<table>
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<th>Question</th>
<th>Yes</th>
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<tr>
<td>1. Are the subjects clearly informed about:</td>
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<tr>
<td>- Nature and purposes of the study?</td>
<td>Yes</td>
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<td>- Discomfort to subjects?</td>
<td>Yes</td>
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<td>- Psychological risks?</td>
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<td>2. Have the procedures to be followed, including consent forms, been reviewed and approved by the appropriate institutional review board?</td>
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<td>3. Are there risks and benefits of study clearly described in the consent form?</td>
<td>Yes</td>
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<td>4. Will subjects be paid or reimbursed?</td>
<td>Yes</td>
<td>No</td>
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<td>5. Will all subjects be informed of their right to withdraw from the study at any time without penalty?</td>
<td>Yes</td>
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<td>6. Will confidential records be maintained?</td>
<td>Yes</td>
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<td>7. Is there a plan for the disposal of all materials used in the study?</td>
<td>Yes</td>
<td>No</td>
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**Study Title:**

**Investigator:**

**Project:**

**Date:**

**Supporting Agency:**

**Abstract Summary:**

**Informed Consent Form:**

**Protocol:**
APPLICATION FOR PROJECT GRANT

1. PRINCIPAL INVESTIGATOR: Dr. Sunday Abraham Aliabi

2. OTHER INVESTIGATORS: Dr. M. John Albert
Dr. Firdausi Qadri
Dr. A.S.G. Faruque
Dr. A.S.M. Hamidur Rahman
Dr. R.B. Sack

3. TITLE OF PROJECT: An evaluation of immune response in Aeromonas-associated diarrhoea, and characterization of strains for some important virulence markers

4. STARTING DATE: November, 1994

5. COMPLETION DATE: April, 1995 (18 months)

6. TOTAL BUDGET REQUESTED: US$ 35,000

7. FUNDING SOURCE

8. HEAD OF PROGRAMME: Dr. R. Bradley Sack
Associate Director
Laboratory Sciences Division

9. AIMS OF THE PROJECT

a) General aim

This study aims at investigating the enteropathogenic role of Aeromonas species in children in Bangladesh. This will be done by evaluating immune responses during Aeromonas-associated diarrhoea as an indirect proof of enteropathogenicity. Isolates will also be characterized for some important virulence markers to provide a clue to their possible pathogenic mechanism(s).
b) Specific aims

1) To measure the level of specific serum and secretory antibody responses to *Aeromonas* species during *Aeromonas*-associated diarrhoea in patients and in age-matched healthy controls.

2) To examine the *Aeromonas* isolates for some properties such as cell surface hydrophobicity, haemagglutination, autoagglutination and resistance to antimicrobial agents, which have been associated with virulence in some *Aeromonas* species and other established enteropathogens.

3) To examine the ability of isolates producing significant antibody titres and potent virulence properties to cause diarrhoea in the Removable Intestinal Tie Adult Rabbit Diarrhoea (RITARD) model.

4) Assess the data obtained to determine the aetiological role of *Aeromonas* species in acute diarrhoeal episodes in children in Bangladesh.

c) Significance

Although it is now generally accepted that *Aeromonas* strains are capable of causing acute diarrhoea in humans, a lot of controversies still surround this claim. There are unusual variations in results of studies conducted in different geographical locations, the mechanism(s) of infection remains very largely unknown, and the Koch's postulates are yet to be conclusively proved for *Aeromonas* as a diarrhoeagenic agent. Hence, these organisms are subject of current and intensive research efforts worldwide. *Aeromonas* species are frequently isolated from diarrhoea patients admitted to the hospitals of
ICDDR.B, and results of this study will throw more light on their clinical significance and characteristics. It is expected that significant antibody responses in diarrhoeal patients and not in the healthy controls as well as the possession of virulence properties, including the ability to cause diarrhoea in rabbits (RITARD) might help to elucidate their pathogenic role. Furthermore, the ICDDR.B provides an excellent facility for the type of case-control study envisaged in this protocol and results obtained might be applicable in most other developing countries where diarrhoea is endemic.

10. ETHICAL IMPLICATIONS

a) This study aims at investigating the role of *Aeromonas* species in acute diarrhoeal disease in Bangladesh. Children under the age of five years will be involved in the study because this age-group is the most vulnerable to diarrhoeal attack. Also, children above this age group are likely to have been exposed to *Aeromonas* infections earlier in life, making it difficult to demonstrate that an increase in specific immune response is due to a current infection.

b) A child’s parent or guardian will be properly briefed on the aims of the study and that it constitutes no health hazard to the child. Consent (preferably written) of parent/guardian will be obtained before a child is included in the study. The following specimens will be collected from each of the children enrolled as cases, for analysis:

- **Stool**: On the day *Aeromonas* species was isolated (Day 1), and 6-8 days after onset of diarrhoea (Day 2).

- **Saliva**: As above

- **Blood (200 µl by finger prick)**: From willing cases on the day *Aeromonas* species was isolated (Day 1), and 14-21 days after onset of diarrhoea (Day 2).
For the controls, only stool and saliva will be collected once on the day the child is enrolled in the study as a control. Finger prick blood will be collected only from willing participants.

**NOTE:** Stool and saliva are normal physiological exudates, while the volume of blood to be collected will not be harmful to the children. A physician who is also a co-investigator in the study, will assist with the collection of blood samples and in assessing the suitability of a given child to participate in the study. The physician research fellow recruited for this protocol will collect the blood samples, and assist with the management of cases admitted to the CRC wards. The Community Health worker will collect the stool and saliva samples, and assist in the identification of appropriate control children in the community.

c) The study will not interfere with the management of patients and the confidentiality of subjects will be maintained.

11. BACKGROUND, RESEARCH PLAN AND BIBLIOGRAPHY

A) BACKGROUND

The genus *Aeromonas* belongs to the family *Vibrionaceae* and consists of organisms that are gram-negative rods, facultatively anaerobic, non-sporeulating and are oxidase positive. In humans, the mesophilic *Aeromonas* group comprising mainly *A. hydrophila*, *A. sobria* and *A. caviae* have been associated with a wide spectrum of diseases, such as soft tissue infections, septicemia, meningitis and food poisoning (Davis *et al.*, 1978; Agbonlahor *et al.*, 1982; Freij, 1984; von Graevenitz and Altwegg, 1991). However, the most frequently reported human infections are gastrointestinal diseases varying from self-limiting watery diarrhoea to acute and persistent dysentery occurring in both children and adults (Holmberg *et al.*, 1986; Gulskin *et al.*, 1992).

