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APPLICATION FOR PROJECT GRANT

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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, 199
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
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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 diseases 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 <i>Aeromonas</i> 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 <i>Aeromonas</i> 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-sporulating 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 diarrhoeal syndromes in many countries, including Australia

(Burke *et al.*, 1983), the United States (Holmberg and Farmer, III, 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 O: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 this surface array proteins may play a similar role for serogroup O: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*.

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(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.

α = Probability of detecting a significant difference (5%)

β = 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 (Titertek, 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 (A_{610}) yielding 1×10^9 to 3×10^9 CFU/ml. A 10- μ l portion of this suspension will be mixed with an equal volume of various molarities of ammonium sulphate $(\text{NH}_4)_2\text{SO}_4$ (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 $(\text{NH}_4)_2\text{SO}_4$ 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 Alserver'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 10^9 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.

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- 5) *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

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

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12. PUBLICATIONS OF PRINCIPAL INVESTIGATOR

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7. Alabi SA, Odugbemi T. Biochemical characteristics and a simple scheme for the identification of *Aeromonas* species and *Plesiomonas shigelloides*. J Trop Med Hyg 1990; 93:166-9.
8. Alabi SA, Odugbemi T. Antimicrobial susceptibility pattern of *Aeromonas* and *Plesiomonas* strains isolated from patients with diarrhoea in Nigeria. Cent Afr J Med 1990; 36:174-6.
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11. Adegbola RA, Akinkuade FO, Alabi SA, Coker AO, Odugbemi T. Prevalence, biotypes and serogroups of *Campylobacter jejuni* and *C. coli* in domesticated animals in Nigeria. Trop Vet 1991; 9:43-5.
12. Alabi SA, Mustapha A, Obi CL, Idigbe O. A possible scheme for biotyping clinical isolates of *Escherichia coli* in developing countries. Nig J Microbiol 1993 (in press).

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. Hamidur 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	US\$ 4,500	
	- Specimen bottles		
	- Glasswares		
	- Pipettes and tips		
	- Syringes and needles		
	- Nunc tubes		
	- Nitrocellulose, etc.		
ii)	Chemicals and reagents	US\$ 6,500	
	- Enzyme conjugates		
	- Cell lines		
	- Nunc plates for ELISA		
	- Tris-HCl		
	- SDS, PBS, etc.		
iii)	<i>Aeromonas</i> ATCC strains	US\$ 1,500	
iv)	Media	US\$ 5,000	
v)	Animal Resources	US\$ 3,150	
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
c)	Capital equipment		US\$ 700
d)	Travel (Local and International)		US\$ 5,000
3)	Computing		US\$ 500
	TOTAL		US\$ 35,000

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 diarrhoea in children as well as adults. However, it is not yet clear if they can actually cause diarrhoea or how they are able to cause it. *Aeromonas* are frequently isolated from stool samples of children with diarrhoea at the CRC, and this study is to determine if *Aeromonas* are causing the diarrhoea 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 diarrhoea. The results of this study will provide a better understanding of this type of diarrhoea. It will also provide necessary information required for a better management of children with *Aeromonas*-associated diarrhoea 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 diarrhoea episode (if applicable).
- 2) Stool, saliva and blood (200 µl) specimens will be collected twice (different occasions) from your child (if he/she has diarrhoea) for further investigation. If your child has no diarrhoea, 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 diarrhoea (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

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র
মহাখালী, ঢাকা-১২১২

সম্মতি পত্র

এ্যারোমোনা (Aeromonas) নামক জীবাণু ঘটিত উদরাময়ের গবেষণা:

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্রের চিকিৎসা কেন্দ্র বহু দিন ধরে এ্যারোমোনা নামক প্রজাতির জীবাণু শিশু ও পূর্ণবয়স্ক লোকদের ডায়রিয়া বা পাতলা পায়খানা রোগের জন্য দায়ী বলে সন্দেহ করা হচ্ছে। এ্যারোমোনা প্রজাতি জীবাণু কিভাবে মানুষের পাতলা পায়খানা করছে এমনো বৈজ্ঞানিকদের কাছে স্পষ্ট নয়। অথচ এ্যারোমোনা প্রজাতি জীবাণু প্রায়ই শিশুদের পাতলা পায়খানার সাথে পাওয়া যাচ্ছে।

