hment 1.

Date 3-7-1994

E SHEET) ETHICAL REVIEW COMMITTEE, ICDDR,B.

ipal Investigator Dr. S.M. Akramuzzaman Trainee Investigator (if any)

ication No. 94-012 Supporting Agency (if Non-ICDDR,B) Commission of the European Union

e of Study Study of immune disruption caused by measles and it's association clinical progress in Dhaka, Bangladesh

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

le the appropriate answer to each of the following (If Not Applicable write NA).

Source of Population:
(a) Ill subjects (Yes) No
(b) Non-ill subjects (Yes) No
(c) Minors or persons under guardianship (Yes) No

Does the study involve:
(a) Physical risks to the subjects Yes (No)
(b) Social Risks Yes (No)
(c) Psychological risks to subjects Yes (No)
(d) Discomfort to subjects (Yes) No
(e) Invasion of privacy Yes (No)
(f) Disclosure of information damaging to subject or others Yes (No)

Does the study involve:
(a) Use of records, (hospital, medical, death, birth or other) (Yes) No
(b) Use of fetal tissue or abortus Yes (No)
(c) Use of organs or body fluids (Yes) No

Are subjects clearly informed about:
(a) Nature and purposes of study (Yes) No
(b) Procedures to be followed including alternatives used (Yes) No
(c) Physical risks Yes No Not applicable
(d) Sensitive questions Yes No
(e) Benefits to be derived (Yes) No
(f) Right to refuse to participate or to withdraw from study (Yes) No
(g) Confidential handling of data (Yes) No
(h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure Yes No Not applicable

5. Will signed consent form be required:
(a) From subjects Yes No
(b) From parent or guardian (if subjects are minors) (Yes) No
6. Will precautions be taken to protect anonymity of subjects (Yes) No
7. Check documents being submitted herewith to Committee:
___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
✓ Protocol (Required)
✓ Abstract Summary (Required)
✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
___ Informed consent form for subjects
✓ Informed consent form for parent or guardian
✓ Procedure for maintaining confidentiality
___ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Ctte. for review.

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

Akram
Principal Investigator

Trainee

ENTERED 2 1 JUN 1998

SECTION I - RESEARCH PROTOCOL

Title: Study of immune disruption caused by measles and it's association with clinical progress in Dhaka, Bangladesh.

Principal investigator/Co-Principal Investigators:

Dr. S. M. Akramuzzaman, International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B)
Dr. Felicity Cutts, London School of Hygiene & Tropical Medicine (LSHTM), U.K.
Prof V. ter Meulen, University of Wurzburg, Germany

Co-investigators:

Ms. Leanne Unicomb, ICDDR,B
Mr. M. A. Wahed, ICDDR,B
Dr. Anwar Hossain, ICDDR,B

Consultant: DR. Dilip Mahalanabis, ICDDR,B

Starting date: As soon as possible

Completion date: Two years from the starting date

Funding source: Commission of the European Union

Total direct cost: US$ 510,248

Name of the Division: Clinical Sciences Division

Signature of the CSD Head: Mahalanabis

Date: 3/7/94

Abstract summary:

This study will investigate different hypotheses for the biological mechanisms underlying the excess delayed mortality after measles or high titre measles vaccines. Morbidity will be monitored after mild measles (among children recruited through community-based surveillance) or complicated measles (recruited at hospital), and compared to controls (a cohort of healthy children and a cohort of children hospitalized with other illnesses). One hundred and ten children will be recruited in each group, and will be examined twice weekly in the first 6 weeks then weekly until 6 months after recruitment. Appropriate laboratory and radiological investigations of episodes of illness will be conducted to identify the causative agent where possible. Measles infection will be confirmed by serology. To investigate the hypothesis that measles virus infection causes a prolonged bias to type 2 helper T cell response (which is predicted to increase the antibody response to infectious agents), the response to vaccination with hepatitis B vaccines 6 weeks after recruitment will be compared between cases and controls. In each analysis, age, sex, socioeconomic status, nutritional status, and levels of serum retinol and zinc will be assessed as possible confounders.