In a number of previous studies, *Aeromonas* species have been epidemiologically linked with acute diarrheal syndromes in many countries, including Australia.
(Burke et al., 1983), the United States (Holmberg, and Farmer, 1984), India (Sanyal et al., 1975), Sweden (Ljungh, and Wadstrom, 1985), Ethiopia (Wadstrom et al., 1976), Nigeria (Alabi and Odugbemi, 1990) and Peru (Pazzaglia et al., 1991). Despite these reports, suggesting enteropathogenicity, some controversies still surround the aetiological role of Aeromonas species in diarrhoeal diseases. In Thailand, for instance, isolation frequencies among the native adult population was reported to be similar in diarrhoeal and control cases (Pitarangsi et al., 1982), whereas among US Peace Corps volunteers in the same country, Aeromonas were found in 30.8% of episodes of diarrhoea but only in 8.5% in healthy controls (Echeverria et al., 1981), thereby suggesting differences in endemicity or immunity.

In a recent review by Janda (1991), a new classification system was proposed based on differences in genotypic and phenotypic characteristics. This classification now includes well-known species of A. hydrophila, A. sobria, and A. caviae, as well as recently described A. veronii (Hickman-Brenner et al., 1987), and A. schubertii (Hickman-Brenner et al., 1988). However, their importance remains largely unknown since the few investigations linking them with diarrhoea have been limited to laboratories in developed countries. This new classification will be used in this study.

Furthermore, Aeromonas strains, particularly A. hydrophila and A. sobria, have been reported to produce a variety of biologically active extracellular substances such as haemolysins (Asao et al., 1986; Singh and Sanyal, 1992), enterotoxins (Ljungh et al., 1977; Chakraborty et al., 1984) and haemagglutinins (Atkinson and Trust, 1980; Singh and Sanyal, 1993). They have also been reported to be invasive in HEp-2 cells and rabbit intestine (Watson
et al., 1985; Pazzaglia et al., 1990). Although these are recognized virulence factors in other established enteric pathogens, such as Escherichia coli, further investigations are required to elucidate their roles in Aeromonas-associated diarrhoea. For instance, in a study involving human volunteers in the USA, the administration of various exotoxin-producing A. hydrophila strains reportedly failed to induce diarrhoea in the majority of the subjects (Morgan et al., 1985). In view of these unusual observations, Altwegg and Geiss (1989) concluded that the pathogenic mechanisms described so far for Aeromonas species do not provide sufficient explanation for diarrhoeal cases associated with these organisms; and as-yet unknown virulence factors may account for a significant proportion of the clinical problem, and, most probably, host factors such as the immune status of the patient, play an important role.

In this regard, some laboratories have identified an important group of highly pathogenic Aeromonas strains that have been linked to a variety of human infections as well as diseases in fish (Dooley et al., 1986; Janda et al., 1987; Dooley and Trust, 1988; Kokka and Janda, 1990). This subgroup has been shown to possess a number of closely associated phenotypic and structural properties, such as the ability to autoagglutinate (or autoaggregate) in broth, the presence of common somatic antigen (Sakazaki and Shimada scheme, group 0:11), and the presence of a surface array protein in the form of an S layer on the outermost surface of the bacterium (Paula et al., 1988; Kostrzynska et al., 1992). In A. salmonicida, the S layer has been shown to be the major virulence determinant of its pathogenicity in fish (Ishiguro et al., 1985), and it is suspected that these surface array proteins may play a similar role for serogroup 0:11 Aeromonas strains in both human and animal
infections. These are possibilities worthy of further evaluation.

The intestinal mucosa is known to act as the first line of defense against pathogens trying to invade the human body, and the local immune mechanisms (particularly secretory antibodies) play a central role in this defense. Following contact with an antigen, activated lymphocytes in Peyer's patches migrate to local lymph nodes to mature and later return to the gut, via lymphatics and blood, to secrete antibodies particularly the IgA isotype (Tomasi Jr., 1983). In a recent study among US students on summer studies in Mexico, Jiang et al. (1991) demonstrated a four-fold or greater secretory IgA titre rise in the faeces of eleven out of twelve students shedding *A. hydrophila* or *A. sobria* but not *A. caviae* during a bout of diarrhoea. They suggested that since strains that merely colonize may not induce a specific host immune response, their findings strongly support aetiological roles for *A. hydrophila* and *A. sobria* but not *A. caviae*. While these seem to agree with the views of most authors on *A. hydrophila* and *A. sobria*, others believe that *A. caviae* could also be pathogenic and that enteropathogenicity is probably strain-specific rather than species-specific (Singh and Sanyal, 1993). Jiang and his colleagues also did not include healthy controls in their study and thereby lack a major basis for comparison. Also, only the secretory immunoglobulin A (sIgA) response was examined.

In Bangladesh, there are a few reports on *Aeromonas* species in relation to enteric infections. Aziz et al. (1986) were the first to report isolation of *A. hydrophila* from acute diarrhoeal illness in rural Bangladesh. Similarly, during a diarrhoeal epidemic between August-November, 1987, *Aeromonas* species were isolated from 24% of cases (Rahim and Kay, 1988). Also in 1991, Alam and his colleagues reported two fatal cases of *Aeromonas* septicaemia in children
hospitalized with diarrhoea at the Clinical Research Centre (CRC) of the ICDDR,B. Furthermore, laboratory reports of stool analysis from the regular surveillance patients admitted to the CRC between January, 1990, and December, 1992, showed that *Aeromonas* species were isolated from about 27% of the patients (unpublished data); and were the only enteropathogen isolated in 20% of these patients. It is also important to note that these organisms are commonly isolated from water sources in Bangladesh thereby providing a natural source of infection (Rahim *et al.*, 1985; Islam *et al.*, 1992).

Therefore, there is the need for a detailed study of these seemingly important organisms in Bangladesh. This case-control study is designed to evaluate the production of the three major classes of immunoglobulins (IgA, IgG and IgM) in children with *Aeromonas*-associated diarrhoea, and to examine the strains for some important virulence properties; in order to establish their clinical significance and provide a better understanding of their enteropathogenicity.

B) RESEARCH PLAN

Subjects

At the Clinical Research Centre (CRC) of the ICDDR,B, more than 70,000 patients with diarrhoea are treated annually. In 1980, a surveillance system was introduced in which every 25th diarrhoeal patient reporting to the hospital is entered into a programme for in-depth clinical, microbiological and demographic investigation (Stoll *et al.*, 1982). Stool specimens of such patients are screened for all established enteropathogens including *Shigella*, *Salmonella*, *Vibrio cholerae*, *Aeromonas*, *Plesiomonas*, diarrhoeagenic *Escherichia coli*, enterotoxigenic
Bacteroides fragilis, Giardia lamblia, Entamoeba histolytica, Cryptosporidium, rotavirus, enteric adenovirus and astrovirus.

