus ssp. jejuni has been implicated on the basis of thorough clinico-epidemiological eividence as an important aetiological agent of diarrhoeal syndromes in man in the developed world, although very little is known about its pathogenic mechanisms. Studies conducted at ICDDR,B indicate that the isolation rate of this organism from both diarrhoeal and non-diarrhoeal individuals is quite high and almost equal in Bangladesh whereas in the other parts of the world the ratio is about 5:1. Moreover, a variety of animals and poultry birds do carry C. fetus ssp. jejuni. In the developing countries including Bangladesh man and domestic animals and poultry birds usually live in close association in rural areas and thus increase the possibility of cross infections amongst them.

2* Title : Studies on the Pathogenic Mechanisms of Campylobacter fetus ssp. jejuni isolated in Bangladesh and their role in the the aetiology of Diarrhoea.

Sub-Title and Inyestigators : Studies on enterotoxicity, enteroinvasion and serologic response of C. fetus ssp. jejuni - by Prof. Suhas C. Sanyal and Dr. Marc Streulens.

Biotyping and pathogenicity of C. fetus ssp. jejuni in chick embryo.
by Dr. S.Q. Akhtar

Serotyping of C. fetus ssp. jejuni
by Dr. M.I. Huq

Epidemiological studies of C. fetus ssp. jejuni.
by Dr. Roger Glass

Studies of the clinical profile of a limited number of patients may elucidate the disease spectrum caused by C. jejuni in Bangladesh. The proposed comprehensive experimental studies using strains isolated from severely ill patients, the diarrhoeal episodes of whom are clearly due to them to be compared with healthy human and animal isolates aim at clarifying the role of this organism in the aetiology of diarrhoea in Bangladesh. As the pathogenic mechanism of C. fetus ssp. jejuni is ill understood finding out suitable models will elucidate whether this organism elaborates either any type of enterotoxin/s or cytoxin/s, and if so, the mode of action of the toxin/s. These experimental studies will also clarify whether this organisms is capable of invading intestinal mucosal cells and/or cross the mucosal barrier and invade the blood tissue. The proposed serological studies by agglutination, complement fixation, bactericidal and immunofluorecence techniques using reference and/or homologous strains in acute diarrhoea or dysenteric patients to be compared with health individuals will provide evidence about the role of the organisms in the aetiology of these diseases in this country and may clarify the reasons for high and almost equal prevalence of them in diarrhoeal and non-diarrhoeal populations. Establishment of the passive haemagglutination or slide agglutination techniques for serotyping and the possible development of a biotyping scheme for C. fetus ssp. jejuni strains will be of immense help in epidemiological studies. These may also be of some aid in distinguishing human and animals strains; further the biotyping may explore the possibility of existence of any correlation between pathogenic and nonpathogenic strains. If some consistent and specific markers are obtained, the possibility of cross infections between man and animal may be explored through a well-designed community based epidemiological study.

9. Reviews:

a. Ethical Review Committee : _____

b. Research Review Committee : _____

c. Director : _____

d. BMRC : _____

e. Controller/Administrator : _____

SECTION II - RESEARCH PLANT

A. INTRODUCTION

1. Objectives:

i) To look for an animal or tissue culture model for detection any virulence factor of C. jejuni strain isolated from enterities patients.

ii) To screen isolates from cases of acute watery diarrhoea and invasive enterocolities from whom C. jejuni strains are isolated as the sole pathogen to be compared with isolates from healthy individuals, domestic animals and poultry birds.

iii) To establish serotyping and biotyping schemes to delineate the distinctive characterstics, if there is any, amongst isolates from cases, healthy individuals and animals.

vi) To test the serologics responses to reference or homologous antigens in diarrhoeal cases and healthy individuals with and without C. jejuni infection to establish the aetiologic role of these organisms.

v) Oncea a consistent marker for specific strains is obtained an attempt will be made through a carefully designed epidemiologic study in a community to establish cross infections of C. jejuni between man and animals.

vi) To define the desease spectrum caused by C. jejuni in Bangladesh.