On a subsample of subjects aged 6-11 months, (n=25 per group), the study will measure the lymphoproliferative response to stimulation with measles virus and measles virus purified membrane proteins, cytokine profiles, and changes in surface molecules, particularly those involved in the measles virus receptor complex, on subpopulations of peripheral blood mononuclear cells (PBMCs). Two groups of measles vaccinees (n=25 per group) will also be studied, one group receiving two doses of standard titre measles vaccines at ages 6 and 9 months, the other a single dose at 9 months, to allow comparison of the effects of wild versus vaccine virus on cellular function. The presence of measles virus in PBMC samples and subpopulations in children with acute measles will be compared to vaccinees at different time points following exposure.

These studies will help to understand the basis for immune suppression after measles, to determine the extent of immunosuppression after measles vaccination, and to raise hypotheses for the mechanism of differences in persistence of immunity after measles or measles vaccination. This information will be invaluable in the development and evaluation of novel measles vaccines.

Research Review Committee _____

Ethical Review Committee _____

Director _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objectives:
To investigate different hypotheses for biological mechanisms for increased mortality after measles or high titre measles vaccines, by obtaining information on the target cells of measles virus and surface molecule changes in natural infection versus vaccination, assessing the kinetics of immune disruption after measles or measles vaccination, and monitoring the incidence of secondary infections among children with measles compared to controls.

2. Background:
The prevention of measles associated mortality in young infants is a major challenge to global measles control. In developing countries, case-fatality ratios (CFR) have varied in community studies from 3% to 15%; even higher mortality being reported during epidemics[1]. CFRs are increased among children under three years of age,[2,3] unvaccinated children,[4] secondary cases,[5,6,7,8] children of low socioeconomic status[6], those with lack of access to care including adequate nutritional management[9,10] and vitamin A supplementation[11,12,13] and those with underlying illness,[14] particularly immunosuppressive diseases such as HIV/AIDS[15]. Complications may affect virtually all organ systems of the body, and have been documented both in the early convalescent phase (the first 4-6 weeks after rash onset), and for several months afterwards.[16,17]

Mortality has been shown to be increased for several months after measles infection[18] particularly when infection occurs in the first year of life[19,20]. Aaby et al have reported measurable increases in overall mortality up to the third year after exposure to measles in the first six months of life in Guinea Bissau.[21,22] There was no difference in mortality among children who had clinical measles (n=13) or exposure without symptoms (n=58). Among children with measles, half of deaths were attributed to diarrhoea, compared to 20% of deaths in controls. Measles vaccines have been available for 30 years, and heat-stable vaccines, appropriate for use in tropical climates, for almost 15 years. Current vaccination programs are estimated to prevent approximately 2 million measles-related deaths globally per year.[23] Nonetheless, almost one million deaths still occur each year, and the improvement of measles control through vaccination is one of the highest priority interventions in the health sector.[24]

Since 1976, the World Health Organization (WHO) has recommended the administration of standard-titre, live, further attenuated measles vaccines in developing countries at age 9 months, or as soon as possible thereafter[25]. Because of the high morbidity and mortality from measles prior to this age[26,27,28], trials of different strains and potencies of live attenuated measles vaccines were conducted among younger infants. High titre

Edmonston Zagreb (EZ) vaccine produced equivalent seroconversion rates among 6 month old infants to those after standard titre Schwarz vaccines among 9 month old infants[29,30,31,32]. In 1990 the Expanded Programme on Immunization (EPI) recommended the use of high titre EZ measles vaccine at 6 months of age in countries in which measles before the age of 9 months is a significant cause of death[33].

