Attachment 1.
(FACE SHEET)

Date 18/2/91

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. Samia K. Saha. Trainee Investigator (if any) _____

Application No. PCC/005/90/(Revised) Supporting Agency (if Non-ICDDR,B) _____

Title of Study Study on the Virulence of Campylobacter fetus and C. coli and their motile and non-motile variants.

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
   (d) Sensitive questions Yes No
   (e) Benefits to be derived Yes No
   (f) Right to refuse to participate or to withdraw from study Yes No
   (g) Confidential handling of data Yes No
   (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure Yes No
5. Will signed consent form be required:
   (a) From subjects Yes No
   (b) From parent or guardian (if subjects are minors) Yes No
6. Will precautions be taken to protect anonymity of subjects Yes No
7. Check documents being submitted herewith Committee:
   ___ Umbrella proposal - Initially submit overview (all other requirements wil be submitted with individual studies)
   ___ Protocol (Required)
   ___ Abstract Summary (Required)
   ___ Statement given or read to subjects 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 summa
   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

APPLICATION FOR PROJECT GRANT

1. TITLE OF THE PROJECT : Study on the virulence of *Campylobacter jejuni* and *C. coli* and their motile and non-motile variants

2. PRINCIPLE INVESTIGATOR : Dr. Samir K. Saha
Dhaka Shishu Hospital (DSH)

3. COINVESTIGATORS : a) Dr. Waqar A. Khan
Dhaka Shishu Hospital

b) Dr. M. John Albert
ICDDR,B

4. CONSULTANT : Prof. M. S. Akbar
Dhaka Shishu Hospital

5. STARTING DATE : October 01, 1990

6. COMPLETION DATE : September 30, 1991

7. TOTAL BUDGET REQUIRED : US$ 5,185

8. APPROVALS

Director
Dhaka Shishu Hospital

Head
Laboratory Sciences Division
ICDDR,B

9. SUMMARY

Recently, a small, sensitive and reproducible animal model has been developed by the principal investigator to test the enterotoxicity of *Campylobacter jejuni* (Saha *et al.*, 1988) which, subsequently, was also found to be suitable to study the colonisation of the organism and the histopathological changes caused by the organism (Saha and Sanyal, 1990; S.K. Saha, Ph.D. thesis, 1990). Use of this animal model for further detailed study of *Campylobacter* should resolve some of the existing discrepancies regarding the pathogenesis of *C. jejuni* and *C. coli* and the role of flagella in the enteropathogenicity of *C. jejuni/coli*.

This proposal aims to study the prevalence and comparative virulence of *C. jejuni* and *C. coli* and their virulence in patients and in experimental animal model. The study also aims to the determination of role of flagella in virulence in rat ileal loop model by using the isogenic non-motile variants of *Campylobacter jejuni/coli*. Virulence in patients will be studied on the basis of the macroscopic and microscopic appearance of faeces, duration of diarrhoea, abdominal pain, vomiting, dehydration, temperature, haemotological values, haemolytic process, etc. Virulence in animal model will be studied on the basis of quantitative assay of enterotoxin, colonising ability, infective dose and relative histopathological changes.

The work will help to identify the prevalent species of *Campylobacter* in Bangladeshi children and to understand the relative virulence of *C. jejuni* and *C. coli* and their motile and non-motile forms, in patients and experimental animal model.

Study on motile and non-motile variants will be of paramount significance to understand the role of flagella in virulence and whether the flagellin epitope(s) should be considered as potential targets against which the host antibodies has to be directed for immunoprophylactic measures against *Campylobacter* enteritis.

10. AIMS OF THE PROJECT

a) General aims

1) Determination of prevalence of *Campylobacter jejuni* and *C. coli* in diarrhoeal and non-diarrhoeal patients.

2) Study on the relative level virulence of *C. jejuni/C. coli* in clinical settings and in experimental animal model. And the comparative virulence of motile and non-motile strains in animal model.

b) Specific aims

1) *Campylobacter* strains will be isolated and identified to see the prevalence of *C. jejuni* and *C. coli* and in diarrhoeal and non-diarrhoeal patients.

2) Comparative virulence of the strains in patients will be determined on the basis of the following clinical symptoms:

   i) macroscopic and microscopic appearance of stool

   ii) duration of diarrhoea

   iii) abdominal pain

   iv) vomiting

   v) dehydration

   vi) temperature

   vii) haematological values, i.e. TC, DC, ESR, Film for haemolytic process (HUS), joint pain, etc.

