ttachment 1.

Date March 31, 1982

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Revised Date: April 30,198

rincipal Investigator Dr. S. K. Roy

Trainee Investigator (if any) 8C

pplication No. 82-014 (Rev)

Supporting Agency (if Non-ICDDR,B)

itle of Study Characteristics of iarrhoea in Typhoid Fever (Revised protocol)

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

ircle 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)

. 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 - N.A
(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 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.

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

S.K.Roy

Principal Investigator [redacted] 15 JUN 1998 Trainee

82-014 (Rev)
6/5/82

SECTION 1 - RESEARCH PROTOCOL

1. TITLE: CHARACTERISTICS OF DIARRHOEA IN TYPHOID FEVER.

2. PRINCIPAL INVESTIGATOR: Dr. S. K. Roy

CO-INVESTIGATORS: Dr. T. Butler, Dr. P. Speelman, Dr. B. Stoll, Mr. A.R.M.A. Alim & Dr. A.S.M. Hamidur Rahman.

3. STARTING DATE: May 31, 1982.

4. COMPLETION DATE: May 30, 1983.

5. TOTAL DIRECT COST: US $ 23,241

6. SCIENTIFIC PROGRAMME HEAD: This protocol has been approved by the Pathogenesis and Therapy Working Group.

Signature of Scientific Programme Head: Thomas Butler

Date: 31/3/82

7. ABSTRACT SUMMARY:

A total of fifty patients of 6 months to 60 years age will be studied in the study ward of the ICDDR,B after selection from the registration centre. Patients with history of continuous fever for more than 4 days with diarrhoea and suggestive clinical signs and symptoms for enteric fever will be entered into the study. On admission a complete medical history will be taken and thorough clinical examination will be performed. Samples will be collected for stool, blood, and urine. Everyday follow-up chart will be maintained for clinical changes, vital signs, stool frequency, purging rate, stool characteristics, output and intake of fluids, sample collection and investigations. Stool examinations with special preparations will be done for microscopy, protein concentration and quantitative analysis of culture isolates for S. typhi. Patients will be rehydrated with isotonic I.V. solution. Patients will be treated with chloramphenicol for 2 weeks. The usual hospital diet will be allowed. Patients will be kept in hospital for 2 weeks. Fifteen isolates of S. typhi will be examined by the Sereny test and culture filtrates for ST and LT assay. The study will be able to characterise diarrhoea caused by S. typhi, its invasiveness and the mechanism of diarrhoea in the gut.

8. REVIEWS:

(a) Research involving human subjects: _____

(b) Research Review Committee: _____

(c) Director: _____

(d) BMRC: _____

(e) Controller/Administrator: _____

[redacted] 15 JUN 1998

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objectives:

(a) To describe the characteristics of diarrhoea in typhoid fever. Whether it is due to toxin or invasion or otherwise.

(b) To look for the possible roles, toxin production and tissue invasiveness by S. typhi in the pathogenesis of diarrhoea.

2. Background:

Typhoid fever is a human infection caused by Salmonella typhi (1). Typhoid is rare in the developed countries now-a-days but still a predominant cause of morbidity and mortality in the developing countries including the Indian Subcontinent (2). From the very early period typhoid fever was known to be recognised by its prolonged continued fever which is still the important feature (4). The common features of typhoid include fever, diarrhoea, vomiting and abdominal pain. Typhoid fever runs a course of about 3-6 weeks in untreated cases. During first week there are few specific features. The onset is insidious with mounting fever, headache, vague abdominal pain and cough. In the second week patients become some what dull and apathetic with sustained fever and slightly tender and distended abdomen with doughy feeling. Diarrhoea is common at this stage (2). The spleen is palpable in 75 per cent of patients (2). Rose spots appear between 7-12 days but these are less visible in Asian people than Europeans. Again diarrhoea may dominate the picture from the onset (2). In the ch ·ldren diarrhoea often dominates the clinical

picture from the beginning(4). As to the severity of typhoid fever much depends on previous health and nutrition as 25 per cent mortality in children was noted in a South African study (5).