2. Background:

An increasing number of reports from a large number of countries have linked a diarrhoeal syndrome in man with infection by

Campylobacter fetus ssp. jejuni (King, 1957; Wheeler and Borchers, 1961; Dekeyser et al, 1972; Cooper and Slee, 1971; Butzler et al, 1973; Skirrow, 1977; Bruce et al, 1977; Severin, 1978; Lindquist et al, 1978; Blaser et al, 1979; Steele and McDermott, 1978; Karmali and Fleming, 1979; De Mol and Bosmans, (1978),Guerrant et al, 1978; Bokkenheuser et al, 1978; McGechie, 1978; quoted by Butzler and Skirrow,1979; Maki, M. et al, 1979; Itoh et al, 1980; Ringert et al, 1980; Blaser et al, 1980; Blaser et al, 1981; Glass et al, 1981). This organism may indeed be one of the most common bacterial agent of infectious diarrhoea in man (Prescott and Karmali, 1978;). Campylobacter fetus ssp. jejuni has been implicated as a causative agent of enteritis based on the following criteria:

a. High degree of correlation between the presence of this organism in the faeces and clinical enteritis.

b. The organisms have been isolated from the blood of patients with enteritis as well as from both stool and blood in some cases.

c. Patients were demonstrated in certain cases to develop high titres of specific antibody to their infecting organisms.

d. Erythromycin, an antibiotic inactive against enteric bacteria and viruses, usually effects a rapid remission of symptoms.

However, very little is known about their pathogenicity factors. Enteropathogenic bacteria may cause disease in the following ways:

i. Enterotoxigenic bacteria elaborate either heat-labile and/or heat-stable exotoxins which cause fluid out-pouring in the gut. Vibrio cholerae produces only heat-labile toxin, some of the Escherichia coli strains can produce either heatl-labile or heat-stable or both and Plesiomonas shigelloides produce both types of toxins (Sanyal et al, 1980).

ii. Locally invasive infection is caused by Shigellae. Some strains of Shigellae may produce enterotoxin and thus increase the severity of the disease. Clostridium perfringens and V. parahaemolyticus may act in a similar manner.

iii. In case of invasive type of infection the ingested organims pass through the mucosa and may invade the blood stream. Salmonella and Yersinia enterocolitica are examples of this type of infection.

Although there is growing evidence that enteritis due to Campylobacter fetus ssp. jejuni may fall into the last category,

no convincing experimental proof has yet been advanced in support of this concept.

Few reports indicate that certain strains of C. jejuni can produce ST (Butzler and Skirrow, 1979; Huq et al, 1981). Gubina et al (1981) demonstrated production of LT by certain strains of C. jejuni. Newell et al (1981) studied supernates of broth culture of three strains isolated from cases diarrhoea which were found to be cytotoxic in He la cell monolayers.

Epithelial adhesion and penetration using chicken embryo cell culture and He La cell line were demonstrated by scanning and transmission electron microscopy (Butzler, 1981 ; Newell et al, 1981). Davidson and Solomon 1981). Davidson and Solomon (1981) demonstrated invasive capability of C. jejuni in chorioallantoic membrane of chick embryo. Butzler and Skirrow (1979) reported invasion of blood streams and cecal mucosal cells using 8-day old chicks. Fitzgeorge et al (1981) reported colonozation of of the gut and bacterimia in rhesus monkeys. Macartney et al (1981) observed invasion of gut mucosa and blood after oral feeding in dogs. These data indicate that very little study has been done on pathogenic mechanism.

Several serotyping schemes are currently being developed using passive haemagglutination (Penner and Hennesy, 1980; Lauwers, 1981), slide agglutination (Lior, 1981), tube agglutination (Butzler 1974; Abbott et al, 1980; Bryner et al, 1981), coagglutination (Kosunen et al, 1980), and bactericidal test (Abbott et al, 1980).