Implementation of that recommendation was delayed because of inadequate supplies of high titre vaccine. Subsequently, data which suggested increased mortality among children who received high titre (HT) vaccines at an early age[34,35] led the WHO to rescind the recommendation to use high titre vaccines[36].

The finding of increased mortality after HT vaccines raised several questions about the biology of measles and measles vaccination. Several aspects of the mortality increase have parallels in the complications of natural measles. The increase in mortality was associated more with the high titre than the strain of vaccine. Increased infectious dose of wild virus has been postulated to account for the higher mortality among secondary cases than primary cases seen in measles, although this could also reflect an increased risk of secondary infections in these cases. The mortality excess was delayed (manifest 2 or more years after vaccination); and was only documented in studies in countries with high background mortality; no increased mortality was found in studies in the Philippines, Mexico, and Peru, where higher socioeconomic status populations were vaccinated.[35] This pattern is similar to the observation of excess delayed mortality following measles in lower socioeconomic populations in developing countries, but not in populations of higher socioeconomic status. The increased mortality was nonspecific, deaths being attributed to the usual spectrum of diseases in those countries (respiratory infection, diarrhoea, malaria and malnutrition). Thus, the HT vaccines may mimic natural measles in disrupting immune function so as to increase susceptibility to mortality from multiple causes.

The increased mortality following high titre vaccines occurred primarily among females; there was little difference in mortality between male recipients of high or low titre vaccines. In natural measles, studies have not shown a consistent effect of gender on mortality, once cultural differences in care of male and female infants have been controlled for.[37,38,39,40,41,6] Aaby analyzed data from Senegal[42] and Kenya[43] and suggested that increased mortality among girls was due to more intense exposure associated with cross-sex transmission, but others point out that differences in care, such as feeding practices, could not be excluded, even though patterns of health centre consultations did not differ between boys and girls.[44]

Different hypotheses have been put forward to account for the increased mortality after high titre measles vaccines. Griffin[45] and co-workers postulate that, like natural measles infection, HT vaccines cause an imbalance in the helper T cell response to

antigenic challenge, with a prolonged bias towards a type 2 T_H response.[46]

Functional types of CD4 T cells can be distinguished by their state of differentiation and the types of cytokines they produce. After initial stimulation by antigen, naive CD4 (T_0) cells produce mainly Interleukin-2 (IL-2). After restimulation two types of memory CD4 cells emerge: T_H1 and T_H2. The majority of T_H1 (77%) but only a minority of T_H2 (18%) clones exhibit cytolytic activity in a PHA-dependent assay. T_H1 cells activate macrophages leading to DTH responses (IFN-γ), stimulate lymphocyte proliferation (IL-2) and MHC class-II restricted cytotoxicity (TNF-β). In contrast, T_H2 clones are important for macrophage deactivation (IL-4 and IL-10) and are more effective in inducing antibody synthesis of all classes in the presence of specific antigen (IL-4, IL-5, IL-6 and IL-10), and the degree of response is proportional to the number of T_H2 cells added to B cells. These two subtypes of CD4 T cells are cross-regulatory; IFN-γ inhibits proliferation of type 2 CD4 cells, whereas IL-4 and IL-10 inhibit cytokine production by type 1 CD4 cells.

The cytokine profile during measles is consistent with an increase in CD8 and type 1 CD4 T cell activity before rash onset (elevated IFN-γ), and of T_0 cells and type 1 CD4 cells during the rash (elevated IL-2). Clearance of measles virus from tissues begins with the onset of the rash and is associated with the appearance of measles-virus specific cytotoxic CD8 T cells,[47] soluble CD8 and IFN-γ in plasma,[48] and infiltration of lymphocytes into areas of viral replication.[48] These data suggest an important role for CD8 T cells in clearance of virus-infected cells from tissues by cytolytic mechanisms.