Pathogenesis of typhoid fever:

It is accepted that S. typhi enters the body by mouth. The earlier idea of entry to the blood stream through the tonsil and lymphoid tissue of pharynx was not eventually accepted (6). Incubation period of typhoid fever begins from the entrance of organisms through mouth till appearance of clinical symptoms and signs by the tenth to fourteenth day. Appearance of clinical illness is dependant on several factors like the dose of the organisms, host resistance factors, and virulence of the strain. Some S. typhi bacilli after crossing the gastric acid barrier penetrates the mucous mumbrane of jejunum (7). They enter the epithelium near the peyer's patches by pinocytosis and thereafter settle in the lamina propria and finally enter into endothelial cells of the peyer's patches. There they stimulate mononuclear cell response and then are phagocytosed by macrophages of peyer's patches where multiplication of bacteria occurs for a few days. In every 20-30 minutes they double in number. The macrophage cells and some plasma cells carry these organisms via lymphatics and blood stream to the reticulo-endothelial system (R.E. system), especially to spleen, liver, bone marrow, lymph glands of the intestine and mesentery. Rapid multiplication occurs in the R.E. system (6,8) during the incubation period. Being overwhelmed with the invading organism, cells of the R.E. system pour them into the blood stream. The following bacteremia is continuous

and clinical signs of profound illness set in, such as chill, fever, headache, generalised ache, abdominal discomfort and cough (6). At this stage most of the organisms multiply more in other tissues in comparison to blood. Spread through blood occurs to almost all organs of the body including spleen, bone marrow and gall bladder. The spleen plays an active role for relapse(in 10-15 per cent cases) of fever after the first illness. The bone marrow is the main seat of the organisms after the disappearance from blood and other tissues with treatment by Chloramphenicol. Culture of bone marrow often yeilds positive isolation. The gall bladder plays an important role in long term carrier state and provides the main bulk of bacteria in the gut which are usually found in stool culture duing the late 2nd and 3rd week of typhoid fever (9). Excretion in stool is not influenced by chloramphenicol treatment (1).

In some cases S. typhi causes enteritis(1) with mild diarrhoea in the first one or two days after ingestion of bacteria, when the local mucous membrane is affected. However, the mild diarrhoea may also be due to other organism ingested through infected water or food. Stool culture may be positive during the 1st 72 hours of ingestion but it remains negative till 2nd week of progress of disease. All gram-negative bacilli contain endotoxin within themselves. The production of exotoxin has not been documented. The nature of endotoxin has been studied by many investigators. The role of endotoxin in typhoid fever has not been established to cause the toxemia of the disease (10). Endotoxin tolerance is developed by repeated increased dose of endotoxin injection (6,8). Hyper-reactivity to endotoxin was found by subsequent administration after primry challenge of intravenous endotoxin. Endotoxin

produced "O" antibody in blood but failed to protect the volunteers from subsequent oral challenge of virulenet S. typhi organisms. Marked hyper-reaction to norepinephrine occurred after administration of endotoxin showing that endotoxin may play some role in pathogenic mechanism of typhoid fever (11, 12).

In one study the limulus test was negative for circulating endotoxin in typhoid fever (10). Pathological lesions of very mild to severe degree occurs in almost all organs of the body. Changes in mucous membrane of intestine varies from simple shedding of epithelial cells to mild hyperemia and oozing of blood is common in later weeks. According to the host resistance and other defence mechanisms of the body, the pathophysiological process by the invading organism advances which involve some lesions in the intestine. Proliferation of S. typhi mainly in endothelial cells and the local reaction of inflammatory process cause the enlarged peyer's patches to ulcerate at the overlying epithelel tissue and cause exudations. The amount of bleeding increases when it erodes adjacent blood vessels. Peyer's patches sometime extend to serosal surfaces to cause perforation of the intestinal wall leading to fatal outcome, though the incidence has come down from that of the preantibiotic era. Mesenteric lymph glands are involved in causing characteristic doughy feeling. Liver and gall bladder are involved; in some cases hepatitis is common (13). Stones in the gall bladder play a part in the chronic carrier state (1). In the kidney, disseminated intravascular coagulation occurs in typhoid fever may be produced by endotoxin acting locally in lymphoid tissue (10). Endotoxin stimulated pyrogen liberated from host tissue cells such as polymorphs and Kuffers cell of liver is responsible to cause fever in typhoid.