The following methods for serology have been used with variable success: tube agglutination with formolised antigen (Butzler, 1978), with boilde antigen (Jones, 1980); complement fixation using sonicate (Butzler, 1978); indirect immunofluorescence (Blaser, 1979), bactericidal test (Karmali and Fleming, 1979). All these methods require autologous isolates and therefore, of little value for sereepidemiology apart from outbreak situations. Two recent methods using pools of antigens that seem to be species specific are promising. These are: a simplified modification of the ELISA test performed by diffusion in ge, DIG-ELISA (Elwing and Nilsson, 1980) which was found to be reproducible during epidemic as well (Svedhem et al, 1981). This method utilises a pool of glycoprotein preparations from microcapsules (McCoy et al, 1975) of strains of C. fetus ssp. jejuni isolated from the study population. Complement fixation test (Mosimann et al, 1981) using a basic extract of a pool of five strains has shown reproducible results.

The isolation rate from diarrhoeal children in Bangladesh differes very little from that of non-diarrhoeal ones (Blaser et al, 1979) whereas one report from Belgium (Butzler et al, 1973) indicate the ratio to be 5:1. Later reports from the developed world indicate that isolation from diarrhoeal cases ranged from 4.3 to 13.9 per cent whereas from healthy controls the isolation rate varied from 0-1.3 (Blaser et al, 1981).

In five countries of the developing world similar observations like those in the developed countries were made (Butzler, 1973; De Mol and Bosmans, 1978; De Mol, 1981; Ricciardi and Ferreira, 1980; Ringertz et al, 1980; Billingham,1981), however, in Bangladesh and South Africa high and similar prevalence of C. jejuni was observed in healthy and diarrhoeal children (Bokkenhauser et al, 1978; Blaser et al, 1979).

Glass et al, (1981) suggested several hypotheses for the different epidemiological picture in Bangladesh: one is that Campylobacter, like Escherichia coli, represents a large family of organisms of which only selected members are pathogenic, second to explain the high prevalence of asymptomatic infection is the possible role of immunity in a hyper-endemic setting and third that inoculum size determines illness versus carriage. It is important to note that this organism is widely distributed in animals like dogs, sheeps, pigs etc. and in chickens, and human infections from these sources are not uncommon (Butzler and Skirrow, 1979; Blaser, 1981). Water (Tiehand and Vogt, 1978) and milk borne (Robinson et al, 1979) outbreaks caused by this organism are also known. According to Skirrow and Benjamin (1980) in Britain C. jejuni biotype 1 is most commonly isolated from cases of enterocolitis and this is the predominant type found in cattle and sheep; C. jejuni biotype 2 and C. coli which are occasionally found in human cases, are the dominant

types in poultry and pigs respectively and the NARTC strains are common in wild birds but not associated with illness in man. Recently Lauwers, (1981) and Lior, (1981) using serotyping reported that dogs, cows, swine and chicken may be the sources of infection. Another important aspect need be noted in Bangladesh that people in this country are used to live in close association with domestic animals and poultry birds which might lead to wide spread cross infections between man and animals.

3. Rationale:

This study has the possibilities of exploring the pathogenic mechanisms of C. fetus ssp. jejuni which are still unknown. There is also the possibility of establishing the importance of this organism in the aetiology of diarrhoea in man in Bangladesh. It also envisages to establish serological techniques and serotyping and biotyping schemes at ICDDR,B to clarify some of the epidemiologic factors in the context of developing countries like Bangladesh.

B. SPECIFIC AIMS:

1. To find a suitable experimental model to idenitfy pathogenic factors of C. jejuni as an enteropathogen in man:
   a. Production of enterotoxin/s
   b. Production of cytotoxin/s

c. Local epithelial invasion

d. Systemic invasion.

2. In case they are enterotoxic and/or cytotoxic, to explore the mode of action in experimental models.

3. To look for any correlation between identified virulence factor/s with sympotmatic versus asymptomatic infections in man.

4. To look for the same virulence factor/s in animal and environmental isolates.

5. To set up serotyping and biotyping schemes for strains to be used as markers.

6. To compare the distribution of thes markers in isolates from symptomatic and asymptomatic individuals.

7. To compare the distribution of strain specific markers amongst the human and animal isolates available in the laboratory to identify the major reservoir of C. jejuni infection in Bangladesh.