আমাদের গবেষণার উদ্দেশ্য হচ্ছে নিশ্চিত ভাবে জানা এ্যারোমোনা প্রজাতির জীবাণু পাতলা পায়খানা করছে। এবং পাতলা পায়খানা করার ক্ষমতা এই জীবাণুর কতটা আছে। এই গবেষণার ফলাফলের মাধ্যমে ডায়রিয়া চিকিৎসা আরও সহজতর হবে। বাংলাদেশ এবং পৃথিবীর অন্যান্য দেশ এই গবেষণার ফলাফলে উপকৃত হবে।

সেজন্য আপনার শিশুকে আমাদের গবেষণায় অংশগ্রহণের জন্য পরামর্শ দিচ্ছি। যদি আপনি রাজী থাকেন, নিম্নলিখিত নিয়মানুসারী অনুসারে আপনার শিশুকে গবেষণায় অন্তর্ভুক্ত করে নেয়া হবে।

- ১। আপনাকে বহু প্রশ্ন সম্বলিত (ডায়রিয়া ও অন্যান্য স্বাস্থ্যগত বিষয়) একটা ফর্ম পূরণ করতে হবে।
- ২। আপনার শিশুর পায়খানা, মুখের লালা (saliva), কয়েক ফোটা রক্ত (200 ul) দুই বারে নেয়া হবে যদি তার পাতলা পায়খানা থাকে। যদি পাতলা পায়খানা না থাকে শুধু পায়খানা ও মুখের লালা নেয়া হবে। এতে আপনার শিশুর কোন ক্ষতি হবে না। শুধুমাত্র আপনার শিশু সামান্য একটু ব্যথা পাবে কয়েক ফোটা রক্ত (200 ul) নেয়ার সময়।
- ৩। আপনার শিশুর চিকিৎসা কেন্দ্র আসার জন্য যানবাহন খরচ বহন করা হবে।
- ৪। এই গবেষণা আপনার শিশুর পাতলা পায়খানা চিকিৎসার কোন ব্যাহত করবে না।
- ৫। আমরা সব রকম গোপনীয়তা বজায় রাখব।
- ৬। আপনার শিশুকে যে কোন সময় গবেষণা থেকে নিয়ে যেতে পারবেন।
- ৭। আপনার অন্য কিছু জানবার থাকলে আমরা আনন্দের সাথে উত্তর দেব।
- ৮। যদি আপনি আপনার শিশুকে এই গবেষণায় অন্তর্ভুক্ত করতে রাজী থাকেন তবে দয়া করে नीচে স্বাক্ষর করুন।

স্বাক্ষর প্রধান গবেষকের

স্বাক্ষর/বাম বুড়ো আংলুরের ছাগ
মা/অভিযাবক

তারিখ: _____

তারিখ: _____

Simplify the
language

শিশুর
চিকিৎসা
কেন্দ্র
আমরা
জানি
চিকিৎসা
কেন্দ্র
সহায়

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.

ICDDR,B SURVEILLANCE ACTIVITY, CRC, DHAKA.

Patient, s name -----	Father, s name -----	code	column
Case number	/ _ / _ / _ / _ / _ / _ / _ /		1-7
Interviewer	/ _ /		8
Date	/ _ / _ / _ / _ / _ / _ /	d d m m y y	9-14
Age	/ _ / _ / _ / _ / _ / _ /	y y m m d d	15-20
Sex 1=male, 2=female	/ _ /		21
Religion 1=Muslim, 2=Hindu, 3=christ 4=Buddhist 5=Others	/ _ /		22
Use of replacement fluid before arrival: 0=none 1=ORS packet 2=Home made ORS 3=Barly 4=Rice/ 4=soil soup 5=I.V fluid 6=1/2+4 7=1/2+5 8=3/4+5	/ _ /		23
Chemotherapy before arrival 0=none 01=penicillin 02=tetra 03=ampi 04=chlo 05=furox 06=genta 07=sept 08=kana 09=nali 10=metro 11=AMP+Fux 12=Sept+Metro 13=Fux+Nali 14=Selexid 98=Others	/ _ / _ /		24-25
How many persons eat from the same cooking pot	/ _ / _ /		26-27
How many hrs before the onset of diarrhoea meal was taken	/ _ / _ /		28-29
How many children <5 years age in your family.	/ _ /		30
How many members of your family with diarrhoea in past 7 days.	/ _ /		31

