chment 1.

Date 25.08.88

ETHICAL REVIEW COMMITTEE, ICDDR,B.

cipal Investigator Dr. Christne Wanke Trainee Investigator (if any) N/A

ication No. 88-022 Supporting Agency (if Non-ICDDR,B) _____

e of Study Characterization of erence Traits of E. coli and their ociation with Diarrheal Illness in ladesh.

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

le 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: NA
(a) Nature and purposes of study Yes No NA
(b) Procedures to be followed including alternatives used Yes No NA
(c) Physical risks Yes No NA
(d) Sensitive questions Yes No NA
(e) Benefits to be derived Yes No NA
(f) Right to refuse to participate or to withdraw from study Yes No NA
(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: NA
   (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). NA
   _/_ Protocol (Required)
   ___ Abstract Summary (Required) NA
   ___ 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 NA
   ___ Informed consent form for parent or guardian NA
   ___ Procedure for maintaining confidentiality NA
   ___ Questionnaire or interview schedule * NA

* 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.

(PTO)

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

Christine Wanke
Principal Investigator

AUG 29 1988
Trainee

SECTION 1 - RESEARCH PROTOCOL

1. Title : Characterization of Adherence Traits of E. coli and their Association with Diarrheal Illness in Bangladesh.

2. Principal Investigator : Dr. Christne Wanke

   Co-Investigator : Dr. Bradford A. Kay
   Dr. Khaleda Haider
   Dr. Fitzroy Henry
   Dr. Abdul Baqui
   Dr. A.N. Alam
   Dr. Gary Hlady

3. Starting Date : After approval

4. Completion Date : 6 months after starting

5. Total Direct Cost : 12, 220

6. Scientific Program Head :

   This protocol has been approved by the Laboratory Sciences Division.

   Signature of Scientific Program Head : _____

   Date : Aug. 23, 1988

7. Abstract Summary :

Diarrheal disease remains the single most serious threat to the life of children in the developing world, including Bangladesh. Escherichia coli are known to be one of the major pathogens causing diarrhea in this setting; there are four major groups of E. coli which cause diarrhea. These are the enterotoxigenic (ETEC), the enteropathogenic (EPEC), the enterohemorrhagic (EHEC) and the enteroinvasive (EIEC). The ability of E. coli to adhere to the small bowel promotes the diarrheagenic capability of at least three of these groups.

ETEC adhere by means of fimbriae and then deliver toxin. The enteropathogenic E. coli adhere by a poorly understood attaching and effacing mechanism. The enteroinvasive E. coli adhere before they invade mucosal cells. Various bioassays of these mechanisms of adherence are available, including patterns of species specific mannose resistent hemagglutination of red cells for ETEC strains and HEp-2 cell adherence for EPEC strains. In addition, bacterial surface characteristics such as hydrophobicity and net surface charge may correlate with the ability of various E. coli to adhere. We propose to examine 400 E. coli strains isolated from children less than 1 year of age with diarrhea seen at the 4% surveillance system of the ICDDR,B (Appendix A) and 120 E. coli strains isolated from children with prolonged diarrhoea in a community population (Appendix B) for these adherence characteristics. We wish to determine which adherence factors are important in these populations, and wish to identify adherence traits which may be blocked as a means of preventing diarrhea. E. coli bioassays required for this protocols can be readily performed in the laboratories of ICDDR,B. Currently no evaluation of E. coli adherence characteristics is being done at the Center.

8. Reviews :

a. Ethical Review Committee : _____

b. Research Review Committee: _____

c. Director's signature and remark, if any : _____

C. SECTION II - RESEARCH PLAN

A. INTRODUCTION:

1. **Objective :** This protocol has three major objectives:

a. to identify the colonizing traits important in ETEC diarrheal disease seen at ICDDR,B and to characterize new colonizing factors that may be identified.

b. to define the role of the EPEC in diarrheal disease seen at ICDDR,B as determined by HEp-2 cell adherence pattern.

c. to compare the surface characterisitics of apparently non-pathogenic E. coli recovered from children with diarrhea at ICDDR with known pathogenic strains in an attempt to correlate surface charactersitics with the potential to cause diarrheal disease.