For each of the patients included as cases in the surveillance system, an age-stratum matched healthy control is also identified from the same neighbourhood and included for in-depth investigation as well. In this proposed study, children enrolled in the surveillance system as cases or controls, and who satisfy the inclusion criteria as indicted below, will be included:

(a) Cases

(i) Inclusion criteria:

1. Age less than or equal to 5 years.

2. A current acute diarrhoeal episode not more than 3 days after onset.

3. The only identifiable enteropathogen from stool analysis is Aeromonas.

(ii) Exclusion criteria:

1. All known cases of chronic or persistent diarrhoea

2. Children with evidence of diarrhoea of non-infectious aetiology (e.g. lactose intolerance).

3. Children receiving antibiotic therapy.

(b) Controls:

(i) Inclusion criteria:

1. Age less than or equal to 5 years.
2. No history of diarrhoea 4 weeks before enrollment.

3. Preferably culture-negative for *Aeromonas* and other enteropathogens. However, controls that turn out to be culture positive will be retained in the study and analysed separately at the end of the study.

(ii) Exclusion criterion

1. Evidence of recent antibiotics therapy.

Samples

Stool, saliva, and blood (200 μl) (optional) will be collected twice (acute and convalescence) from each of the children enrolled as cases, while only stool and saliva will be collected once from the controls. These specimens will be processed to detect the presence and level of specific antibodies (IgA, IgG and IgM) to *Aeromonas* species isolated from that particular child as well as standard ATCC strains. For each of the cases, a set of samples will be collected first on the day an *Aeromonas* spp. was isolated, and then 6-8 days post diarrhoea onset for stool and saliva, but 14-21 days post onset for serum. Stool and serum samples will be collected separately in appropriate sterile containers. For the collection of saliva, a sterile plastic dropper will be gently inserted into the mouth of infants to draw out saliva, while older children will be given sterile cotton wool to chew and this will be drained into a sterile container.

Sample size

The required number of study children (N) for each of the *Aeromonas* species being investigated in this study was calculated using the following formula:
\[ N = \frac{P_1 \times Q_1 + P_2 \times Q_2}{(P_1 - P_2)^2} \times f(\alpha, \beta) \]

where,

- \( P_1 \) = Proportion expected to demonstrate rising antibody titre among cases (40%) in view of diarrhoea endemicity in Bangladesh, hence possible previous exposure to *Aeromonas* infection.

- \( P_2 \) = Estimated proportion (5%) of controls that could also show rising antibody titre due to asymptomatic *Aeromonas* infection.

- \( \alpha \) = Probability of detecting a significant difference (5%)

- \( \beta \) = The risk of a false negative result (10%)

- \( 1-\beta \) = Power to detect a difference (90%)

- \( Q_1 = 1 - P_1 \); \( Q_2 = 1 - P_2 \)

Therefore:

\[
N = \frac{0.40 \times 0.60 + 0.05 \times 0.95}{(0.40 - 0.05)^2} \times 10.5
\]

\[
= 24.6
\]

This represents approximately 25 positive cases for each of the *Aeromonas* species. However, results from other parts of the world have shown that the recently reported *Aeromonas* species (*A. veronii* and *A. schubertii*) are less frequently recovered from clinical specimens than *A. hydrophila*, *A. sobria*, or *A. caviae* (Janda, 1991). Since the situation is not expected to be significantly different in Bangladesh, this study will involve a total of 210 \((25 \times 3 \times 2 + 15 \times 2 \times 2)\) children to be made up of 25 cases and 25 controls for each of the three main *Aeromonas* species; as well as 15 cases and 15 controls for each of the two recently reported but less frequent species.
Demonstration of significant antibody titre rises in the majority of the cases and not in the strictly matched controls will be a strong indication of specific immune response to the *Aeromonas* strains involved.

**Bacterial strains**

Stool specimens of cases and controls will be screened for *Aeromonas* species using sheep blood agar, both by direct plating and enrichment in alkaline peptone water. Isolates will then be identified and speciated by standard biochemical tests as recommended by Janda (1991). It is envisaged that isolation and identification of *Aeromonas* spp. will be completed within 48-72 hr of specimen collection. In view of recent reports on the isolation of multiple *Aeromonas* species from single patients' stools (Pazzaglia *et al.*, 1991), 3 suspected *Aeromonas* colonies will be selected per specimen for identification and speciation. If they belong to the same species, one will be randomly selected for further investigations. If they happen to belong to different species, each will be investigated separately in subsequent tests.

**Control strains**

ATCC reference strain of *Aeromonas* species will be obtained and used as positive controls in the various biochemical and immunological tests.

**Preparation of LPS**

Lipopolysaccharide (LPS) antigen will be prepared from the control strains using the phenol-water extraction method (Westphal and Jan, 1965). The LPS will then be tested against the stool, saliva and sera samples from each of
the subjects (cases and controls). The LPS is being used in this study because previous studies by Jiang et al. (1991) have shown the LPS as the immunodominant antigen in *Aeromonas* species.

**Extraction of immunoglobulins from samples**

Immunoglobulins (IgA, IgG, and IgM) will be extracted from stool, saliva and sera samples of all the subjects (cases and controls) included in the study. Extraction of IgA from stool samples will be by the method of Winsor et al (1986) as adapted by Jiang et al (1991) and from saliva as described by Schultz et al (1992). IgG and IgM will be extracted from the samples using the method of Lindberg et al (1984). All extracted samples will be stored at -20°C until assayed.

**ELISA procedures**

ELISA will be carried out as described by Schultz et al. (1992). Briefly, wells of disposable microtiter plates (Flow Laboratories Inc., USA) will be coated with 100 μl LPS (25 μg/ml) in phosphate buffered saline (PBS), pH 7.2. The LPS-coated plates will be incubated overnight at room temperature and thereafter will be washed 3 times with PBS containing 0.1% Tween-20. Plates will then be incubated with PBS-Tween containing 0.1% bovine serum albumin for 1 hr at 37°C and washed again. Each of the extracted immunoglobulin samples will then be added to different set of wells in two-fold dilutions in PBS-Tween starting with a 1:2 dilution, and the plates will be incubated again at 37°C for 2 hr. After washing, anti-human IgA, IgG or IgM immunoglobulin (conjugated to alkaline phosphatase and diluted 1:500) will be added and the plates further incubated at 37°C for 1 hr. ELISA will then be completed by adding substrate containing paranitrophenylphosphate (Sigma, MO, USA).
(1 mg/ml) in diethanolamine buffer at pH 9.8 and incubating for 1 hr at 37°C.

Optical density of the reaction will be measured at 405 nm (fitertek, Multiscan, Finland). Samples from each subject will be tested within the same assay in duplicate or triplicate and both positive and negative control samples will be included in each microtiter plate. The antibody titres will be determined as the means of the interpolated dilutions of the samples giving an A_405 of 0.2 above background.

Total and specific IgA, IgG and IgM titrations

Both total and specific IgA, IgG and IgM titres of the extracts from the samples will also be determined as described by Winsor et al (1986).