3) Comparative virulence of the strains in rat ileal loop model will be determined on the basis of the following criteria:

   i) comparative infective dose (cfu)

   ii) ability to cause the histopathological changes in rat gut

   iii) ability to colonise the rat gut

4) The non-motile isolates/variants of *C. jejuni* and *C. coli* will be serially passaged through rat gut to see whether the strains will revert to motile form.

11. SIGNIFICANCE

The above mentioned works on *Campylobacter* will help to identify the predominant *Campylobacter* species in Bangladeshi children and the comparative virulence of *C. jejuni* and *C. coli* in causing enteritis.

The detailed comparative study on motile and non-motile variants will facilitate better understanding of pathogenesis of *Campylobacter*.

Finally, the study shall have a paramount significance to determine the role of flagella in virulence and whether the flagellin epitopes should be considered as the potential targets against which the host antibodies have to be directed for immunoprophylactic measures against *Campylobacter* enteritis.

11. ETHICAL IMPLICATION

No major ethical issues are involved.

12. BACKGROUND

Campylobacter jejuni/coli is now well-established as one of the commonest causes of enteritis in man (Walker *et al.*, 1986). The two species, *C. jejuni* and *C. coli*, are very closely related. Studies have shown that the prevalence of the species in man is not the same at different parts of the world (Skirrow, 1984; Georgecurbot *et al.*, 1986).

Furthermore, two studies, from developed countries, regarding the virulence of these two species in human being is in contrast to each other, one group claimed *C. jejuni* as more virulent and another group, *C. coli* and *jejuni* having the same degree of virulenceas (Papovic-Uroic *et al.*, 1988; Figura and Guglielment, 1988). These studies were only clinical-symptom-oriented. However, there is no such data from the developing countries.

The exact mechanism of the pathogenicity of *Campylobacter* is still in obscurity. There are both clinical and experimental evidences to suggest that invasion (Butzler and Skirrow, 1979) and/or production of enterotoxin (Ruiz-Palacios *et al.*, 1983; Saha *et al.*, 1988; Saha and Sanyal, 1990) play an important role in the pathogenesis of *Campylobacter*. In certain established enteropathogens, such as *Vibrio cholerae* (Attridge and Rowley, 1983) and *Salmonella typhimurium* (Carsiotis *et al.*, 1984), motility has been established as an important virulence factor. The reports on the possible role of motility in the virulence of *Campylobacter* are scanty. Two groups of workers (Morooka *et al.*, 1985; Newell *et al.*, 1985) observed a significant role of motility for the pathogenesis of the organism but another group (Field *et al.*, 1986) failed to correlate the virulence of *Campylobacter* with its motility. Two available reports regarding the reversion of non-motile *C. jejuni/coli*

strains to a motile phenotype *in vivo* is also in contrast to each other (Caldwell *et al.*, 1985; Field *et al.*, 1986).

All these discrepancies in the studies on pathogenicity of *Campylobacter* could be attributed to the absence of a suitable animal model (Walker *et al.*, 1986). However, recently we have reported that an ileal loop model using C-F rats (Saha *et al.*, 1988, Saha and Sanyal, 1990), is excellent in sensitivity and reproducibility and can be used further for studying the pathogenicity of *C. jejuni* and *C. coli*.

13. RESEARCH PLAN

Rectal swab/stool samples will be collected from diarrhoeal (watery/bloody) patients of age groups of 3 months to five years and age-sex matched non-diarrhoeal controls admitted in the Dhaka Shishu Hospital (DSH). *Campylobacter* strains will be isolated on *Campy*-BAP (Blaser *et al.*, 1979) following the method of Saha and Sanyal (1989) and will be identified by the method of Skirrow and Benjamin (1980). Stool specimens will be simultaneously cultured on MacConkey, *Salmonella-Shigella* and TCBS agar to exclude the presence of other enteropathogenic bacteria. The iodine preparation of stool specimens will be microscopically examined to exclude the presence of parasite(s). Presence of rotavirus, ETEC (Svennerholm *et al.*, 1986) and EPEC (Albert *et al.*, submitted) will be excluded by the ELISA test.

Clinical

Fifty cases, from which *Campylobacter jejuni/coli* will be isolated as a sole pathogen, will be studied on the basis of the clinical features, such as watery diarrhoea, bloody mucoid diarrhoea, mucoid diarrhoea without blood, abdominal pain, vomiting, dehydration, peak temperature, white blood cell count, etc.

Blood specimens routinely referred to our pathology laboratory will be used to do the haematological tests mentioned in the proposal.