2. **Background :**

Escherichia coli: E. coli are one of the most common causes of diarrheal illness in pediatric populations in the developing world. (1, 2) Although E. coli are a part of normal flora in a majority of individuals, there are E. coli which are clearly capable of causing diarrheal disease in humans and animals by at least four well described mechanisms (3,4,5). The ETEC produce secretory toxin (heat labile and heat stable) which cause net isotonic fluid secretion into the gut (3). The EPEC attach to and efface the small bowel mucosa by a mechanism as yet unknown. EHEC produce a cytotoxin which causes bloody diarrhea, and EIEC invade the colonic mucosa to produce a dysenteric illness.

ADHERENCE: The ability of a microorganism to adhere to the tissue on which it causes disease is a well recognized first step in the pathogenesis of infectious diseases. The adherence of organisms is associated with disease in multiple systems throughout the human body (6,7). The ability of oral microflora to attach to teeth and dental plaque is recognized as a major step in the development of dental caries. The ability of E. coli to adhere to renal epithelium by means of P or S fimbriae can distinguish those E. coli capable of causing upper urinary tract infections or pyelonephritis from those limited to lower tract disease. Microorganisms capable of causing bacteremia or meningitis (such as E. coli, Pseudomonas, Staphylococci, and Candida) are known to have surface adherence characteristics that mediate their ability to invade the blood stream or cross the blood-brain barrier. Recently recognized adhesins of Hemophilus influenza may be pertinent to the abilitiy of these organisms to cause respiratory tract disease. In organisms capable of causing diarrheal illness, the ability to adhere to intestinal mucosa has often been recognized as an important early step in pathogenesis. Adherence is recognized in parasites such as Giardia, Entaemeba histolytica, and probably cryptosporidiosis. Adherence traits are important in the initiation of diarrheal disease by such diverse bacterial pathogens as Campylobacter, Vibrio cholerae, E. coli and Shigella (9,10).

E. COLI ADHERENCE:

ETEC: Both human and animal ETEC strains often possess, in addition to one or more enterotoxins, fimbriae capable of attaching to enterocytes of the small bowel. In ETEC infections in animals, these fimbriae are relatively species -specific and include porcine K88, 987 and bovine K99 and F41 (11).

The Colonizing Factor Antigens (CFA) of human ETEC were described in the 1970's by Evans and Evans (12). CFA's have been characterized by their ability to hemagglutinate specific species of red blood cells; by their appearance on negative stain under electron microscopy; and by their antigenic composition. CFA I agglutinates bovine red blood cells and is a 7 nanometer fimbria which is antigenically homogeneous. CFA II agglutinates bovine, chicken and human A red blood cells, has both 7 nm and 2 nm fimbriae and fibrils and appears to be antigenically heterogeneous. It is composed of subunits called coli surface (cs) antigens (1,2,3). A third colonizing fimbriae was described by Rowe and is known as E8775. This fimbriae also agglutinates bovine, chicken and human A red blood cells and has both 7 and 2 nm fimbriae which are antigenically heterogeneous (composed of cs 4,5,6). It is antigenically distinct from CFA II (13). In 1984, Honda and Miwatani described a fimbrial appendage seen by electron microscopy on E. coli strains isolated from a diarrheal outbreak in Japan. These strains did not hemagglutinate and appeared antigenically distinct from CFA I, II and E8775 (14). In 1987 Knutton described a 3 nm fibril on

ETEC strains seen to adhere to small bowel epithelium (15). Both of these may be additional colonizing factors.

Whether these colonizing factors are essential for the production of ETEC disease remains controversial. Studies which have examined ETEC for colonizing factor antigens from diarrheal outbreaks or in endemic areas have reported from 0% of strains to 86% of strains to be positive for CFA I and II (16,17). In 1985, Gothefors surveyed ETEC strains from the ICDDR,B surveillance program and from children hospitalized at ICDDR,B by immunodiffusion for CFA I and II and found 75% to be positive (18). Diarrhea appeared to be clinically more severe in those children whose E. coli had both enterotoxins and CFA's.