Antisera production

Antisera to the five ATCC Aeromonas strains being used as controls in this study will be raised in rabbits. Each of the rabbits will be injected intravenously weekly for 5 weeks with increasing amounts of a given Aeromonas antigen as follows: 0.25, 0.5, 1.0, 1.5, and 2.0 ml of antigen suspensions containing approximately 10^9 cells per ml of physiological saline. After the 3rd antigenic dose, test sera samples will be collected from each of the rabbits to determine antibody titres. The animals will then be bled to death one week after the final injection. Merthiolate (0.001%) will be added to the sera, and stored at 4°C. The antisera produced will be used as positive controls for the ELISA assays; and also for ELISA inhibition experiments as described by Granfors et al (1981) to determine the specificity of the assays.
Characterization of isolates for some virulence-associated properties

All the *Aeromonas* isolates will be tested for the following properties which have been reported to be associated with virulence in *Aeromonas* species:

1) **Cell surface hydrophobicity**: The adherence of enteric bacteria to intestinal epithelial cells is regarded as a vital step in the initiation of infection and the disease process. In this study, adherence properties of isolates will be assessed by measuring their relative cell surface hydrophobicities, using the salt aggregation test (SAT) as described by Lindahl *et al.* (1981). Isolates will be grown on BHI agar overnight at 37°C, harvested and washed twice in 0.002 M phosphate buffer (pH 6.8) and then standardized to an OD (A₆₁₀) yielding 1 x 10⁹ to 3 x 10⁹ CFU/ml. A 10-μl portion of this suspension will be mixed with an equal volume of various molarities of ammonium sulphate (NH₂)₂SO₄ (4 to 0.0625 M) on glass slides. These will be properly mixed and observed for agglutination within 1 min. The SAT value of a given isolate will be the lowest molarity of (NH₂)₂SO₄ which caused a visible clumping (agglutination) of that isolate.

2) **Haemagglutination assay**: The method of Atkinson and Trust (1980) will be used. Briefly, human group O erythrocytes will be collected by venipuncture and stored in Alsever's solution at 4°C. Before use, they will be washed three times in PBS (0.04 M, pH 7.4) and then a 3% (v/v) suspension will be prepared in PBS. Overnight broth cultures of each isolate (about 10⁹ bacteria/ml) will be prepared, centrifuged and washed twice in PBS.
Haemagglutination (HA) tests will then be performed at room temperatures by mixing 20 µl of erythrocyte suspension with 20 µl of bacterial suspension on a slide. This will be rocked gently by hand, and strains will be considered HA-positive if agglutination occurs within 5 minutes. Sensitivity of HA to D-mannose, L-fucose, or D-galactose will also be determined. A positive HA reaction will be regarded as sensitive (S) to a given sugar (e.g. D-mannose) if the reaction becomes negative in the presence of the sugar, and resistant (R) if it remained positive.

3) Autoagglutination: Isolates will be screened for the autoagglutination (AA) marker as described by Janda et al. (1987). Briefly, each isolate will be inoculated into a 6-ml tube of brain-heart infusion broth (BHIB) and incubated at 37°C overnight (18 to 24 hr). Each tube will then be gently vortexed to resuspend any pellet growth and immediately placed in a boiling water bath for 1 hr. Isolates showing visible evidence of precipitation from broth will be considered as AA positive.

To differentiate AA positive strains from those exhibiting the suicide phenomenon earlier reported by Namdari and Bottone (1989), cell sediments obtained from duplicate BHIB cultures of each isolate will be assessed for cell viability by inoculating onto sheep blood agar.

4) Resistance to antimicrobial agents: The susceptibility of isolates to various antimicrobial agents will be determined by the disk diffusion method as described by Bauer et al. (1966) using commercially available disks (BBL Microbiology Systems). From the results it will be possible to extrapolate resistance pattern of isolates to commonly used drugs.
3) **Ability to cause diarrhoea in rabbits**: Selected isolates will be tested for their ability to cause diarrhoea using the removable intestinal tie adult rabbit diarrhoea (RITARD) model as described by Spira et al. (1981).

**Storage of Aeromonas isolates**

- **Short-term**: 15% (v/v) glycerol-broth (Trypticase soy broth or nutrient broth) culture in Nunc tubes at -20°C or at -70°C
- **Long-term**: Lyophilization

**Data analysis**

Data generated from this study will be analyzed for significance or otherwise, using appropriate statistical methods, such as the SPSS programme which is widely used in the Centre. Antibody titres will be compared between the case and control groups; and correlation between the various properties, immune response and ability to cause diarrhoea in rabbits will be assessed.

**C) BIBLIOGRAPHY**


12. PUBLICATIONS OF PRINCIPAL INVESTIGATOR


3. Odugbemi T, Coker AO, Dosunmu-Ogunbi O, Oyerinde JO, Alabi SA, Uzoma K, 
Macaulay SA, Ogunsanya T, Alonge AA. Study on etiology of infective 
diarrhoeal diseases in Nigeria. In: Proceedings of Nigeria/Japan 

4. Olukoya DK, Daini AO, Alabi SA, Coker AO, Odugbemi T, Akinrimisi EO. 
Antimicrobial resistance patterns and plasmids of enteropathogenic 

5. Coker AO, Olayiw B, Obi CL, Alabi SA. Characterisation and antibiotic 
sensitivity of Campylobacter jejuni and C. coli isolated from children 
at the Lagos University Teaching Hospital (LUTH), Lagos, Nigeria. 

6. Alabi SA, Odugbemi T. Occurrence of Aeromonas species and Plesiomonas 
shigelloides in patients with and without diarrhoea in Lagos, Nigeria. 

7. Alabi SA, Odugbemi T. Biochemical characteristics and a simple scheme 
for the identification of Aeromonas species and Plesiomonas 

8. Alabi SA, Odugbemi T. Antimicrobial susceptibility pattern of Aeromonas 
and Plesiomonas strains isolated from patients with diarrhoea in Nigeria. 

9. Alabi SA, Odugbemi T. Plasmid screening amongst Aeromonas species and 
Plesiomonas shigelloides isolated from patients with diarrhoea in Lagos, 

10. Adegbola RA, Alabi SA, Akinwande FO, Coker AO, Odugbemi T. Correlation 
between human and animal bio-serogroups of Campylobacter isolates in 

11. Adegbola RA, Akinwande FO, Alabi SA, Coker AO, Odugbemi T. Prevalence, 
biotypes and serogroups, of Campylobacter jejuni and C. coli in 

clinical isolates of Escherichia coli in developing countries. Nig J 
13. FLOW CHART (sequence of tasks within time frame)

(a) First 3 months: November 1993 - January, 1994

During this period, the protocol will be conducted on a pilot scale to involve standardization of techniques, meeting sampling targets, and assessing overall modalities for the protocol.

(b) Next 12 months: February 1994 - January, 1995

The research project will be carried out in full as outlined in the protocol. This will involve sample collection, isolation and identification of Aeromonas species, measurement of antibodies, and characterization of isolates for virulence factors.