8. To establish cross infections of C. fetus ssp. jejuni between man and animals in Bangladesh by carefully designed epidemiologic studies in a community once it is possible to have consistent antigenic or biochemical markers for specific strains.

9. To establish, through serological response, the aetiologic role of the organism in causation of diarrhoea. This part will be performed starting with sera collected by Dr. Glass as per his protocol on epidemiologic study.

10. To study the disease spectrum caused by C. jejuni in Bangladesh.

C. METHODS OF PROCEDURE:

Clinical data collection and selection of strains: Fifty strains of C. fetus ssp. jejuni isolated from diarrhoeal patients and if available a similar number from healthy individuals, animals and poultry birds from stock cultures maintained in ICDDR,B laboratory will be retested for their identity and subjected to the tests noted below. The strains from diarrhoeal cases will be picked up after screening the preserved clinical history sheets of the hospital surveillance system to have a good correlation with the degree of severity of illness. A chart will be made for each new patient of the hospital surveillance study that will depict name, age, sex, address and different clinical parameters including presenting symptoms with emphasis on febrile predromal period, abdominal pain, volume of fluid loss and requirement of fluid, extent of dehydration, presence of blood in stool, duration of illness etc. Another 50 freshly isolated strains from such diarrhoeal patients that seem to be severely ill and clearly due to that organism will be tested similarly to be compared with fresh isolates from healthy individuals, animals and poultry birds, if possible. The isolation and identification procedures as set up in Microbiology Branch, ICDDR,B

will normally by followed in this study. In addition, we plan to set up rapid presumptive diagnosis by phase contrast microscoic examination of fresh stool specimens.

Studies on Pathogenicity: Although a large number of test are listed below because we do know so little about the pathogenic mechanism of C. jejuni most likely that not all need be done. To begin with each test will be done with a small number of selected strains to give significant results, in case a test is found useful it will be persued, otherwise dropped. Special efforts will be made to mimic the disease in experimental animals, rather that adapting in vitro techniques.

1. Tests of enterotoxicity:

    1. Heat-labile enterotoxin :

        a. Adult rabbit ileal loop tests with both live cells and culture filtrates will be performed in duplicate (Sanyal et al, 1980) In case there is no fluid accumulation in the first series of tests with live cells, serial consecutive three passages in loops will be done with a representative groups of strains to see if there is enhancement of toxigenicity (Singh and Sanyal, 1978). If there is fluid accumulation on passaging, culture filtrates will be prepared from the passaged strain for loop tests.

        b. Skin permeability assay with the culture filtrates will be done following the method described by Craig (1970) and (Dubey and Sanyal, 1978).

        c. Chinese hamster ovarian cell culture assay with culture filtrat will be done by the method described by Guerrant et al (1974).

d. Mouse adrenal cell culture (Y-1) assay with culture filtrates will be performed following the method of Donta et al (1974) and Sack & Sack (1975).

e. If possible Vero cell culture test will also be done as per the method described by

2. Heat-stable enterotoxin:

a. Ileal loop tests with culture filtrates held at temperature of 56°C, 60°C, 65°C and 100°C for 10,20 and 30 min. (Dubey and Sanyal, 1978).

b. Time course of fluid accumulation in ileal loops (Evans et al 1973; Sack, 1975; Sanyal et al, 1980) with a representative group of strains.

c. Suckling mouse assay following the method as described by Dean et al (1972) and Sanyal (1980).

II. Tests to be done to examine the mode of action in case they produce on enterotoxin:

a. Prostaglandins synthesis (Declofenac sodium and Indomethacin) and receptor (SC 19220 in Tween 80) blockers will be administered in adult rabbits and then ileal loop test with

culture filtrates will be performed to examine if there is any inhibition of fluid accumulation.

b. 5' hydroxytryptamine synthesis (Parachlorphenylalanine, PCPA) and receptor (Cyproheptadine) blockers will be tested in the same way.

c. cAMP inhibitor like chlorpromazine will be tested similarly. All the above three tests will be performed following the methods of Sanyal et al (1978) and Agarwal and Sanyal (1981).

d. Membrane stabilising agents such as chloroquine will also be tested.

e. Heat-stable enterotoxin of Escherichia coli has been reported to act through mediation of cGMP (Hughes et al, 1978). If C. fetus ssp. jejuni produces a heat-stable enterotoxin an attempt will be made to see if Atropine sulfate or some other drug known to inhibit cGMP can reduce fluid accumulation in ileal loops, although Guerrant et al (1980) a,b recently reported that E. coli ST activity on cGMP is not affected by Atropine. However, prostaglandin synthesis blockers may prove effective and these drugs may be tried.