Various theories are used to explain strains which do not have CFA's. These include the possible loss of plasmid DNA coding for the CFA, the existence of unrecognized colonizing factors, as well as the possibility that CFA's are not essential for the production of ETEC diarrhea. It is known that plasmids can be lost from ETEC strains after serial subculturing (19). The description of colonizing fimbriae other than CFA I,II,III by Honda and Knutton suggests that there may be additional unrecognized colonizing factors. Various studies have been done to evaluate the necessity of CFA in production of disease. Smith and Linggood (11) in 1971 prepared a series of porcine ETEC strains which were enterotoxin positive-CF (K88) positive; enterotoxin negative-CF positive; enterotoxin positive-CF negative and enterotoxin negative-CF negative. They found that

20/25 piglets given enterotoxin positive-CF positive strains developed diarrhea, 0/8 piglets given enterotoxin positive-CF negative strains and 0/11 piglets given enterotoxin negative-CF negative strains developed diarrhea. Satterwhite used CF positive-toxin positive strains in human volunteers and found that 6/7 volunteers receiving 8 logs of the toxin positive-CF positive strains developed diarrhea as opposed to 0/6 volunteers receiving the same dose of toxin positive-CF negative strains (20). In 1980, Levine found that 2/2 volunteers given CF positive-toxin positive strains and 4/5 volunteers given CF negative-toxin positive strains also developed diarrhea, suggesting that CF may not be essential in ETEC disease production (21). Levine's study did not examine the possibility that unrecognized CF's were present on the non CFA I or II strains which caused diarrhea.

EPEC: The enteropathogenic E. coli/enteroadherent E. coli have been associated with diarrheal illness since the 1950's. However, only in the 1970's did intestinal biopsy specimens of children with persistent diarrhea reveal the closely adherent-mucosal effacing nature of the association of EPEC and the gut (22). Attempts to associate other virulence factors such as hemolysins or to consistently associate EPEC seotypes with diarrheal disease have been largely unsuccessful.Work done by Scaletsky (23) with EPEC adherence to HeLa cells and more recently by Cravioto and Nataro (24,25) with HEp-2 cells has revealed an association between the ability of E. coli in EPEC

serogroups to adhere focally to tissue culture and the production of diarrheal disease. More recently, an additional pattern of adherence, agglutinating (or adherence in the pattern of "stacked bricks") has been associated with EPEC and ETEC serogroups able to produce diarrheal disease. EPEC strains which adhere diffusely to HEp-2 cells do not appear to be associated with diarrheal disease in these studies (25,26). How the EPEC cause diarrhea remains unknown. Some strains of EPEC have been shown to produce low levels of vero-cytotoxin and theoretically, the EPEC organisms could deliver this toxin directly to the mucosa when closely adherent. No enterotoxins or secretory toxins have been isolated from EPEC strains (27).

ADHERENCE OTHER THAN EAF AND THE PRODUCTION OF DIARRHEA

As there is controversy about the necessity of CF for the production of ETEC diarrhea, there is also controversy about the ability of E. coli strains which have colonizing ability or pili to produce disease when enterotoxins are not present. This potential was first identified by Smith and Linggood in 1971 (11) in their engineered porcine strains. 30% of strains which had the K88 fimbrial CF and did not have enterotoxin produced diarrhea in piglets. Levine in 1983 reported 10% of human volunteers given a CFA II positive-toxin negative ETEC developed mild diarrhea. Wanke and Guerrant used this same isogenic strain pair (1392 with CFA 11 and without toxin and 1392 without CFA 11 and without toxin) in the reversible ileal tie model in weanling rabbits. This study demonstrated the consistent association of a high level of small bowel colonization with the development of

diarrhea. Diarrhea did not develop in any animal given the CFA negative strain (29). Sack noted the development of diarrheal illness in 50% of rabbits given 1392 with CFA and no diarrhea in the animals given 1392 without CFA (30). 30% of the rabbits given the CFA positive strain developed lethal diarrhea, but there was no significant difference in the degree of small bowel colonization between the animals given the 1392 with CFA and 1392 without CFA strains in this study.

The mechanism by which this diarrhea occurs is not known. There are no apparent changes in villus structure by light microscopy (29), no differences in levels of cyclic AMP, cyclic GMP or brush border enzymes. between diarrheal animals and control animals (Wanke and Guerrant, unpublished data.) E. coli do not deconjugate bile acids and lead to the production of osmotic diarrhea by malabsorpsion of fat. The fluid recovered from the small bowels of these animals is isotonic, an addditional fact not consistent with osmotic diarrhea.