Immunology:

There are different pictures of antibody present in human blood in endemic and non-endemic areas. In the non-endemic areas and in unvaccinated cases titre of "O" antibody $\geq$ 40 and H $\geq$ 80 is diagnostic (14). In endemic areas children below 10 years having titre of "O" antibody $\geq$ 40 is diagnostic (14). Rising antibody titres in blood at 4-5 days interval is more suggestive. There is no study establishing the present blood level of O & H antibody for Bangladesh. For older ones 3 to 4 fold increase in "O" titre is suggestive.

Stimulated by immunization antibodies formed are mostly IGM (25). Oral live vaccines produce secretory IGA in intestinal epithelium which provides some effect. A galactose epimerase (Gal E) mutant strain of S. typhi (Ty 21 a) was used for oral live vaccine which has been safe, stable and protective against typhoid fever for at least three years (24, 16). The development of acquired resistance to S. typhimurium in mouse depends on a non-humoral mechanism capable of destroying organisms from endogenous or exogenous sources (26). It may occur through thymus dependant T lymphocytes.

Cell mediated immunity: Immunity to typhoid fever does not depend wholly on humoral respones. Celluler immunity as measured by lymphocyte Migration Inhibition (LMI) is an important feature of most infections with S. typhi (11, 12).

Endotoxin stimulated antibody could not protect from subsequent oral challenge of S. typhi in volunteer. The relapse in typhoid fever occur after 1-3 weeks of 1st attack but was not protected by high circulating antibody titre of "O", H & Vi. It is demonstrated that absence of Vi antibody offers less resistance to subsequent S. typhi infection (15).

Evidence of diarrhoea in typhoid fever:

About 10 per cent of the patients of enteric fever die in untreated cases and recovery follows in 3-6 weeks of prolonged fever and debilitating illness. Diarrhoea occurs in one third of the cases. Though diarrhoea has been described in enteric fever, detailed knowledge about its nature, mechanism and toxingenic origin are not clearly known.

A number of diarrhoeal episodes in enteric fever were reported by Jonson (3) in the study children upto 12 years. About 44 per cent of that group complained of diarrhoea out of which 38 per cent had clinical dehydration.

In 62 per cent of those cases stool was foul smelling, 33 per cent stool was watery with mucous, 31 per cent stool contained blood and mucous and only watery in 26 per cent and almost pure blood in 10 per cent. In that group fever lasted for an average period of 8.8 days, abdominal pain for 7.6 days. The average duration of vomiting and anorexia were 5.3 days and 6.5 days respectively. In certain cases diarrhoea was associated with tenesmus. Tenderness of abdomen was found in 69 per cent cases, absent or diminished bowel sounds in 67 per cent cases and distension in 40 per cent cases. Dehydration was mild in 25 per cent cases, moderate in 55 per cent and severe in 20 per cent cases.

Najwakhuri Bulos from Jordan (17) reported in a study of children suffering from S. typhi (40 per cent) and S. paratyphi A and B (60 per cent) that diarrhoea was present in 72 per cent of children below 2 years and 33 per cent in children above 2 years. He noted dehydration in 56 per cent cases of below 2 years age group and 17 per cent in children above 2 years. Mean duration of diarrhoea was 5.8 days and 1.4 days in these two groups respectively. Nasrullah (18) has described the infected area of gut in enteric fever to show oedema and exudations in the distal 15-20 c.m. of the terminal ileum but diarrhoeal episodes were not marked in that group. Ulcerations in the ileum in haemorrhaegic patients was not associated with diarrhoea in some other series.

Diarrhoeal episodes accompany relapse of typhoid fever. Relapse of typhoid fever is increasing these days in Chloramphenicol treated cases being in the range of 10-20 per cent when it was 8-10 per cent in preantibiotic era (19). Intestinal haemorrhage and intestinal perforation often occur after diarrhoeal episode has been advanced in typhoid fever. Diarrhoea with tender abdomen is often common in the second week of enteric fever. Stool cultures are often positive even during the 1st week though the isolation rate rises in the late 2nd and 3rd weeks. Positive stool culture are quite common in convalescent period but rapidly declines during the second month. Three per cent of the patients remain faecal excretors by the end of the year and remain such for the rest of life (2). The pathogenesis of diarrhea in typhoid fever is not still clear. Any toxin mediated secretory nature is not established.