III. Tests for Cytoxicity

a. Shigella like cytotoxin will be looked for using HeLa cell monolayer incubated with crude culture filtrate. The cytotoxicity will be quantitated by dye exclusion method and by microtiter spectophotometric method of Gentry and Dalrymple (1980).

b. If HeLa is not sensitive to the cytotoxin, if present in culture filtrate, other cell lines will be tested similarly.

Tests for local invasiveness :

a. Sereny's (1957) tests will be performed to see if the C. fetus ssp. jejuni strains can produce keratoconjunctivitis in rabbits or guinea pigs eyes with a dose of 10^7 as suggested by Sereny or a high dose of 10^{9-10} live cells (Gemski et al, 1980).

b. Invasion of the epithelial cells of HeLa monolayer culture by live bacteria will be tested following the methods described by La Brec et al (1964) and Sanyal et al (1980) and Newell and Pearson (1981).

c. Intraduodenal administration of a dose of about 10^{8-9} live cells in adult rabbit without ligation of loops will be done with a representative group of strains and invasion of the mucosal cells will be examined after sacrifice of a pair of animals by macroscopic as well as histopathological examination of sections of gut tissues at 1,2,4,8 and 18 h. The rabbits will also be

observed throughout for development of diarrhoea (Canty and Blake, 1977).

d. Mucosal invasion will also be observed in ligated rabbit ileal loop following the method of Gianella et al (1973).

e. Invasion of chicken embryo cells. Primary in vitro cultures of 10-day old chicken embryos will be inoculated with a representative group of the test strains following the method of Butzler and Skirrow (1979). The final concentration of the organisms in the tissue culture medium will be 10^8 per ml. The C. fetus ssp. jejuni strains are expected to invade the cells within 24 hours and kill them by 36 hours. All the uninoculated control cells are expected to remain normal.

f. Invasion in chick embryo model as done in the laboratory following the method of Davidson and Solomon (1981).

IV. Tests to examine the passage of bacteria through the intestinal mucosa :

a. Intragastric or oral or intraperitoneal inoculations in Swiss albino mice (18-20 g) followed by culture of faeces and specimens from different organs especially liver and spleen according to the method of Collins (1970) and Gemski et al (1980). Prior to the experiments mouse gut will be free of Campylobacter.

B. Inoculation of eight-day old chicks in groups of five will be inoculated with 10^8 organisms of each strain from a representative group keeping a group of five chicks to serve as control which

will receive culture medium following the method of Butzler and Skirrow (1979). One week later faeces and heart boood will be cultured, the chicks will be sacrificed and cultures will be made from liver and bowel. If the organism is invasive, cultures from liver and blood are expected to be positive. Signs for development of diarrhoea will be noted daily.

V. Maintenance of pathogenicity of C. fetus ssp. jejuni after isolation :

This organism does not survive usually more than 72 h unless forzen or lyophilized. Veale et al's modification of Arco's Guinea pig plastic chamber model may be tried for survival of the organisms and maintenance of virulence factors. This chamber may provide ideal microenvironment for them. Further tests for pathogenicity may also be done with the organisms if they survive and multiply in the chamber.

VI. Tests for serologic responses :

For tests of serologic responses the same acute diarrhoeal patients from the hospital surveillance study will be used that we shall be sampling from and on whom clinical informations are available and the episodes are clearly due to C. fetus ssp. jejuni. Control subjects will comprise of similar number of matching non-diarrhoeal individuals from the ICDDR,B staff clinic. Blood specimens will be collected by capillary tube method as used at ICDDR,B from young children and by syringe in 2 ml quantities from older children and adults during convalescence.