While the mechanisms of this presumably secretory process related to colonization are not understood, there are well known clinical associations between diarrhea, malnutrition and small bowel overgrowth (31,32,33,34). Organisms recovered from children in Australia included fecal gram negative rods; in Mexico, Klebsiella and E. coli; in the Gambia, Klebsiella, E. coli, Enterococcus, Pseudomonas and Bacteroides species. While on occasion, some of the small bowel organisms have belonged to EPEC serotypes, rarely are recognized pathogens isloated from small

bowel fluid. None of these organisms have been studied for their adherence characteristics, which may play a role in the production of diarrhea.

OTHER BACTERIAL SURFACE CHARACTERISTICS THAT ARE ASSOCIATED WITH ENTERIC ADHERENCE:

The previously discussed fimbrial CFA and the adhering and effacing nature of the EPEC E. coli are well described. There remains a variety of surface characteristics of bacteria that are associated with adherence in other systems that have not been systematically evaluated in E.coli recovered from diarrheal stools. Campylobacter jejuni and Vibrio cholerae (10) have demonstrated outer membrane proteins, surface hydophobicity and surface charge that are associated with the ability to adhere (35,36). The lipopolysaccharide capsule of H. influenza has recently been examined as a potential adherence trait.

3. Rationale :

As there is little to no data available about the adherence characteristics of diarrheagenic E. coli in Bangladesh, this study can provide valuable information in this regard. The distribution of adherence traits in hospital and community based populations can help define the role that E. coli with these traits play in clinical disease. Correlation of these traits with the clinical description of illness can provide useful data about the full spectrum of E. coli disease. Systematic screening of E. coli for colonizing fimbriae can provide useful information abut new colonizing factors associated with ETEC disease.

Screening E. coli for tissue culture adherence will provide valuable information about the role of the EPEC and the newly described auto aggluting E. coli in Bangladesh. The ability of strains with no currently recognized virulence traits to cause diarrhea in animals can provide useful information about new pathogenic mechanisms of diarrheal disease by E. coli. All of this adherence data will be of value in the development of antiadherence vaccines, in the development of non-antibiotic-anti adherence agents for the treatment of diarrheal disease and in studies of the pathogenesis of diarrhea.

B. SPECIFIC AIMS:

In parallel with the protocol by Strockbine et al. using DNA probes for focal and diffuse adherence to evaluate E. coli isolated from children less than 1 year seen at the ICDDR,B surveillance program, we propose to examine the bioassay and biochemical evidence of adherence traits by:

Hemagglutination with bovine, chicken, guinea pig and human type A red blood cells.

HEp-2 cell adherence for focal, diffuse and agglutinating patterns.

Hydrophobic Interaction Chromatography and/or salt aggregation.

Surface charge by ionic chromatography.

Ability of strains with adherence potential but no recognized entero or invasive toxin to cause diarrhea in the rabbit reversible ileal tie model.

C. METHODS

Population: stools and strains are being collected from 400 (Appendix A) children less than 1 year who are screened by ICDDR,B surveillance. These strains will be studied routinely by ELISA for LT/ST and by DNA probes for adherence and invasive potential.

In addition, selected strains collected by Dr. Fitzroy Henry from his prospective study of prolonged diarrhea in Zinzira and Mirzapur will be studied (Appendix B).

Strained selected by Dr. Abdul Baqui from his prospective community study of the risks for prolonged diarrhea in Matlab will also be studied (Appendix B).

These three populations will allow a survey of the potential differences in occurrence of adherence traits between hospitalized and community populations and between acute and prolonged diarrhea.

ASSAYS

Hemagglutination assays will be performed with red blood cells of bovine, guinea pig, chicken and human origin to determine the patterns of agglutination that are consistent with CFA I, II and E8775 fimbriac as well as type 1 pili.

Hemagglutination assays will be performed in glass, with a 1:4 dilution of citrated red blood cells, less than 5 days old. Bacteria for assay will be freshly grown on CFA agar to maximize the production of fimbriae. 1% mannose will be added to the RBC solution to determine if the hemagglutination is sensitive or resistent to mannose.

EAF:

HEp-2 cells will be grown in minimal essential media, plated on sterile glass coverslips in 24 well Costar tissue culture plates at the time of assay. Prior to reaching confluence, the cells will be incubated with 100 microliters of test bacterial suspension with 1% mannose for 3 hours, washed five times with phosphate buffered saline, fixed with methanol, stained with Giemsa and examined by light microscopy for evidence of the 3 patterns of adherence.Known positive controls and known non HEp-2 adherent bacteria will be included in each assay.