A study in Sudan in 143 adult patients of typhoid fever demonstrated diarrhoea in 86 per cent of cases. Some of the cases were associated with Schistosomiasis, and in stool Giardia lamblia was present in 14.6 per cent and Shigella in 13.2 per cent cases (21).

At the ICDDR,B about 100 hospitalized patients per year are diagnosed to have typhoid fever (22). Because of its being a diarrhoea hospital most of the patients presenting with diarrhoea in enteric fever attends ICDDR,B. In a retrospective review of 62 patients with typhoid fever Stoll et al (23) found that 94 per cent of the patients had diarrhoea (table 1). The nature of the diarrhoea in these patients was difficult to elucidate from the retrospective survey.

3. Rationale:

Many patients with typhoid fever suffer from diarrhoea to a great extent which may lead to dehydration and need be managed in diarrhoeal hospitals like ICDDR,B. As there are gaps in knowledge about the characteristics of this diarrhoea and the pathogenesis of diarrhoeal episode in enteric fever, it is essential to understand those phenomena more clearly. Management and treatment of diarrhoea now a days depend mostly on the specific disease process. By using knowledge from this study, diarrhoea in typhoid fever will be understood more clearly which may contribute to better treatment in the future.

4. Specific Aims:

(a) To describe carefully the clinical nature of diarrhoea in Typhoid fever.

(b) To examine the mechanism of diarrhoea by testing S. typhi for invasiveness and toxin production.

METHODS OF PROCEDURE:

A total of 50 patients will be studied. All of the study patients will be selected from ICDDR,B Out Patient Department and will be sent to the Study Ward. They will be studied for 2 weeks in the hospital which is the usual treatment period for typhoid fever.

Selection of patients:

Patients of 6 months to 60 years of age with a medical history suggestive of enteric fever and diarrhoea will be selected for the study. A patient with history of continuous fever for 4 days or more with diarrhoea of stool of any character unlike clear dysentery symptoms will be the primary set for selection. Clinical examinations will be performed to search for signs of typhoid fever like temperature $\geq 101^{\circ}F$, lower pulse rate in relation to temperature, splenomegaly, hepatomegaly, presece of rose spots, distended and/or tender abdomen prior to final selection. Patients who were treated previously by Chloramphenicol or co-trimoxazole will not be selected for study.

Sample size:	Fifty in number
Age group:	6 months to 60 years.
Sex:	Both male and female.

Clincal procedure:

Patients selected for the study will be put in study beds. Then a complete medical history will be obtained from the patients and/or attendant. A thorough clinical examination will be done. All samples for study will be taken afterwards. Assessment of nutritional standard will be done by weight-hight and age.

Therapy: patients will be rehydrated with isotonic intravenous fluid in case of clinical dehydration. Oral rehydration solution will not be used. Antibiotic will be started after establishment of enteric fever by blood culture or stool culture or widal test (titre $\geq$100 for adults and $\geq$40 below 10 years) except when clinical judgement indicates earlier treatment in severe illness. Chloramphenicol will be started by 48 hours of admission in a dose of 60 mg/kg body weight divided in 4 equal doses.

A follow-up data collection chart will be filled out every day which will include medical history, clinical findings, vital signs, output-intake of fluid and stool and results of investigations.

The following investigations will be done on the first day:

Blood: A quantity of 6 ml venous blood will be obtained.

1. Total WBC count, differential counts, Hct.
2. Blood culture - 2 separate samples.
3. Widal test.
4. Electrolytes, urea, creatinine.

Stool:

1. M.E. will be done for E. histolytica, Giardia lablia, and other parasites.
2. M.E. for number of W.B.C. and R.B.C. per ml (quantitative) with differential counts according to standard technic of ICDDR,B.
3. pH and concentration of protein will be measured.
4. Occult blood test will be done.
5. Electrolytes of stool will be measured.
6. Two fresh specimens will be sent for culture of S. typhi, Shigella, V. cholerae, Enterotoxigenic E. coli, Campylobacter and ELISA test for Rotavirus in children below 4 years.
7. S. typhi count per gram of stool will be done by quantitative culture in dilutions of 10^{0}-10^{-6} in appropriate media.
8. S. typhi isolates from 15 patients with moderate to severe degrees of purging will be tested for invasiveness by the Sereny test and for LT and ST toxins by CHO cell and suckling mice assays. The rest of isolates will saved for any future need.