The separated sera will be diluted 1 in 10 in normal saline distributed in 0.5 ml aliquots, whereever possible in small vials and preserved frozen

at 70°C until tested for agglutination, bactericidal, immunofluorescence techniques with reference strains and/or homologous strains following the methods of Butzler and Skirrow (1979), Jones et al (1980) and Watson (1981).

Alternatively, a simplified modification of the ELISA test performed by diffusion in gel, DIG-ELISA (Elwing and Nilson, 1980) which was found to be reproducible during an epidemic (Svedhem et al 1981) will be done using a pool of glycoprotein preparations from the microcapsules (McCoy et al, 1975) of strains of C. fetus ssp. jejuni isolated from the study population. Complement fixation test (Mosimann et al, 1981) using a basic extract of a pool of five strains has shown reproducible species specific result may be used.

VII. Setting up of a Serotyping method :

The passive haemagglutination technique for serotyping developed by Penner and Hennessy (1980) and Lawers (1981) and the slide agglutination technique of Lior (1981) appear to be the most promising ones. The type strains of the first method have been obtained from Dr. Penner by Dr. M.I. Huq and we shall acquire the other sets from Dr. Lawers and Dr. Lior. Antisera will be raised against those strains in this laboratory with the aim to set-up either the passive haemagglutination or the slide agglutination technique of serotyping as early as possible. A set of 100 strains isolated from cases of diarrhoea and their environment including animals will be brought out from ICDDR,B stock and tested for serotyping. Extension of

the typing scheme will be made with the untypable strains that are isolated at ICDDR,B to make it more efficient, useful and relevant to the epidemiology of the disease in Bangladesh.

VIII. Biotyping :

The thermophilic strains of Campylobacter are associated with diarrhoeal illness in man. Different workers have tried with various extent of success to type them on the basis of their growth requirements, biochemical characters, anti-microbial sensitivity etc. (Veron and Chatelain, 1973; Butzler and Skirrow, 1979; Skirrow and Benjamin, 1981; Weaver et al 1981). In this study an attempt will be made to find out a set of tests based on various physicochemical characters and anti-microbial sensitivity which might be of help to distinguish between strains isolated from man and animals and elucidate the existence, if there is any, of correlation between enteropathogenicity and biotypes of the strains of C. fetus ssp. jejuni.

Epidemiology:

If it becomes possible to have consistent antigenic or biochemical markers for specific strains an attempt will be made to explore the possibility of cross infections between man and animals. For this purpose carefully designed epidemiological studies will be carried out in a small rural community maintaining household animals and poultry birds. The details of the surveillance program of the community which will comprise of humans and their environment will

be planned later on when the markers are established following standard procedures (Marwah et al, 1975).

D. SIGNIFICANCE

C. fetus ssp, jejuni has been implicated as an important aetiological agent of diarrhoea in the developed world, although its exact pathogenic mechanism still remains unclear. It has been established in those countries in the aetiology of the disease mainly on the basis of thorough clinico-epidemiological investigations. However, studies conducted at ICDDR,B indicated that the clinico-epidemiological features of this organism might differ in these countries from those of others. The isolation which is fairly high (about 20 pecent) from healthy individual almost equals to that of diarrhoeal cases in Bangladesh, whereas in most of the developing and developed countries the ratio is about 1:5. Moreover, a variety of animals and poultry birds do carry C. fetus ssp. jejuni. In the developing world including Bangladesh man and domestic animals and poultry birds usually live in close assocaition in rural areas and thus increase the possibility of cross infections. The proposed study will elucidate whether this organsim elaborate either heat stable or both types of enterotoxin, if so, the mode of action of the toxins whether acts through mediation of prostaglandins or 5 hydroxytryptamine or adenylate cyclase or guanylate cyclase or otherwise. The study will also clarify whether this organism is locally invasive i.e. whether it is capable of invading the intestinal mucosa cells. It will also be clear from this study if it can cross the intestinal mucosal barrier