(c) Last 3 months: February - April, 1995

This period will be used for the analysis of results and write-up of findings for publication.

Note: Periodic preliminary analysis of results will be carried out to monitor the progress of the protocol during implementation.

14. SPECIFIC TASKS FOR EACH LISTED INVESTIGATOR

Dr. Sunday A. Alabi

As the Principal Investigator, will carry out the study with the assistance of the listed collaborators.

Dr. M. John Albert

The study is to be carried out in the Enteric Bacteriology/Immunology Laboratories of the Laboratory Sciences Division. As Head, Department of Laboratory Research, Dr. Albert will supervise and see to the day-to-day progress of the study.

Dr. Firdausi Qadri

As an immunologist, will assist with the ELISA and other immunological tests.

Dr. A.S.G. Faruque

As clinician in the Clinical Sciences Division of ICDDR,B, will assist with the clinical part of the project, e.g. checking of patients and collection of blood samples.
Dr. A.S.M. Hanifur Rahman

As veterinary doctor in the Animal Resources Branch, he will assist with animal experiments.

Dr. R. Bradley Sack

As the Associate Director of Laboratory Sciences Division, ICDDR,B, is the preceptor of Principal Investigator and will handle the overall supervision and coordination of the study.
15. BUDGET (for 18 months)

a) Personnel

(i) One Physician Research Fellow ($150 x 18) US$ 2,700
(ii) One Community Health Worker ($125 x 18) US$ 2,250
(iii) One Laboratory Assistant ($100 x 18) US$ 1,800

b) Operating cost

i) Materials
   - Specimen bottles
   - Glasswares
   - Pipettes and tips
   - Syringes and needles
   - Nunc tubes
   - Nitrocellulose, etc. US$ 4,500

ii) Chemicals and reagents
   - Enzyme conjugates
   - Cell lines
   - Nunc plates for ELISA
   - Tris-HCl
   - SDS, PBS, etc. US$ 6,500

iii) Aeromonas ATCC strains US$ 1,500

iv) Media

v) Animal Resources

vi) Lyophilization of isolates US$ 200

vii) Xerox US$ 200

viii) Stationary and correspondence US$ 200

ix) Incentives to patients US$ 400

x) Medical Illustration US$ 400

US$ 22,050

US$ 700

US$ 5,000

US$ 500

US$ 35,000

TOTAL
INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
CONSENT FORM

An investigation of the diarrhoeagenic role and characteristics of Aeromonas species in Bangladesh

The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) is planning to undertake a study in the Clinical Research Centre (CRC), Dhaka. Over the years, a group of bacteria known as Aeromonas have been suspected of causing diarrhea in children as well as adults. However, it is not yet clear if they can actually cause diarrhea or how they are able to cause it. Aeromonas are frequently isolated from stool samples of children with diarrhea at the CRC, and this study is to determine if Aeromonas are causing the diarrhea seen in such children. Aeromonas organisms isolated from these children will also be tested to identify peculiar characteristics that may enable them to cause more diarrhea. The results of this study will provide a better understanding of this type of diarrhea. It will also provide necessary information required for a better management of children with Aeromonas-associated diarrhea in Bangladesh, and possibly be applicable to other countries of the world as well.

Therefore, we request you to allow your child to participate in this study. If you agree, the following procedure will be followed:

1) You will be expected to provide answers to a set of questions (Questionnaire) on your child's background, and the duration and nature of the present diarrhea episode (if applicable).

2) Stool, saliva and blood (200 µl) specimens will be collected twice (different occasions) from your child (if he/she has diarrhea) for further investigation. If your child has no diarrhea, only stool and saliva will be collected once. This will be at no risk to your child. However, your child will feel a little pain when the finger prick is being done to collect the small quantity of blood (200 µl) required.

3) We shall bear the cost of transportation for your follow-up visit to the CRC, if required.

4) The study will not in any way interfere with the management of your child's diarrhea (if applicable).

5) We will maintain the confidentiality of the information given us.

6) You may withdraw your child from the study at anytime if you wish.

7) If you have any other question to ask, we shall be happy to answer them.
8) If you agree to allow your child participate in this study, please sign below.

Signature of the Principal Investigator

Signature or left thumb impression of parent/guardian

Date

Date
গবেষণার নাম: এরোমোনাস (Aeromonas) নামক জীবাণু প্রথিত উরায়ের গবেষণা:

এরোমোনাস (Aeromonas) নামক জীবাণু প্রথিত উরায়ের গবেষণা কেন্দ্রের চিকিৎসা দেশে অন্যদিন থেকে এরোমোনাস নামক গবেষণার জীবাণু নিয়ম ও পণ্য বিয়োলোজিকের চাইরিয়া বা পাত্রী পায়বাহন রোগের জন্য দায়ী হয়। এরোমোনাস গবেষণা জীবাণু বিভিন্ন মানুষের পাত্রী পায়বাহন রোগ এর জন্য বিদ্যমান। এটি এরোমোনাসের প্রাপ্তি জীবাণু প্রায়ই শিষ্টদের পাত্রী পায়বাহনার সাথে পাওয়া যায়।

আমাদের গবেষণার উদ্দেশ্য হলো শিষ্টের জীবাণু এরোমোনাসের প্রাপ্তির জীবাণু পাত্রী পায়বাহন করে। এবং পাত্রী পায়বাহনের মাধ্যমে জীবাণু চিকিৎসা আরো সহজতর হবে। বাংলাদেশ এবং পৃথিবীর অন্যান্য দেশ এই গবেষণার ফলাফলে উত্তীর্ণ হবে।

যেন্না আপনার শিষ্টকে আমাদের গবেষণায় অংশগ্রহণের জন্য প্রস্তুত দিনত। যদি আপনি রাজি থাকেন, নিম্নাপন্ন নিয়মানুসারে আপনার শিষ্টকে গবেষণায় অংশগ্রহণ করে নেয়া হবে।

১। আপনাকে বহুল সময় দানকর (ডায়রিয়া ও অন্যান্য স্বাস্থ্যগত বিষয়) একটি কমপক্ষে পরিষ্কার করতে হবে।

২। আপনার শিষ্ট পায়বাহন, মসৃণঘলা (saliva), কয়েক কাটা রক্ত (200 ul) দুই বারে নেওয়া হবে যদি তার গায়ত্রী পায়বাহন থাকে। গলি পাত্রী পায়বাহন না থাকে সেটা পায়বাহন ও মসৃণঘলা নেওয়া হবে। এতে আপনার শিষ্টকে কোন রোগ না। মূল্যায়ন আপনার শিষ্ট সামান্য একটি বাধা পায় কয়েক কাটা রক্ত (200 ul)