A. Sereny's test for invasiveness

According to ICDDR,B special publication No.3 working manual, tests will be performed. Fifteen culture isolates of S. typhi from heavy purging cases of the study patients will be identified and confirmed by inoculating onto Kligler Iron Agar Slant, Motility Indole Urea medum and citrate medium. Fresh smooth strains are stocked onto Blood agar base slant. A culture suspension is

made in sterile normal saline from a lawn of bacteria from blood agar plate grown one day before. Suspension of 10 organism per ml is made and .05 ml is instilled in one eye of a guineapig. One guinea pig will be used for testing each strain. Control positive and negative strains will be used. Following inoculation the animal will be examined every 24 hours for 72 hours.

B. Toxin test:

S. typhi of the 15 heavy purging cases of the study will be tested for ST & LT.

S. typhi strains to be tested will be inoculated into 3.5 ml of Trypticase soy broth +0.6% yeast extract medium in tubes suitable to fit in a roller drum. For each run 2 control toxin positive and 2 control toxin negative strains will be inoculated. Cultures will be incubated at 37°C and run 22-25 circles per minute. After 20-22 hours incubation the culture broth will be centrifuged and the supernate will be divided in two 1 dram vials. One drop of 5 mcg. gentamicin sulphate will be added to one tube and used for ST assay in suckling mice. The other tube will be kept frozen at -70°C and used for chinese hamster ovary cell (CHO) assay for LT.

B1. Infant mouse assay for ST

For each strain to be tested, 2 of 2-4 days old newborn swiss albino mice are taken. The stomach full with milk is seen from outside. Intragastric, percutaneus injection of 0.1 ml of crude culture filtrate with 2% Evan's Blue is given to each of the mouse and kept at 25°C. They are killed after 4 hours by ether. The

The whole intestine is brought out by forceps. The weight of the gut and the rest of body is taken and ratio of the gut to body weight is determined. If that is below 0.070, considered as negative and 0.070 - 0.085 as borderline and those are repeated while ratio over 0.085 are considered positive.

B2. Chinese hamster ovary (CHO) Cell assay for LT:

The assay will be done in the following way: a monolayer of Chinese hamster ovary cell is grown in a 25 CM^2 tissue culture flask under 4.5 ml of Fizmedium with 10% Fetal calf serum (FCS) and antibiotics (100 units/ml Penicillin G and 100 mcg/ml Streptomycin). When a measured quantity of the prepared toxin is added to the cell suspension in a microtitre plate and incubated overnight, a toxin positive preparation will cause enlongation of cells. The number of elongated cells seen under the inverted microscope in each 100 cells may be counted and recorded.

Interpretation: A negative is equal to or less than 9.3 elongated cells/100 cells and a positive is equal to or more than 13.6 elongated cell/100 cells. Tests with 9.3 to 13.6 elongated cells/ 100 cells are border line and should be repeated.

Urine:

1. Analysis will be done from fresh specimens
2. Culture will be done following standard procedure

The following clinical records will be obtained during study period:

1. Pulse - rate, volume - 8 hourly
2. Rectal temperature - 8 hourly
3. Rate of respiration - 8 hourly
4. Blood pressure - 8 hourly
5. Stool - character, colour, consistency, frequency, quantity by weight - hourly
6. Vomiting - 8 hourly
7. Urine output - 8 hourly
8. Fluid intake quantity - 8 hourly
9. Body weight - daily

The following laboratory tests will be performed as scheduled:

1. Stool culture - on the 1st, 7th and 14th day. For those positive a quantitative culture will be done.

   Stool ME with differential cell counts/per cc - 1st, 7th, 145h day
   Stool - pH and protein concentration - 1st, 7th, 14th day
2. Blood for widal test: 1st and 14th day
3. Blood for culture: 1st day