As the rash subsides, plasma levels of IL-4 rise and remain elevated for several weeks, suggesting a prolonged activation of type 2 CD4 cells. The long-term effects of measles are consistent with a lasting bias towards a type 2 T_H response. The depression of DTH skin responses [49,50,51] and of measles-virus specific lymphoproliferative responses[52] during measles are consistent with the lack of long-term activation of type 1 CD4 cells. The presence of long-term activation of type 2 CD4 cells is consistent with the suppression of macrophage activation and DTH responses, polyclonal B cell activation[53] and increased IgE present during the acute phases of measles,[54] and with predominance of measles-virus specific IgG1 and IgG4 antibody. The depression of macrophage activity induced by a T_H2 bias (IL-4 and IL-10) would lead to increased susceptibility to a broad range of infections.

Another potential mechanism for immune disruption is via MV(measles virus)-induced changes in cell surface molecules. Although the initial target cell subpopulation of PBMNCs for MV infection has not been positively identified, recent experiments suggest that MV specified sequences can be detected in peripheral macrophages isolated from acute measles cases[55]. Although this finding has to be reconfirmed and extended to vaccinated

children, the infection of macrophages would be particularly interesting. Besides potential consequences for the secretion of cytokines (see above) and potential dysregulation of surface proteins (see below), infection of macrophages may provide the basis for persistent extraneural MV infections. Tissue culture experiments indicate that an ideal intracellular environment is provided by macrophages as host cells to favor the establishment of persistent infections in restricting viral transcription and translation[56],[57]. It was observed that the expression of the IFN-ß inducible MxA protein was followed by a selective downregulation of MV glycoprotein synthesis in stably transfected macrophages but not in brain cells.[58] In addition it was found that in the course of MV infections cell surface changes occur on infected target cells. Whereas the expression of LFA-1 (leucocyte function antigen 1) is enhanced in the MV-infected human monocyte cell line (U937),[59] CD46 and moesin are downregulated.[60],[61] These latter two surface molecules represent parts of the MV receptor. CD46 (membrane cofactor protein, MCP) is a member of the "regulators of complement activation (RCA)" protein family, that serves critical functions by protecting cells from non-specific complement mediated lysis by inactivating complement factors[62]. Moesin (membrane organizing extension spike protein) belongs to the family of cytoskeletal proteins[63]. The consequences of downregulating these molecules on the surface of primary or persistently infected tissue culture cells on cell function and, most importantly, their expression and regulation in an acute measles infection or in a vaccinee will provide a new and exciting experimental approach to relate MV infection with impairments of leucocyte functions as encountered during MV induced immunosuppression.

3. Rationale:

The unexpected finding of increased mortality after the use of high titre vaccines in young infants leads to the obligation to understand better the mechanism of action of measles vaccines. Direct study of HT vaccines in humans is no longer ethical, but because of the parallels between the response to these vaccines and to natural infection, further study of natural measles infection using modern immunobiological techniques is of paramount importance to increase our understanding of the basis of immune disruption after measles and the method of action of different vaccines. This information will be invaluable in the development, and evaluation of safety and efficacy of novel measles vaccines.

B. SPECIFIC AIMS

1. To compare changes in measles-specific antibody profile and cytokine profile after measles or measles vaccination, and determine the duration of abnormalities.

2. To trace the expression of measles virus (MV)-specific RNAs in different subpopulations of peripheral blood mononuclear cells (PBMCs) in order to identify the target cells of MV in natural infection versus vaccination.

3. To analyze surface molecule changes (including RCA and LFA-1) on lymphocyte populations as a result of measles virus infection or measles vaccination.

4. To document the natural history of measles and investigate the hypothesis that measles induces a prolonged T_H1/T_H2 bias, by comparing the incidence of secondary infections and the immune response to challenge with hepatitis B vaccines among children in the convalescent phase of measles and controls.

METHODS AND MATERIALS

Study design:

A prospective cohort study will be conducted. The following cohorts of children will be recruited:

Measles cases:

(1) A community-based cohort of acute measles;
(2) A hospital cohort of complicated measles cases.