৩। আপনার শিষ্ট চিকিৎসা দেশে আসার জন্য সারাদিন বহন করা হবে।

৪। এই গবেষণা আপনার শিষ্টের গায়ত্রী পায়বাহনী চিকিৎসার কোন ভারীত করবে না।

৫। আমারা সব ক্ষমতায় আপনার রাখতে।

৬। আপনার শিষ্টকে বে কোন সময় গবেষণা করতে নিয়ে যেতে পারবেন। হুষ্ট্য তিউরেসিন আপনার অসুস্থতার সাথে উঠতে দেবে।

৭। আপনার অন্য কিছু জানার আগে আমরা আমাদের সাথে উত্তর দেব।

৮। যদি আপনি আমন্ত্রণের শিষ্টকে এই গবেষণায় অংশগ্রহণ করতে রাজি থাকেন তবে আমা করে নিচে স্বাক্ষর করুন।

স্বাক্ষর প্রধান গবেষকের
স্বাক্ষর/বাম বুঝড়া জানান্ত্রির হাঁস

তারিখ: ____________________

তারিখ: ____________________

Simplify (language)
ABSTRACT SUMMARY FOR ETHICAL REVIEW

1. This study aims at evaluating immune response in *Aeromonas*-associated diarrhoea in children in Bangladesh, and characterization of isolates from these children for some important virulence markers. That *Aeromonas* strains are capable of causing acute diarrhoea is controversial, though they are more often isolated from patients with diarrhoea than from controls. This study intends to use production of significant immune responses during *Aeromonas*-associated diarrhoea as a proof of their enteropathogenicity. Isolates will also be tested for some virulence factors to give a clue to their pathogenic mechanisms. The study is limited to children less than 5 years old because this is the age-group most vulnerable to diarrhoea attack, older children would have had previous exposure to *Aeromonas* infection thereby making it difficult to associate immune response with current infection.

2. The study involves no potential risk to the children.

3. The children will be under constant observation of physicians and other health workers.

4. Informed consent in a consent form will be obtained from authorized guardian/parent of a child before he/she is included in the study. Guardian/patients will be fully informed about the study and a freely given consent will be obtained, preferable in writing.

5. During data analysis only the identification numbers of children will be used and confidentiality will be maintained.

6. Interview of guardian/parents will be conducted at the hospital (CRC) and households by female interviewers to get baseline information on the child and the current diarrhoea episode (if applicable), as indicated in the questionnaire. Each interview will be between 15-20 minutes.

7. The children will be provided with the standard treatment for diarrhoea and they will be followed at households and hospital. Results of this study will provide proof of *Aeromonas* enteropathogenicity and potential pathogenic mechanisms. This will have a major impact on the importance and management of *Aeromonas*-associated diarrhoea in Bangladesh and other countries of the world.

8. The study requires only the use of stool, saliva and a small quantity of blood (200μl) which will be at no risk to the children.

102/ABS2.SAA:
ICDDR, B SURVEILLANCE ACTIVITY, CRC, DHAKA.

Patient's name ------------------ Father's name ---------------------

Variable                        Code                        Column

case number

interviewer

date

age

sex

= male, 2 = female

religion

= Muslim, 2 = Hindu, 3 = Christian

= Buddhist, 5 = Others

type of replacement fluid before arrival: 0 = none, 1 = ORS packet

= Home made ORS, 3 = barley, 4 = Rice, 5 = fluid soup, 6 = I.V fluid, 7 = I/2/4, 8 = 3/4/5

chemotherapy before arrival

= none, 01 = penicillin, 02 = tetracycline, 03 = ampicillin, 04 = chloramphenicol, 05 = furox, 06 = gentamycin, 07 = tetracycline, 09 = nalidixic acid, 10 = metronidazole, 11 = Fux, 12 = Sept + Metro, 13 = Fux + Nalidixic acid, 14 = Selexid, 15 = Others

how many persons eat from the same cooking pot

how many hrs before the onset of diarrhoea meal was taken

how many children <5 years of age in your family

how many members of your family had diarrhoea in past 7 days.
No of deaths in the family in last 5 years from diarrhoea.

Feeding (up to 3 yrs of age)
1=BM 2=BM+CM/PM 3=BM+Rice/Ata powder
4=CM/PM 5=Rice/Ata gruel/powder
6=3+4 7=4+5 8=Family food

Education of patient's father:
0=none 1=maktab 2=1-3 yrs 3=4-5 yrs
4=6-10 yrs 5=10-12 yrs 6=>12 yrs

Education of patient's mother:
(categories as above)

Self education (categories as above)
for patients >15 yrs of age:

Income of household (from all sources)
1=Up to Tk 500 2=500-999 3=1000-1499
4=1500-1999 5=2000-2999 6=3000-4999
7=>5000

Sources of water for drinking:
=Tap 2=TW 3=pond/river/ditch
4=1+2 5=1+3 6=2+3 7=1+2+3

Sources of water washing/bathing
(categories as above)

Place of defecation:
=Sanitary 2=semi-sanitary 3=service
=duhole (with ring) 5=open pit
=Hanging 7=No fixed place

Vitamin A capsule:
=none 1=within 3 months 2=4m-6m
=7m-12m 3=>12m

Temperature:
=Up to 36.6 1=36.7-37.7 2=37.8-38.8
3=38.8

Duration of diarrhoea before arrival:
1=day 2=1-3 days 3=4-6 days 4=7-9 days
5=10-12 days 6=12-14 days 7=>15 days

Texture of stool:
0=watery 2=non-watery
Stool contents
0=usual 1=Mucus 2=Blood 3=MU+BL

No of stools in 24 hrs:
0=3-5, 1=6-10, 2=11-15, 3=15-20, 4=>20

Abdominal pain:
0=no, 1=yes

Vomiting in last 24 hrs:
0=no, 1=<10 times, 2=>10 times

History of cough with diarrhoea:
0=none, 1=1-7 days, 2=8-14 days, 3=15-20 days, 4=>20 days

History of measles:
0=none, 1=measles in past 3m, 2=measles in >3m-6m, 3=present history of night blindness, 4=past history of night blindness, 5=measles+night blindness

History of convulsion:
0=none, 1=convulsion within 12 hrs, 2=convulsion within 13-24 hrs, 3=convulsion >24 hrs

Other diseases (specify)

How long do you live in Dhaka city:
0=<1yr, 1=1-2yrs, 2=3-5yrs, 3=>5yrs, 4=seasonal

Present location (Thana/area):
0=Basti, 1=Common housing area, 2=Residential area, 3=Village area, 4=Others
PHYSICAL EXAMINATION:

Thirst
0=normal, 1=mild, 2=moderate
3=severe (for adult)

General Condition
0=normal, 1=restless, 2=lethargic but
irritable when touched, 3=Drowsy/cold
& sweating extremities, 4=coma

Radial pulse
0=normal rate & good volume,
1=Rapid & weak, 2=Rapid & feeble/sometimes
impalpable, 3=not palpable