   Blood - C.B.C. - 1st, 14th day
4. S. typhi sensitivity to drugs: 1st positive isolate
5. Urine culture: 1st day

Analytical frame work:

1. Means + S.D. of quantitative analysis of stool
2. Means + S.D. of electrolytes of stool and blood
3. Frequency of positive cultures of blood, stool and urine
4. Means + S.D. of fever, diarrhoea, positive culture of stool
5. Variation in clinical picture according to age and sex
6. Comparing means + S.D. of frequency of diarrhea in patients with S. typhi alone in stool and patients of typhoid with other pathogens.
7. Comparing with patients with S. typhi in stool with S. typhi in blood alone.
8. Statistical tests: as required.

D. SIGNIFICANCE:

The results of this study will be able to show whether the diarrhea is a major component of clinical presentation of enteric fever and whether it follows a course of definite nature. The mechanism of diarrhea will be known from this study which will directly add to our present knowledge in this field. The future management of diarrhea of enteric fever may be improved as a result of this study.

E. FACILITIES REQUIRED:

I. II. II, IV, & V - All will be available from the existing set up and mentioned in budget section.

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SECTION III - BUDGET

A. DETAILED BUDGET:

1. Personnel Services:

Name	Position	% of effort	Project Requirement Taka	Dollar
Dr. S.K. Roy	Principal Investigator	25%	15000	-
Dr. T. Bulter	Co-Investigator	10%	-	6700
Dr. P. Speelman	do	5%		1850
Dr. B. Stoll	do	5%		1100
Mr. A.R.M.A. Alim	do	10%	6000	
3 Senior Staff Nurses		10%	7000	
2 Aid Nurses		5%	2000	
1 Sr. Lab. Technician (Cl. Path.)		20%	6000	
1 Statistician		5%	3500	
Programer (Computer)		5%	4000	
Dr. A.S.M. Hamidur Rahman	Co-Investigator	5%	2500	
			Total Tk. 46000	US$ 9650

2. Supplies and Materials:

(a) Clinical supplies. Needles, gloves, syringe, testube, etc US$ 500

(b) Lab. test

Blood:	Cost	Total
C.B.C. - 3	Tk. 4.20	Tk. 630
Widal - 2	Tk.29.00	Tk.1450
Culture - 2	Tk.15.00	Tk.1500
Electrolytes - 2	Tk. 12.00	Tk.1200

Stool	Cost Tk.	Total Tk.
Microscopy - 3	2.50	350.00
Culture - 3	15.00	2250.00
Electrolyte with protein - 2	11.00	1100.00
Quantitative S. typhi culture - 1	10.00	500.00
Elisa - 1	50.00 X 30	1500.00
Urine:		
Analysis - 2	3.00	300.00
Culture - 1	7.50	375.00
Toxin Assay:		
Sereny test - 1	50.00 X 15	750.00
LT, ST test - 1	30.00 X 15	450.00
	Total Tk.	12355.00

C. Stationaries 3000.00

3. Equipment Nil

4. Patient hospitalization: 50 X 14 X Tk.150 105000.00

5. Out patient care - Nil

6. ICDDR,B Transport - Nil

7. Travel and Transportation of persons: Attending International Seminar on typhoid fever. US $ 2000.00

8. Travel and Transportation of things: Nil

9. Rent communication and utilities: 1 Filing Cabinet Tk. 2000.00

10. Printing and Reproduction: US $ 500.00

11. Other contractual services: None

12. Construction, Renovation, Alteration: None.

B. BUDGET SUMMARY:

	Taka	Dollar
1. Personnel services	46000	9,650
2. Supplies and Materials	15355	500
3. Patient hospitalization	105000	-
4. Travel and Transportation	-	2000
5. Rent, Communication, Utilities	3200	-
6. Printing and Reproduction	-	500
Total	Tk. 169555	US $ 12650

(1 US $ = Tk. 20) = US $ 8478

Total US $ = 21128

Incremental cost 10% = US $ 2113

Grand Total = US $ 23241

Table - 1

Salmonella isolates of ICDDR,B Hospital, 1981

1981:	R/S	S. typhi	Sal(other)	Total
January	244	13	3	16
February	208	10	3	13
March	293	13	1	14
April	440	8	3	11
May	347	19	3	22
June	312	8	3	11
July	272	6	2	8
August	307	6	4	10
September	279	8	3	11
October	236	6	7	13
November	260	3	4	7
December	282	2	5	7
	3580	102	41	143
1982:				
January	277	5	2	7
February	265	10	5	15