Children between 6 months to 12 years of age with measles identified in the study community or seen in the hospital will be included in the study.

Controls (age matched to cases):

(3) A community-based control group of healthy children;
(4) A hospital-based control group of children attending with diseases other than measles.

Measles vaccinees:

(5) A group of children vaccinated with standard titre measles vaccine at age 9 months.
(6) A group of children vaccinated at ages 6 and 9 months.

2. Study site:

The study will be conducted in a peri-urban poor community of Dhaka city, capital of Bangladesh, which is served by a weekly community clinic of the Clinical Sciences Division of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), and is located about 10 km from the ICDDR,B hospital. A community of approximately 1,000 families was censused in 1990; the census will be updated and the number of families increased for the purpose of this study. Measles vaccination coverage is approximately 50%, and measles incidence has been about 10-15% per year among children in this area. Acute measles cases will be recruited into the study from this population. Severe measles cases are not seen here because of the ready access to health care and referral to hospital of children with complications. The Clinical Research Centre of the ICDDR,B is a large, busy hospital which acts as a primary care hospital as well as a referral centre for diarrhoea and other infectious diseases (particularly acute respiratory tract infection) and malnutrition. It is the site the ongoing study of nosocomial transmission of measles. This hospital will be the site for recruitment of complicated measles cases.

3. Recruitment of cohorts:

Informed consent will be obtained from parents for the participation of all cohorts.

Cohorts 1 and 3 (community cases and controls): In the study community, health workers will visit each household every 3 days so that cases can be detected soon after the onset of measles rash. There will also be passive surveillance through the community clinic. A standard definition for measles will be followed for identification of measles cases:[61] Generalized

maculo-papular rash of 3 days duration, and history of fever (hot to touch), and at least one of the following: cough, coryza or conjunctivitis. For each case detected, a healthy control child within the age categories of 6-8, 9-11, 12-23, 24-35, 36-59 and 60-144 months will be randomly selected from the study community. Cohorts 2 and 4 (hospital cases and controls): In the hospital, active surveillance of children attending outpatient department has already been established for the study of Nosocomial Transmission of Measles. A measles case presenting within 6 weeks of onset of measles rash will be recruited by a Research Physician involved in the study. The next child within the same age-category (as above) as the case, presenting with another illness of similar severity will be be recruited as a control. Preliminary work is being conducted to have an idea on mother's perceptions of severity of different diseases by comparing a sample of measles cases and children with other diseases with regard to distance travelled, time taken to reach the hospital and the duration of the episode prior to reporting.

Cohorts 5 and 6 (vaccinees): Children attending the ICDDR,B vaccination clinic for third dose DPT/polio at age 4-6 months will be randomized to receive either measles vaccine at ages 6 and 9 months or a single dose at age 9 months.

For this study, hospital cases and controls, and vaccinees living within a reasonable distance from the hospital which permits home visits will be enrolled.

4. Clinical examination and follow-up:
A standard questionnaire will be administered to all cohorts. For cases, information on exposure, sequence of measles cases within the household to identify primary and secondary cases, present and past illness, immunization status, family size, birth order, and socioeconomic status will be sought. Clinical examination and anthropometry will be conducted. For other cohorts, similar information on present and past illnesses, immunization status etc will be obtained.

All cohorts will be examined twice-weekly for the first 6 weeks and then weekly for detection of subsequent infectious diseases for 6 months post-onset. The dietary management of cases in the home will be monitored. Anthropometry will be conducted weekly on all cohorts. Children will be asked to return immediately in the event of any acute illness at any time during follow-up. If secondary infection is detected the child will be brought to the ICDDR,B hospital for further investigations (eg chest x-ray, microscopy and culture of stool samples, blood and urine culture, and culture of nasopharyngeal secretions if there is upper respiratory tract infection).