Bacteriological

The non-motile variants of 12 representative motile isolates of *Campylobacter* (6 *C. jejuni* and 6 *C. coli*) will be screened by using 0.5% agar plates (Field *et al.* 1986) just after isolation. The non-motile strains of *C. jejuni* and *C. coli* will be further confirmed by flagellar staining (Leifson's method).

EXPERIMENTAL

Enterotoxins

Six *Campylobacter jejuni* and six *C. coli* strains (both their motile and non-motile isogenic variants), with least possile subculture, will be tested in rat ileal loop model (Saha *et al.*, 1988). The comparative enterotoxicity of the strains will be interpreted by measuring the ml of fluid accumulated per cm of rat gut.

Infective dose

To determine any differences in the infective doses [the minimum number of bacteria that cause full-blown reaction (0.5% w/v)] of *C. jejuni* and *C. coli* and their motile and non-motile variants, graded doses (cfu) of the strains will be injected into rat ileal loops.

Reversion

Non-motile strains will be serially passaged through the rat gut by the method described earlier (Saha *et al.*, 1988), and any change in their motility will be tested after each passage. Observation of any reversion of a non-motile strain to a motile one, on soft agar plates, will be confirmed by flagellar staining, and.

Colonisation/Adherence

Comparative ability of motile and non-motile strains of *Campylobacter* to colonise/adhere the rat gut will be evaluated by determining the cfu of the organism in mucus scrappings of rat gut after inoculation with same number of bacteria of both, motile and non-motile, variants. Lumen of the gut will be gently washed with normal saline before taking the mucosal scrappings.

Histopathology

Neighbouring rat ileal loops, inoculated with similar cfu of motile and non-motile variants, will be processed (after

18 hr of inoculation) for histopathological study. In brief, the microtome sections of respective gut loop will be stained by Haemotoxylin and Eosin method (Luna, 1968). The stained sections of the loops with motile/non-motile variants of *C. jejuni/coli* will be comparatively examined for any change in crypt:villi ratio, cellular infiltration of epithelium, sloughing of mucosal cells, etc.

14. BIBIOLOGRAPHY

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15. PUBLICATIONS OF PRINCIPAL INVESTIGATOR

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2) Akbar MS, Naila Khan and Saha SK. (1986) Evaluation of chloramphenicol and co-trimoxazole in the treatment of enteric fever. Bangladesh J Child Health 10(1):11-14.

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11) Saha SK and Sanyal SC. (1990) Production and characterisation of *Campylobacter* enterotoxin in a synthetic medium and its assay in rat ileal loops. FEMS Microbiol. Lett. 67:333-338.