Respiration
0=normal, 1=faster than normal
2=deep, 2 rapid

Clinical assessment of dehydration
0=no dehydration, 1=mild, 2=moderate
3=severe

Convulsion
0=no, 1=yes

OTHER PHYSICAL FINDINGS:

Vitamin A deficiency
0=normal, 1=conj. xerosis, 2=Bitot spot
=corneal ulcer, 4=carotomolacia
=1+2, 8=3+4, 7=corneal scar

Ear - Otitis media
0=absent, 1=otitis media present

Eye - mouth
0=normal, 1=angular stomatitis
=gingivitis 3=Pharyngitis
=Thomitis

Nose
0=clear, 1=Rhonchi, 2=crepitation
=both

Vagina
0=normal sounds present, 1=Distended sounds present
=Distended sounds sluggish 3=Distended sound absent
=Distention with tenderness.
Liver and spleen:
0=not palpable, 1=liver enlarged
2=spleen enlarged, 3=liver & spleen enlarged

Rectum prolapse
0=None, 1=yes

Extremities:
0=Oedema absent, 1=Oedema present

Diagnosis:
0=Uncomplicated diarrhoea
1=Complicated diarrhoea

Note: Complicated diarrhoea admitted in medical ward

Disposition:
0=Discharged from examination desk
1=ORP, 2=TC, 3=TC to ward, 4=ward
5=Study ward, 6=Referred to another hospital
7=Death on arrival

Duration of stay:
0=days/hours

Outcome:
0=Cured, 1=Illness continuing
2=Died, 3=Absconded, 4=Others
- Rehydration method used
  0 = none, 1 = ORS only, 2 = IV only, 3 = ORS to IV, 4 = IV to ORS, 5 = others

<table>
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<tr>
<th>Treatment</th>
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<tr>
<td>Tetracycline</td>
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<td>Ampicillin</td>
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<td>Septrin</td>
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<td>Furoxone</td>
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<td>Penicillin/Crystaten V</td>
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<td>Metronidazole</td>
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<td>Gentamycin</td>
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<td>Nalidixic Acid</td>
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<tr>
<td>Chloramphenicol</td>
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<tr>
<td>Others</td>
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<tr>
<th>Weight on Admission</th>
<th>__________</th>
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<tr>
<td>Weight on Discharge</td>
<td>__________</td>
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<tr>
<td>Height (cm)</td>
<td>__________</td>
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<tr>
<td>Arm Circumference</td>
<td>__________</td>
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### ICDDR, B SURVEILLANCE ACTIVITY

### ADDITIONAL QUESTIONNAIRE

1. Patient I.D. number

2. Date of interview

3. Total distance covered (miles)

4. Total time taken to reach (h/min)

5. Birth order of the child
   (< 5 yrs)
   1st=01, 2nd=02, 3rd=03,
   9+th=99, Adopted=88

6. Any diarrhoea of this child in last 7 days, other than this episode (Yes=1, No=2)

7. Any diarrhoea of this child in last 14 days, other than above episodes (Yes=1, No=2)

8. Any diarrhoea of this child in last one month, other than above episodes (Yes=1, No=2)

9. Any cough/fever/sneezing/running nose/ rapid respiration/breathing difficulty/ ear discharge/husky voice in last 14 days (Yes=1, No=2)

10. Any other disease of this child in last 7 days (Yes=1, No=2)

11. Any other disease of this child in last 14 days (Yes=1, No=2).

12. Any other disease of this child in last one month (Yes=1, No=2)

13. Father's age (years): 
   (<=15 years)
   Not applicable=99

14. Mother's age (years): 
   (<=15 years)
   Not applicable=99
15. Floor structure
1 = Cemented
2 = Noncemented

16. Wall structure
1 = Brick
2 = Bamboo fence
3 = Ordinary tin
4 = Corrugated tin
5 = Straw
6 = Jute stick
7 = Mixed
8 = Mud
9 = Other

17. Roof structure
1 = Concrete/pucca
2 = Bamboo fence
3 = Ordinary tin
4 = Corrugated tin
5 = Straw
6 = Polythene
7 = Mixed
8 = Other

IMMUNISATION HISTORY (children <5 yrs):

18. BCG : / 1 = Yes, 2 = No, 3 = Don't know, 9 = Not applicable

19. DPT : / 1 = 1st dose, 2 = 1st+2nd, 3 = 1st+2nd+3rd,
4 = No, 5 = Don't know 9 = Not applicable

20. Polio : / - do -

21. Measles : / 1 = Yes, 2 = No, 3 = Don't know, 9 = Not applicable

22. Is the child breastfed now : / 1 = Yes, 2 = No,
(<5 years) 9 = Not applicable

23. If yes frequency of breastfeeding : / / from 6 A.M. to 6 P.M.
Adopted child=88, Not applicable=99

24. How long did you predominantly breastfeed the child (months)
(< <3 years of child)
(<1 month=00, 1 month=01,
adopted child=88,
not applicable=99)
25. At what age, did you stop breast feeding (totally) your child (months) (<6 years)
(<=1 month=00, 1 month=01, adopted child=88, not applicable=99)

26. Primary occupation of father:
(<=15 years of child) or self employment of male patient
(>15 years).

1. Farmer
2 = Day labour
3 = Rickshaw/push car
4 = Taxi/bus/truck/tempo driver
5 = Skill worker
6 = Office executive
7 = Petty business
8 = Big business
9 = Street vendor
10 = Other

27. Any gainful employment of mother:
(<=15 years of child) or self employment of female patient
(>15 years)

1 = Yes, 2 = No,
Not applicable=9

28. Income of father (last month) (<=15 yrs):
Not applicable=9
999999

29. Income of mother (last month) (<=15 yrs):
Not applicable=9
999999

30. Mother reads newspaper (<=15 years): 1 = Yes, 2 = No,
or she reads (>15 years) 9 = Not applicable

31. If yes, how many days in a week: 1 = 7 days in a week,
2 = < 7 days in a week
9 = Not applicable

32. Mother watches T.V. (<=15 years): 1 = Yes, 2 = No,
or she watches T.V. (>15 years) 9 = Not applicable

33. If watches, How many days in a week: 1 = 7 days in a week,
2 = < 7 days in a week
9 = Not applicable

34. Mother listens to radio (<=15 years): 1 = Yes, 2 = No,
or she listens (>15 years) 9 = Not applicable

35. If listens, How many days in a week: 1 = 7 days in a week,
2 = < 7 days in a week
9 = Not applicable
36. Father reads newspaper (<=15 years) : __/
or he reads (>15 years)