No of deaths in the family in last 5 years from diarrhea.	/ /	32
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	/ /	33
Education of patient, s father: 0=none 1= maktab 2=1-3yrs 3=4-5yrs 4=6-10yrs 5=10-12yrs 6=>12yrs	/ /	34
Education of patient, s mother: (categories as above)	/ /	35
Self education (categories as above) (or patients >15 yrs of age.	/ /	36
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.	/ /	37
Sources of water for drinking: 1=Tap 2=TW 3=pond/river/ditch 4=1+2 5=1+3 6=2+3 7=1+2+3	/ /	38
Sources of water washing/bathing (categories as above)	/ /	39
Place of defecation : 1=Sanitary 2=semi-sanitary 3=service 4=dughole (with ring) 5=open pit 6=Hanging 7=No fixed place.	/ /	40
Used vitamin A capsule : 1=none 2=within 3 months 3=4m-6m 4=7m-12m 5=>12m	/ /	41
Temperature 1=Upto 36.6, 2=36.7-37.7, 3=37.8-38.8 4=38.8	/ /	42
Duration of diarrhoea before arrival 1=1 day 2=1-3days 3=4-6days 4=7-9days 5=10-12days 6=12-14days 7=>15days.	/ /	43
Character of stool 1=watery 2= non-watery	/ /	44

Stool contents 0=usual 1=Mucus 2=Blood 3=MU+BL	/ /	45
No of stools in 24 hrs: =3-5, 2=6-10, 3=11-15 =15-20, 5=>20	/ /	46
Abdominal pain : =no, 1=yes	/ /	47
Vomiting in last 24 hrs : =no, 1=<10 times, 2=>10 times	/ /	48
History of cough with diarrhoea : =none, 1=1-7 days 2=8-14days =15-20days, 4=>20days	/ /	49
History of measles =none, 1=measles in past 3m =measles in >3m-6m =present history of night blindness =past history of night blindness =Measles+night blindness	/ /	50
History of convulsion : =none, 1=convulsion within 12 hrs =convulsion within 13-24 hrs =convulsion >24 hrs	/ /	51
Other diseases (specify)	/ /	52
Since how long do you live in Dhaka city =never, 1=<1yr, 2=1-2yrs, 3=3-5yrs = >5yrs, 5=seasonal.	/ /	53
Present location (Thana/area) =Basti, 02=Common housing area =Residential area, 04=Village area =Others.	/ / / / /	54-57

PHYSICAL EXAMINATION :

Thirst	/_/_/	58
0=normal, 1=mild, 2=moderate 3=severe (for adult)		
General Condition	/_/_/	59
0=normal, 1= restless, 2=Lethergic but irritable when touched, 3=Drowsy/cold & sweating extremities, 4=coma		
Radial pulse	/_/_/	60
0=normal rate & good volume, 1=Rapid & weak, 2= Rapid & feeble/sometimes impalpable, 3=not palpable.		
Respiration	/_/_/	61
0=normal, 1=faster than normal 2=deep & rapid		
Clinical assesment of dehydration	/_/_/	62
0=no dehydration, 1=mild, 2=moderate 3=severe		
Convulsion	/_/_/	63
0=no, 1=yes		
OTHER PHYSICAL FINDINGS:		
Vitamin A deficiency	/_/_/	64
0=normal, 1=conj. xerosis, 2=Bitot spot 3=corneal ulcer, 4= caratomalacia 5=1+2, 6=3+4, 7=corneal scar.		
Ear - Otitis media	/_/_/	65
0=absent, 1=otitis media present		
Mouth	/_/_/	66
0=none, 1= angular stomatitis 2= glossitis 3=Pharyngitis 4= Tonsillitis		
Lungs	/_/_/	67
0=clear, 1=Rhonci, 2=crepitation 3=both		
Abdomen	/_/_/	68
0=normal sounds present 1=Distended sounds present 2=Distended sounds sluggish 3=Distended sound absent 4=Distention with tenderness.		