and invade the blood-stream. The studies on serology as envisaged in the proposal will provide evidence about the role of C. fetus, ssp. jejuni in the aetiology of diarrhoea in this country. It may also provide an insight into the riddle of high and almost equal prevalence of the organism in diarrhoeal and non-dairrhoeal populations. Further, this study will provide an opportunity to evaluate a number of serological techniques and thus to find out the most reproducible, sensitive and easy technique that is suitable for this laboratory. Setting up of a Serotyping method at ICDDR,B will provide a basic epidemiological facility and typing of the strains isolated from different sources will provide some baseline data in respect of certain epidemiological parameters and may be of some aid in distinguishing strains of human and animal origin. The proposed biotyping of the strains isolated from various sources may be of great help in distinguishing between strains isolated from man and animals. There may also exist some correlation between enteropathogenicity and biotypes of the strains of C. fetus ssp. jejuni which may be clear after this study. In case some antigenic and/or biochemical markers are obtained from these investigations a well-designed community based small but intensive epidemiological study may throw some light on the possibility of cross infections between man and animal. Further, through the clinical study the disease spectrum in Bangladesh may be clearly defined.

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SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

NAME	POSITION	% OR NO. OF DAY	ANNUAL SALARY	PROJECT REQUIREMENT TAKA	DOLLAR
Prof. S.C. Sanyal	Visiting Prof.	50%	-	-	22,500
Dr. (Mrs.) S.Q. Akhtar	Asst. Scientist	35%		31,890	-
Dr. M. Struelens	Fellow Scientist	25%	-	-	4,360
Dr. M.I. Huq	Head, Microbiol.	15%	-	-	7,800
Dr. Roger Glass	Scientist	5%	-	-	2,000
Dr. A. Al Mahmood	Asst. Scientist	15%	-	20,364	-
Dr. Iqbal Kabir	Sr. Med. Officer	10%	-	6,000	-
Mr. P.K.B. Neogy	Sr. Res. Officer	50%	78,427	39,213	-
To be named	Res. Officer*	50%	25,272	12,636	-
To be named	Lab. Technician*	50%	18,036	9,018	-
Md. Safiullah	Animal Care Taker	15%		6,352	-
Md. Ishaque	Lab. Attendant	50%	18,096	9,048	-
To be named	Field Assistant	50%	19,200	9,600	
To be named	Secretary	50%	25,000	12,500	-
				156,621	36,660

2. SUPPLIES AND MATERIALS

ITEM	UNIT COST	AMOUNT REQUIRED	PROJECT REQUIREMENT TAKA	DOLLAR
New Zealand adult albino rabbits	120.00	300	36,000	-
Suckling mice 200 tests	14.00	200	2,800	-
Swiss albino mice	5.00	100	500	-
Infant chicks (8 day old)	10.00	250	2,500	-
Guinea pigs	35.00	100	3,500	-
CHO cell culture)	14.50	200	2,900	-
Y_1 cell culture)		200		
HeLa cell culture)	20.00	200	4,000	-
Vero cell culture)		200		
Chick embryo culture (10 days old)	4.00	200	800	-
Bacteriological culture	14.00	2000	28,000	

* At present no Research Officer and Lab. Technician available since all are engaged with research activities. These can be recruited against the budget of this protocol.

ITEM	UNIT COST	AMOUNT REQUIRED	PROJECT REQUIREMENT TAKA	DOLLAR
Serological tests : 200 tests				
Agglutination) Bactericidal) Immunofluorescence)	24.00	200	4,800	-
Stationary, office supplies			6,000	-
			91,800	
3. EQUIPMENT				
Membrane filters		1		1,000
Membrane filter holders (autoclavable plastic)		24		480
Egg Incubator		1		2,000
				3,480
4. PATIENT HOSPITALIZATION	150.00	50	7,500	
5. OUTPATIENT CARE - None				
6. ICDDR,B TRANSPORT				
1000 mile transport @ Tk. 4.00/mile			4,000	
7. TRAVEL AND TRANSPORTATION OF PERSONS - None				
8. TRANSPORTATION OF THINGS				300
9. RENT, COMMUNICATIONS AND UTILITIES - None				
10. PRINTING AND REPRODUCTION			5,000	
11. OTHER CONTRACTUAL SERVICES - None				
12. CONSTRUCTION, RENOVATION, ALTERATION - None				

: tool for studying the disease pattern in this country.