HYDROPHOBICITY

Either bacterial surface proteins or bacterial fimbriae may be hydrophobic. The hydrophobic interaction between bacterial cells and gut mucosa formed by the displacement of water molecules may be a mechanism of adherence. Any E. coli with pili will be hydrophobic so all strains which hemagglutinate will be screened for the degree of hydrophobicity by adherence to phenyl sepharose microcolumns. Strains which do not hemagglutinate but have enterotoxins will also be screened by HIC for potential new colonizing factors.

Strains which do not have toxin and do not hemagglutinate will first be screened by salt aggregation (SAT) with a range of salt concentration from .05 M to 4.0 M ammonium sulfate for hydrophobicity. Those that are hydrophobic by SAT will be further characterized by phenyl sepharose HIC using ammonium sulfate concentration values lower than the SAT value. Since E. coli strains become more hydrophobic when they became rongh, aggregation will also be checked in physiological saline.

Strains will be grown on CFA agar for maximum production of pili. Known hydrophobic and nonhydrophobic strains will be included as controls in each assay.

SURFACE CHARGE:

The negatively charged surface proteins of bacteria may mediate ionic interactions with bowel mucosa, providing another mechanism of adherence. Charge of surface proteins of E. coli strains can be determined by % adherence to ionic chromatography microcolumns. DEAE sepharose will be used to determine % adherence (relative negative surface charge) of strains with toxin which have no HA pattern consistent with a CFA; strains without toxin, and strains that fall into the EPEC groups by tissue culture assay.

ANIMAL STUDIES

The reversible ileal tie model will be used in post weanling rabbits. E coli strains which do not have LT or ST enterotoxins, invasive potential (as determined by probe) and which do have

surface characteristics suggestive of adherence (more than 70% adherence to phenyl sepharose and DEAE sepharose) will be tested in the reversible ileal tie model for ability to cause diarrhea and small bowel colonization.

The reversible ileal tie was first descibed by Sack and Spira in adult rabbits and was used for the production of antibody to colonizing bacteria. Later this method was used by Wanke and Guerrant in weanling rabbits to evaluate the association between the small bowel colonization and the production of diarrhea by nontoxigenic E. coli with colonizing ability. 70% of rabbits given the colonizing nontoxigenic E. coli developed watery diarrhea. Any rabbit that did develop diarrhea was colonized with at least 8 logs of the same E. coli per sq. cm of mucosa. Any rabbit that was colonized with 8 logs of organisms developed diarrhea. Rabbits given the noncolonizing nontoxigenic strain of the isogenic pair did not colonize and did not develop diarrhea.

Under general anesthesia, the blind cecum of the weanling rabbit will be ligated. 10 cm proximal to the ileal cecal junction a slip knot will be placed around the small bowel. 10 cc of test E. coli strain at 8 logs of organisms/cc in broth culture will be inoculated into the proximal ileal lumen. The abdominal incision will be loosely closed and anesthesia maintained for 4 hours. After four hours the slip knot will be removed from the distal ileum, the abdominal incision completely closed, and the rabbit monitered for the development of diarrhea.

At 72 hours or if the rabbit becomes debilitated from diarrhea, the animal will be sacrificed and quantitative cultures of 1 cm of small bowel mucosa will be done by serial dilution to determine the extent of colonization.

D. SIGNIFICANCE

The results of these bioassays will be analyzed and compared with the information about the three populations.

1. Routine information available on surveillance patients including age, sex, presentation, type of diarrhea, pathogens isolated, nutritional status, treatment history and outcome.

2. Information as made available by Dr. Henry and Dr. Baqui as to the duration of diarrhea and when during the course of diarrhea the specimen was collected.

The results of the bioassays will also be compared with the results of the DNA probes and the results of the invasive bioassays.

This information will be used to assess the following:

1. the frequency of adherence traits in these populations with ETEC diarrhea.

2. Possible epidemiologic and clinical correlations of diarrhea with the adherence characterisitics.

3. The association of E. coli with only adherence characterisitics in the presence of diarrhea and the ability of these strains to produce diarrhea in an animal model. Thus investigating the possible pathophysiologic significance of adherence traits of E. coli.

4. The correlation of the bioassays for tissue culture adherence with the DNA probes.

5. The overall significance of various adherence traits of E coli in these populations.

E. FACILITIES REQUIRED:

All work will be done in existing laboratory space or the animal house of the ICDDR,B.