liver and spleen :
0=not palpable, 1=liver enlarged
2=spleen enlarged, 3= liver & spleen enlarged

/ _ /

69

Rectum prolapse
0=none, 1=yes

/ _ /

70

Extremities
0=oedema absent, 1=oedema present

/ _ /

71

Diagnosis
0=Uncomplicated diarrhoea
1=Complicated diarrhoea
note : complicated diarrhoea
admitted in medical ward)

/ _ /

72

Disposition
0=discharged from examination desk
1=ORP, 3=TC, 4=TC to ward, 5= ward
6= study ward, 7= Referred to another hospital
8= Death on arrival

/ _ /

73

Duration of stay
(days / hours)

/ _ / _ / _ / _ / " 74-77

Outcome
0=cured, 2=illness continuing
3= died, 4=absconded, 5= others

/ _ /

78



- Rehydration method used 0=none, 1=ORS only, 2= IV only, 3= ORS to IV, 4= IV to ORS 5= others	/_/_/	79
TREATMENT		
0 = no medicine, 1 = one medicine, 2 = two medicine, so on	/_/_/	80
- Tetracycline	/_/_/	81
- Ampicillin	/_/_/	82
- Septrin	/_/_/	83
- Furoxone	/_/_/	84
- Penicillin/Crystapen V	/_/_/	85
- Metronidazole	/_/_/	86
- Genatamycine	/_/_/	87
- Nalidixic Acid	/_/_/	88
- Chloramphenicol	/_/_/	89
- Others	/_/_/	90
Weight on Admission	/_/_/_/_/	91-93
Weight on Discharge	/_/_/_/_/	94-96
Height (cm)	/_/_/_/_/	97-99
Arm Circumference	/_/_/_/_/	100-102

ICDDR,B SURVEILLANCE ACTIVITY

ADDITIONAL QUESTIONNAIRE

1. Patient I.D. number
2. Date of interview
D D M M Y Y
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=09, 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)

36. Father reads newspaper (<=15 years) : ☐ /
or he reads (>15 Years)
37. If yes, how many days in a week : ☐ /
38. Father watches T.V. (<=15 years) : ☐ /
or he watches T.V. (>15 years)
39. If watches, how many days in a week : ☐ /
40. Father listens to radio (<=15 years): ☐ /
or he listens (>15 years)
41. If listens, how many days in a week : ☐ /
42. Mother member of any co-operative : ☐ /
(<=15 years) society, NGO/women
organisation or she herself
(>15 years)
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)
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 : ☐ /
(None=0, Boiling=1, Alum/tablet=2,
Sieving=3, Use filter=4, Other=5)
48. Years of schooling of father (read up to): ☐ / ☐ /

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

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

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

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

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

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

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

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

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 bed room
(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 /

65. Pigeons/birds enter in kitchen:
(Yes=1, No=2, Not applicable=9)
66. Pigeons/birds enter in bed room:
(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 bed room:
(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
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NATIONALITY : Nigerian
MARITAL STATUS : Married
NUMBER OF CHILDREN : Three
HOME INSTITUTION : Research Fellow II
AND POST : National Institute for Medical Research
Yaba, Lagos, Nigeria
CURRENT POST AND ADDRESS : International Health Research Fellow
Laboratory Sciences Division
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ACADEMIC QUALIFICATIONS:

1. Primary School Leaving Certificate - December, 1970
2. Modern III Leaving Certificate - June, 1993
3. West African School Certificate - June, 1978
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 :

1. Microbiologist in a WHO/UNICEF Diarrhoeal Project: 1983-85
2. Graduate Assistant (University of Lagos) : 1986-87
3. Junior Research Fellow (NIMR) : 1988-90
4. Research Fellow II (NIMR) : 1991-date

OTHER INTERESTS :

Current affairs, Scientific discussions,
Sports (table tennis)