8. Use of record : Hospital surveillance study record will be used; if necessary.

B. BUDGET SUMMARY

		TAKA	DOLLAR
1.	Personnel Services	156,621.00	36,660.00
2.	Supplies and Materials	91,800.00	-
3.	Equipment	-	3,480.00
4.	Patient Hospitalization	7,500.00	-
5.	Outpatient Care	-	-
6.	ICDDR,B Transport	4,000.00	-
7.	Travel and Transportation of Persons	-	-
8.	Transportation of Things	-	300.00
9.	Rent, Communication and Utilities	-	-
10.	Printing and Reproduction	5,000.00	-
11.	Other Contractual Services	-	-
12.	Construction, Renovation, Alteration	-	-
	Total :	264,921.00	40,440.00
	Equivalent US$:	16,560.00	

Grand Total : US$ 57,000.00

Conversion Rate US$ 1.00 = Tk. 16.00

ক্যাম্পাইলোব্যাক্টর রোগীর সম্মতি পত্র

হাসপাতালে আপনার/আপনার শিশুর পায়খানা পরীক্ষা করে ক্যাম্পাইলো-ব্যাক্টর নামে এক প্রকার জীবাণু পাওয়া গিয়াছে। এই জীবাণু পায়খানার সাথে অবস্থান করে। এই জীবাণুই আপনার/আপনার শিশুর রোগের কারণ কি না তা দেখার জন্য যদি আপনি রাজী হন তবে আপনার/আপনার শিশুর আংগুল হতে পরীক্ষার জন্য সামান্য রক্ত নেওয়া হবে। আপনার দেহে এই রোগের কোন প্রতিক্রিয়া হয়েছে কি না তা দেখার জন্য আপনার সম্মতিক্রমে দু'সপ্তাহের মধ্যে আবারও সমপরিমান রক্ত নিতে হবে। আপনি যে কোন সময়ে আপনার/আপনার শিশুর নাম প্রত্যাহার করতে পারেন।

আপনার/আপনার শিশুর সম্পর্কিত তথ্যাবলী গোপন রাখা হবে। আমরা আশা করি আপনার সহযোগীতা বাংলাদেশে ক্যাম্পাইলোব্যাক্টর রোগের চিকিৎসা ও দমনে সহায়তা করবে।

রোগী/অভিভাবকের
স্বাক্ষর/টিপ সহি----------

তারিখ----------

ABSTRACT SUMMARY

Campylobacter fetus ssp. jejuni has been implicated on the basis of clinico-epidemiological evidence as an important aetiological agent of diarrhoea, although very little is known about its pathogenic mechanisms. The organisms are prevalent in Bangladesh with almost equal frequency amongst diarrhoeal and non-diarrhoeal individuals creating confusion about the role played by them. This stuudy will be of help in establishing their enteropathogenicity and the exact role played by them in the aetiology of diarrhoea in this country. It may also delineate certain characters of the organisms that might be of help in distinguishing pathogenic non-pathogenic strains as well as isolates frcm diarrhoeal patients and non-diarrhoeal individuals, animals and the environment. This has also the possibility of exploring the existence of cross infection between man and animals and certain other epidemiological aspects of C. jejuni infection in Bangladesh.

1. Subject population : All age groups
2. Potential risks : No potential risks to the subjects involved.
3. Protection against risk : Sterile methodology will be used.
4. Confidentiality : Non applicable
5. Privacy : Not applicable
6. Interviews : Not applicable
7. Benefit : This study will help establishing the pathogenicity of Campylobacter fetus ssp. jejuni. It will help establishing the role played by this organism in the aetiology of diarrhoea in Bangladesh and delineate certain features of the organism that will be of much value as epidemiological

: tool for studying the disease pattern in this country.

8. Use of record : Hospital surveillance study record will be used; if necessary.