Table 1
Diarrhea and Typhoid Fever
ICDDR,B Hospital, Dacca, Bangladesh
August 1, 1979 - July 31, 1980

	N	%
History N=62		
diarrhea	58	94
watery stools	46	74
stool with mucus	23	37
stool with blood	11	18
Stool Examination N=55		
description:		
loose	53	96
formed	2	4
mucus	52	95
blood	3	5
pH:		
acid	9	16
alkaline	46	84
WBC:		
0	1	2
1-10	22	40
>10	32	58
RBC:		
0	28	51
1-10	23	42
>10	3	5
unknown	1	2
Stool Culture N=62		
Negative	48	77
S. typhi only	11	18
Sh. flexneri only	2	3
S. typhi + *Sh. flex*	1	2

From Barua & Stoll
M. Alam

ABSTRACT SUMMARY FOR ETHICAL REVIEW COMMITTEE

1. Young children from 6 month to 12 years suffer from diarrhoea with dehydration and pass mucoid-bloody stool with bacteremia in Typhoid fever. Adults also have diarrhoea of significant amount in typhoid fever. Present knowledge is not adequate in diarrhoea of Typhoid fver. This study proposes to study the nature of diarrhoea which is often found in typhoid fever causing greater illness in sick or toxic patients. The purpose of this study is to learn the diarrhoea of this specific aetiology to improve present status of basic knowledge in the field of diarrhoea. Better management of diarrhoea of typhoid fever may be possible by using knowledge from this study. Rationale for studying this case is to improve the present status of knowledge for better care of patients in future.

2. There are no risk for the patient, except pricks of drawning blood from veins which are regularly done in this hospital with standard aseptic precausious. There is no other alternative method. There is no risk for participation in the study.

3. Early attention of bacterima will help the children. The suffering of the disease will be rather reduced due to special attention of research persons to the patients. There will be no increase of in convenience due to this study.

4. Confidentiality will be maintained with data and informations to be handled by investigators. Study code numbers and patient's number will be used more than specific name. Data will be kept under lock.

5. Signed consent will be taken from the patients or guardian with proper explanation of study procedures and purpose. Compensation is not required. Proper medical care will be taken and dietery arrangement will be done.

6. There is interview of medical history of this illness which will be better helpful and acceptable to the patients for proper care in disease. An interview of about 10 minutes will be taken.

7. Patients will be directly benifited from this study by specific treatment and care by the investigators and thorough investigations. Family members will be benifited to help suffering members in such disease. The benifit to the society will be to a great extent by increasing knowledge about diarrhoeal diseases which may help in developing better strategies for treatment in future. Specific care and early identification of this serious disease will help the patient than little inconvenience of hospitalization.

8. The study will require the use of patient records and blood, stool, urine samples as in this hospital. Fetus and abortus samples will not be used.

CONSENT FORM

(Typhoid - diarrhoea study)

Title: Characteristics of diarrhoea in typhoid fever.

Statement to be read to the patient or guardian before consent is obtained

Typhoid fever is a major problem in all developing countries. It affects children and adults seriously. A patient of typhoid fever sometimes starts passing loose or liquid stool and with mucous and blood in it to make any of us worried for proper care even hospitalization. A present, the knowledge of diarrhoeal problem in case of typhoid fever is not enough and it is needful to undertake research work to know it better for improvement in treatment. In our country, magnitude of this problem is decumented to be high and we are facing increased number of patients with diarrhoea in typhoid fever in the ICDDR,B. You are aware that ICDDR,B is serving you all for treatment of all sorts of diarrhoea and is continuing research activities on the same aspect for improvement of care for suffering humankind. If you agree we shall include your patient/ you in the present diarrhoea study after diagnosis of typhoid fever. If you co-operate us and participate in the study the following samples will be taken.

1. Stool - samples on several days.
2. Urine samples on two occasions.
3. Venous blood samples 6 ml for 2 ways.

All other standard treatment procedures.