1 = Yes, 2 = No,
9 = Not applicable

37. If yes, how many days in a week : __/

1 = 7 days in a week
2 = < 7 days in a week
9 = Not applicable

38. Father watches T.V. (<=15 years) : __/
or he watches T.V. (>15 years)

1 = Yes, 2 = No,
9 = Not applicable

39. If watches, how many days in a week : __/

1 = 7 days in a week
2 = < 7 days in a week
9 = Not applicable

40. Father listens to radio (<=15 years) : __/
or he listens (>15 years)

1 = Yes, 2 = No,
9 = Not applicable

41. If listens, how many days in a week : __/

1 = 7 days in a week
2 = < 7 days in a week
9 = Not applicable

42. Mother member of any co-operative : __/
(<=15 years) society, NGO/women
organisation or she herself
(>15 years)

1 = Yes, 2 = No,
9 = Not applicable

43. If yes, what is the name of that :

44. Father member of any co-operative : __/
(<=15 years) society, NGO/women
organisation or he himself
(>15 years)

1 = Yes, 2 = No,
9 = Not applicable

45. If yes, what is the name of that :

46. Main source of water :

Distance from kitchen (ft) # family user Frequency of collection

Drinking : __/____/  __/____/  __/____/
Cooking : __/____/  __/____/  __/____/
Washing : __/____/  __/____/  __/____/

47. Any treatment of drinking water : __/
(Non=0, Boiling=1, Alum/tablet=2,
Sieveng=3, Use filter=4, Other=5)

48. Years of schooling of father (read up to) : __/
49. Years of schooling of mother (read up to): __/__/ 

50. Numbers of rooms in the family: __/__/ 

51. Number of beds in the family: __/__/ 

52. Family owns fan
   (Yes=1, no=2) __/__/ 

53. Family owns radio
   (Yes=1, no=2) __/__/ 

54. Family owns TV
   (Yes=1, no=2) __/__/ 

55. Family owns Almirah
   (Yes=1, no=2) __/__/ 

56. Family owns luxury cot
   (Yes=1, no=2) __/__/ 

57. Family ordinary cot
   (Yes=1, no=2) __/__/ 

58. Family uses gas for cooking
   (Yes=1, no=2) __/__/ 

59. Cooks in the bedroom
   (Yes=1, no=2) __/__/ 

60. Use of light at night:
    Electric=1, ordinary
    kerosene lamp=2, hurricane=3
    Ordinary kerosene lamp+hurricane=4,
    None=5 __/__/ 

61. Keeps chickens/ducks in:
    Bed room=1, court yard=2,
    corridor=3, other place=4,
    more than one place=5,
    not applicable=9 __/__/ 

62. Chickens/ducks enter in kitchen:
    (Yes=1, No=2, Not applicable=9) __/__/ 

63. Chickens/ducks enter in bed room
    (Yes=1, No=2, Not applicable=9) __/__/ 

64. Keeps pigeons/birds in:
    Bed room=1, court yard=2,
    corridor=3, other place=4,
    more than one place=5,
    not applicable=9 __/__/ 

5
65. Pigeons/birds enter in kitchen:
(Yes=1, No=2, Not applicable=9)

66. Pigeons/birds enter in bedroom:
(Yes=1, No=2, Not applicable=9)

67. Keeps goats/dogs/cats/cows in:
   Bed room=1, court yard=2,
   corridor=3, other place=4,
   more than one place=5,
   not applicable=9

68. Goats/dogs/cats/cows/ enter in kitchen:
(Yes=1, No=2, Not applicable=9)

69. Goats/dogs/cats/cows/ enter in bedroom:
(Yes=1, No=2, Not applicable=9)

70. Disposal of garbage (Court yard=1,
    outside the house=2)

71. Number of cigarettes smoked
    by father per day (<=15 yrs)
    (None=00, Unknown=88,
    Not applicable=99)

72. Number of cigarettes smoked
    by mother or caretaker per day
    (<=15 yrs) (None=00, Unknown=88,
    Not applicable=99)

73. Number of cigarettes smoked
    by the patient per day
    (>15 yrs) (None=00, Unknown=88,
    Not applicable=99)

74. Number of biri/hukka smoked by
    father per day (<=15 yrs)
    (None=00, Unknown=88,
    Not applicable=99)

75. Number of biri/hukka smoked by
    mother or caretaker per day
    (<=15 yrs) (None=00, Unknown=88,
    Not applicable=99)

76. Number of biri/hukka smoked by
    the patient per day (>15 yrs)
    (None=00, Unknown=88,
    Not applicable=99)
77. Since how long do you live in the
   same house (if rented)
   ( <1 yr=00, 1 yr=01, 2 yrs=02,
     3 yrs=03, 4 yrs=04, 10+ yrs=10,
     not applicable=99)
   ___ / ___

78. Since how long do you live in the
   same house (if own house)
   ( <1 yr=00, 1 yr=01, 2 yrs=02,
     3 yrs=03, 4 yrs=04, 10+ yrs=10,
     not applicable=99)
   ___ / ___

79. Admission weight
   ___ / ___ / . ___ / ___

80. Discharge weight
   ___ / ___ / . ___ / ___

81. Admission height
   ___ / ___ / . ___ / ___

82. Discharge height
   ___ / ___ / . ___ / ___

83. MUAC
   ___ / ___ / . ___

84. Tibial length (<5 yrs)
   ___ / ___ / . ___

85. Duration of diarrhoea
   prior to admission (days/hrs)
   (Persistent diarrhoea=15+ days)
   ___ / ___ / . ___ / ___
   D D H H

86. Stool specimen collected (Yes=1, No=2)
   ___ /

87. Number of school going children
   in the family
   ___ /

* Mason, Carpenter, Barber, Washerman.
** Earn < 3000 Tk. in a month.
*** Earn >= 3000 Tk. in a month.
CURRICULUM VITAE OF PRINCIPAL INVESTIGATOR

NAME : DR. ALABI SUNDAY ABRAHAM

DATE OF BIRTH : January 25, 1959

NATIONALITY : Nigerian

MARITAL STATUS : Married

NUMBER OF CHILDREN : Three

HOME INSTITUTION AND POST :
Research Fellow II
National Institute for Medical Research
Yaba, Lagos, Nigeria

CURRENT POST AND ADDRESS : International Health Research Fellow
Laboratory Sciences Division
ICDDR,B, Dhaka, Bangladesh

ACADEMIC QUALIFICATIONS:

1. Primary School Leaving Certificate - December, 1970
4. Bachelor of Science (BSc Microbiology) - June, 1982
5. Master of Science (MSc Microbiology) - June, 1986
6. Doctor of Philosophy (PhD) - December, 1989

SCIENTIFIC PUBLICATIONS: Attached to the protocol section (page 22 and 23)

WORKING EXPERIENCE:

2. Graduate Assistant (University of Lagos): 1986-87
4. Research Fellow II (NIMR): 1991-date

OTHER INTERESTS:

Current affairs, Scientific discussions,
Sports (table tennis)

SAB: mh/L10:ALABI.CV