F. COLLABORATIVE ARRANGEMENTS :

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PS: CW.PRO(050888)

Appendix A

400 HOSPITAL SURVEILLANCE STRAINS (DNA PROBE STUDY STRAINS)

30% of strains presumed ETEC (120 strains)
for hemagglutination to determine patterns consistent with CFA's

Assume 50-80% strains contain CFA's
60-96 strains remain for screening by salt aggregation

Strains which agglutinate <0.5M:
for Hydrophobic Interaction Chromatography
↓
for electron microscopy (when and if available)
to assess for pili

Strains which agglutinate at >0.5-1M:
for DEAE chromatography

Therefore assume 70% strains (280 strains) are non-ETEC
for HEp-2 tissue culture adherence for focal and agglutinating adherence

Appendix B

PROSPECTIVE COMMUNITY STUDIES OF PROLONGED DIARRHEA
(Strains from Drs. Baqui and Henry)

Number of episodes of prolonged diarrhea uncertain;
Estimated 60 episodes per each study

Therefore: 120 strains E.coli
Assume no ETEC from prolonged diarrhea

120 strains for HEP-2 tissue culture adherence assay for focal and diffuse adherence.

Because these E. coli strains will be from prolonged diarrhea of presumed unclear etiology, any type of adherence should be investigated further by HIC and DEAE chromatography as the mechanisms of focal and agglutinating adherence are not known.

ICDDR,B
1986 BUDGET PROPOSAL
(In US $)

AREA DESCRIPTION

Program Name: LABORATORY SCIENCES DIVISION

Project/Protocol/Branch Name: Characterization of Adherence Traits of E. coli ...

Principal Investigator/Branch Head/Program Head: Dr. C. Wanke/I. Ciznar

Budget Code:..... Estimated Beginning Date:.....

Protocol No:..... Estimated Ending Date:.....

EXPENSE CATEGORY		*Column A Total Project Cost	Column B Actual Jan-Sept. 1985	Column C Estimated Whole Yr. 1985	Column D Proposed 1988
A/C No.	Description / Refer Page				
3100	Local Salaries 2				1980
3200	Intl. Salaries 8				0
3300	Consultants 14				3600
3500	Travel Local 15				0
3600	Travel Intl. 16				1500
3700	Supplies & Mat. 18				390
3800	Other Costs 19				100
4800	Inter Deptl. Ser. 20				4650
	Total Direct Operating Cost				
0300	Capital Expenditure Refer Page 21		**	**	0
	TOTAL DIRECT COST				12220

Refers to entire life of project.
* For Finance use only.

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ZIZ-5.

PERSONNEL REQUIREMENT-(LOCAL STAFF) 1986

	No.of Positions	No. of Man Months	$ Amount
Direct Project/Protocol/Branch Staff at 1.1.1986 Sourced from Page 3			
l:			
New Recruitments Sourced from Page 4			
Staff allocated from other area Sourced from Page 5	2	12	1980
(i) Sub-Total	2	6	1980
s:			
Separations Sourced from Page 6			
Staff allocated to other area Sourced from Page 7			
(ii) Sub-Total			
(i)-(ii) TOTAL	2	12	* 1980

*Agrees with Page 1 A/C No.3100 Column D

t86.1-3

MANPOWER-ALLOCATED FROM OTHER AREA (LOCAL STAFF)-1986

Designation	level	Budget Code of Other area	No. of Posit.	No.of Man Months	**Rate Per Month	$ Amount
esearch Officer	GS.5	21 02 10	1	6	220	1320
r. Lab. Attendant	GS.2	21 02 10	1	6	110	660
		TOTAL 2		* 12		* 1980

computer printout for average rate per
h.