Duration of study will be of two weeks.

You will be informed all the results of investigations on request. If you like to refrain from the study at any time, you shall have the right, though your treatment and care will be continued as usual.

You will be acknowledged by all to have contribution in this study.

If you agree to participate in our study please put your signature or lelt thumb impression below.

Signature of the Investigator.

Witness:

1. _____

Signature or L.T.I. of patient or guardian.

Date: _____

Case No. _____

সম্মতি পত্র

(টাইফয়েড ডাইরিয়া গবেষণা)

গবেষণা আরম্ভের পূর্বে রোগী বা অভিভাবককে পড়িয়া শুনাইতে হইবে

উন্নয়নশীল দেশ সমূহে টাইফয়েড রোগ ছোট বড় সকলের জন্যই একটি মারাত্মক ব্যাধি। টাইফয়েড রোগে পীড়িত রোগী বার বার পাতলা পায়খানা বা আমরক্ত সহ পায়খানার পর দুর্বল হয় তখন আমরা উদ্বিগ্ন হই এবং হাসপাতালের শরণাপন্ন হই। এই ধরণের পায়খানায় আমাদের বর্তমান জ্ঞান যথেষ্ট নয় এবং তা আরও সম্পূর্ণ হতে হলে অনেক গবেষণা বা পরীক্ষা নিরীক্ষার প্রয়োজন। পৃথিবীর অনেক দেশে এসম্বন্ধে চিকিৎসা বিষয়ক গবেষণা আরম্ভ হয়েছে। আমাদের দেশে বয়স্ক কিংবা শিশু বহু লোকই এই রোগে কষ্ট পায় - সেজন্য আমাদের এই হাসপাতালে ডাইরিয়া চিকিৎসার সাথে সাথে গবেষণার কাজ হাতে নেওয়া হয়েছে। আপনাদের জানা আছে কলেরা হাসপাতাল-এর সময় থেকে আই, সি, ডি, ডি, আর, বি, এখন পর্যন্ত দীর্ঘ বিশ বৎসর ডাইরিয়া চিকিৎসা ও গবেষণার মাধ্যমে আমরা পীড়িত মানুষের সেবা করে আসছি - যে কারণে আজ এত উন্নতমানের ও সহজ চিকিৎসা পাওয়া যাচ্ছে।

টাইফয়েড রোগের ডাইরিয়া যাতে আরও ভাল করে চিকিৎসা করা যায় সেজন্য আমরা বর্তমান গবেষণায় ব্রতী হয়েছি।

আপনাকে বা আপনার শিশুকে আমরা টাইফয়েড রোগ নির্ণয় করা হলে এই গবেষণায় অংশগ্রহণ করতে আহ্বান জানাই। যদি আপনি রাজী থাকেন তবে নীচের ব্যবস্থাগুলি আপনার জন্য প্রযোজ্য হবে।

আপনাকে ২ সপ্তাহ পর্যন্ত চিকিৎসা ও গবেষনার অধীনে রাখা হবে।

বিস্তারিত পরীক্ষার জন্য সি.সি. রক্ত ২ বার পরীক্ষা করা হবে। কয়েক বার পায়খানা ও প্রস্রাব পরীক্ষা করা হবে। অন্যান্য ঔষধপত্র প্রয়োজনমত দেওয়া হবে। আপনার উপযুক্ত চিকিৎসার সমস্ত ব্যবস্থা নিশ্চিত করা হবে এবং আপনার যে কোন প্রকার অসুবিধা যত্নযোগ সহকারে নিষ্পত্তি করা হবে।

আপনি ইচ্ছা করলে নিজেকে বা আপনার শিশুকে যে কোন সময় এই গবেষণা হতে মুক্ত করতে পারেন - সেজন্য আপনার চিকিৎসা ব্যবস্থার কোন ত্রুটি হবে না।

আপনি যদি এই গবেষণায় অংশগ্রহণে রাজী থাকেন তবে নিম্নে আপনার স্বাক্ষর বা টিপ সহি দিন।

স্বাক্ষর প্রধান গবেষক

তারিখ----------

সাক্ষী----------

স্বাক্ষর/টিপ সহি

নাম----------

রোগী নং----------