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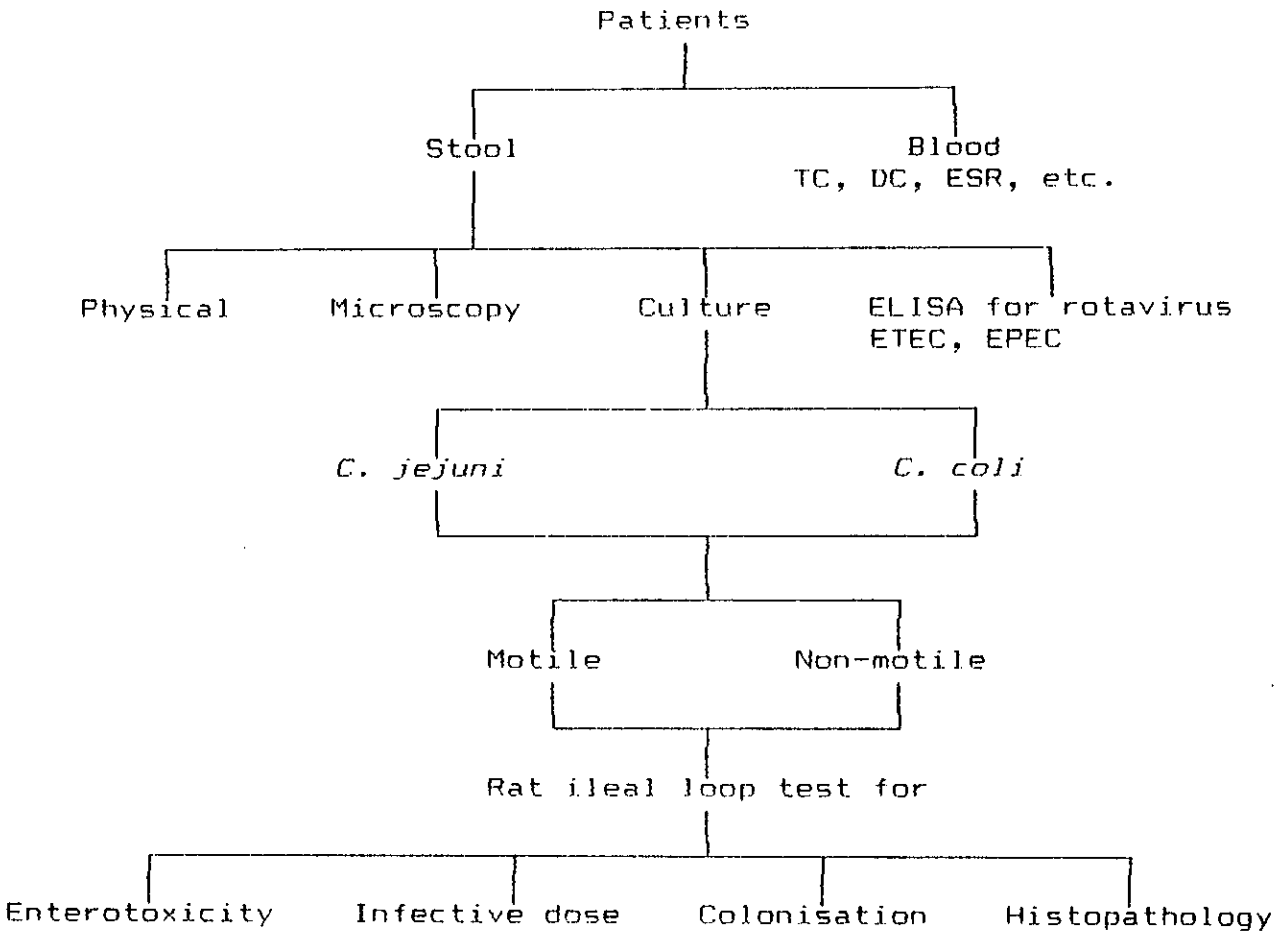
16. FLOW CHART



17. ITEMIZED SPECIFIC TASKS FOR EACH LISTED INVESTIGATOR

a) Dr. Samir K. Saha — Will supervise the whole work and will do the animal experiment

b) Dr. Waqar A. Khan — Will examine the histopathological and haematological slides

c) Dr. M. John Albert — Will supervise and render expert help at the time of animal experiments and ELISA tests at ICDDR,B

d) The data for clinical evaluation will be collected from the hospital record files of the respective patients and Prof. M.S. Akbar will be available for any extra information and/or interpretation.

18. COLLABORATION

1) All the work will be done at Dhaka Shishu Hospital (DSH), except the ileal loop tests and ELISA, which will be done at ICDDR,B.

2) *Campylobacter* isolates will be preserved at $-70^{o}C$ at ICDDR,B.

3) If needed, some *Campylobacter* strains will be obtained from ICDDR,B through proper channel.

19. FACILITIES AVAILABLE AT DHAKA SHISHU HOSPITAL

Dhaka Shishu Hospital, the only children's hospital at national level of Bangladesh, has 350 beds. Isolation of *Campylobacter* and other enteropathogens can be done at DSH. The PI has got the supply of antibiotic supplements and chemicals for the isolation and identification of *Campylobacter*, which he brought with him from India.

Rat ileal loop tests will be done at ICDDR,B, but the colonisation and histopathological studies will be done at DSH. Any reversion of non-motile *Campylobacter* to a motile phenotype, after animal passage, will be tested in DSH. All the facilities, including microtome machine (Reichert-Jung), to do the histopathological studies are available at DSH.

20. BUDGET

a) Personnel

Name	Time input	Cost/annum	
Dr. Samir K. Saha, Ph.D. Principal Investigator	30%	Tk. 36,000	
Dr. Waqar A. Khan, M.Phil. Coinvestigator	10%	12,000	
Dr. M. John Albert, Ph.D. Coinvestigator	5%	-	
Research Officer (MBBS/M.Phil/M.Sc.) (to be recruited)	100%	48,000	
Laboratory Assistant	100%	24,000	
			120,000
Consultant:			
Prof. M. S. Akbar, MRCP Will be consulted when required	-	-	-

b) Supplies

Media and glasswares	Tk. 18,000	
Chemicals and reagents	7,000	
Animals (rats)	7,000	
ELISA tests	7,000	
		39,000

c) Equipment

Refrigerator - one	20,000

d) Overhead/contingencies 5,000

TOTAL PROJECT COST Tk. 184,000

= US$ 5,185