* AGREES WITH PAGE 2 ROW C

p.1186.5

CONSULTANTS 1989

Job Designation	Travel From-To-From	No. of days	** Perdiem Rate	** Perdiem Total	***Honararium Rate	***Honararium Total	**** Travel cost	Total $ Amount
Dr. C. Wanke	Boston-Dhaka-Boston	120	30	3600				3600
							TOTAL	* 3600

** Check with Finance Ext. 281 for perdiem rate.
*** Check with Personnel Tel. 601100 for honorarium rate.
**** Check with Travel Ext. 234 for ticket cost. Travel cost includes transit perdiem, ticket cost and ground

*AGREES WITH PAGE 1 A/C 3300

TRAVEL PLAN (INTERNATIONAL)-1986

Job Designation	Purpose of Travel	Travel From-To-From	Estim. days of travel (A)	** Perdiem Rate (B)	** Perdiem Amount (C=AxB)	*** Transportation Air (D)	*** Transportation Ground (E)	**** Other Cost (F)	Total $ Amount (C+D+E+F)
Dr. C. Wanke	Boston-Dhaka-Boston	2				1500			1500
						TOTAL			* 1500

** Check with Finance Ext. 281 for perdiem rate.
*** Check with Travel Ext.234 for transportation cost.
**** Includes excess baggage, embarkation fee, travel tax etc.

*AGREES WITH PAGE 1 A/C 3600 COLUMN D

Budget86.16

SUPPLIES AND MATERIALS-1986

A/C Code	Item Description	$ Amount
3701	Drugs (used for medication in the hospitals and field stations)	
3702	Glassware (Bottle, beaker, cylinder, petridish, aluminium seal, slides, stopper, tube etc.)	
3703	Hospital Supplies (bandage, gauze, blade, bowl, catheter, cotton, needle, syringe, solution, leukoplast, towel etc.)	
3704	Stationery and Office Supplies (Battery, book register, binders, files, pencil, fastener, paper, ribbon, stapler etc.)	
3705	Chemicals and Media (Acid, reagent, dextrose, sodium, bactoagar etc.)	390
3706	Materials for Uniform (Cloth, button etc. required for making uniforms)	
3707	Fuel, Oil and Lubricants (Diesel, mobil, petrol, Kerosene etc.)	
3708	Laboratory Suplies (Aluminium foil, bag, blade, brush, cap, container, film X-Ray etc.)	
3709	Housekeeping Supplies (Aerosol, battery, wiping cloth, duster, lock and key etc.)	
3710	Janitorial Suppies (Bleaching powder, brush, detol, detergent, insecticide, soap etc.)	
	(Contd. to Page No. 18)	390

SUPPLIES AND MATERIALS-1986

(Contd. from Page No. 17)

A/C Code	Item Description	$ Amount
3711	Tools and Spares (Automobile spares, tyres, tubes, battery, stores required for maintenance services etc.)	
3712	Non-stock Supplies (Materials not normally kept in stock and purchased only against specific requisitions)	
	Sub-Total	
3713	Freight and other Charges Add 30% to above sub-total	
	TOTAL	390

AGREES WITH
PAGE 1
A/C 3700
COLUMN D

Note: For rates please contact Supply Ext.260 (add 10% to rates for inflation)

Budget86.18

OTHER COST-1986

A/C Code	Accounts Description	$ Amount
3800	Repairs and Maintenance (Maintenance and repairs of vehicles, equipments, furniture and building)	100
3900	Rent, communication and utilities (Postage, telephone, telegram, electricity etc.)	
4100	Bank charges	
4200	Legal and professional expenses (Professional membership fee, legal fee, audit fee etc.)	
4300	Printing and Publication (Printing of forms, books, journals, reprints etc.)	
4400	Entertainment, Hospitality & Donation (Guest house accommodation, donations, hospital food, lunch, refreshment etc.)	
4500	Service Charges (Porter, labour, washing, laundry and other misc. exp.)	
4600	Staff Development and Training (Training course fee, training materials, stipend,scholarship, subsistance paid to the staff)	
	TOTAL	100

AGREES WITH
PAGE 1
A/C No.3800
COLUMN D

Budget86.19

**INTERDEPARTMENTAL SERVICES-1986

A/C Code	Service Area	$ Amount
4801	Computer	500
4802	Transport Dhaka	
4803	Transport Matlab	
4804	Water Transport-Matlab	
4805	Transport Teknaf	
4806	Xerox and Mimcograph	
4807	Pathology	
4808	Microbiology Tests	850
4809	Biochemistry	
4810	X-Ray	
4811	I.V. Fluid	
4812	Media	350
4813	Patient hospitalisation study	
4814	Animal Research	2750
4815	Medical Illustration	100
4817	Telex	50
4818	Laboratory Services Charges	50
4830	Transport Subsidy	
	TOTAL	* 4650

** See annexure B for rates.

*AGREES WITH PAGE 1 A/C 4800 COLUMN D

Budget86.20