Ideally, long term follow-up will be conducted to determine immune function 1-2 years after measles infection or vaccination. The long term follow-up will include regular anthropometric measurements to assess the possible role of nutritional status as a potential confounder. A separate proposal will be submitted for

extension of the follow-up on completion of the first phase of the study.

5. Anthropometry:
Height (accurate to the nearest 0.1 cm) will be measured using locally constructed stadiometers (for children >2 years) and length boards (for children <2 years), and weight (accurate to the nearest 20 gms) will be taken using high quality weighing scales, calibrated daily, following methods of standardization described by WHO.

6. Response to Hepatitis B vaccine:
Six weeks after recruitment, the first dose of hepatitis B vaccine will be administered to study children in groups 1-4. The second and the third doses of hepatitis B vaccine will be given at 4 weeks intervals.

7. Sample size:
To detect a difference of 15% in the proportion of children hospitalized during the six months after acute illness (comparing cohort (1) versus (3), and groups (2) versus (4)), with 80% power at the 95% significance level, assuming 5% of controls are hospitalized, 88 children would be required in each cohort. To allow for approximately 20% loss to follow-up, 110 children will be recruited in each of cohorts 1-4.

For detailed studies of surface molecule changes and the expression of MV-specific RNAs in different populations of PMBCs, a subsample of 20 children in each of cohorts (1) through (4) will be studied. To compare surface molecule changes between the primary and secondary cases, cohorts 1 and 4 each will consist of 10 primary and 10 secondary cases so that 20 primary and 20 secondary cases can be studied. To allow for dropout, 25 children will be recruited into this component of the study. For the studies of surface molecule changes among children who receive measles vaccines, allowing 20% loss to follow up, 25 children will be recruited in each of groups (5) and (6).

8. Laboratory assays (see bellow the "Summary table for laboratory assays" for timing of assays):
Laboratories will be blind to the clinical status of the study children. At the ICDDR,B laboratory in Bangladesh, measles-specific IgM and IgG antibody levels will be assayed by ELISA,[65] serum retinol and zinc levels will be determined by high performance liquid chromatography (HPLC)[66] and by atomic absorption spectrophotometry[67] respectively, and anti-HBsAg antibody levels will be assayed by passive haemagglutination (quantitative assay)[68]. The assays described below will be conducted at the University of Wurzburg.

<u>Measures of MV-specific CMI lymphoproliferation:</u> Proliferative responses will be determined in response to anti-CD3-mediated cross-linking of the T cell receptor and to sucrose gradient purified MV (wild type and Edmonston strain) and to purified MV glycoproteins. A modified lymphoproliferative assay will be

conducted according to the low amount of PBMCs available. In order to unmask MV antigen-specific proliferative responses thereby increasing the sensitivity of the assay, submitogenic concentrations (2U/ml) of IL-2 supplemented in the culture medium will be used.

PCR of MV and cytokine specific RNAs: Urine and nasopharyngeal secretions as well as part of the isolated PBMCs (as bulk populations or separated into subpopulations) will be processed for quantitative PCR analysis. Total RNA will be extracted using the RNAsol method and cDNA synthesized using random hexamer priming and AMV reverse transcriptase. For the detection of MV sequences, oligonucleotide primers specific for the MV N and F genes will be used for subsequent amplification. Amplification products will be visualized by ethidium bromide staining on agarose gels, the specificity of the amplified products can be verified by hybridization with the corresponding MV-specific cDNA clones labelled either with ^{32}P or with non-radioactive systems in a biochemical reaction. The remainder of the PCR products will be used in parallel for amplifying cytokine specific RNAs (see below).

ary table for laboratory assays:

	Cases				Vaccinees		Controls			
	Community (Cohort 1)		Hospital (Cohort 2)		6+9 m (Cohort 5)	9 m (Cohort 6)	Community (Cohort 3)		Hospital (Cohort 4)	
	n=110	n=25	n=110	n=25	n=25	n=25	n=110	n=25	n=110	n=25
	M-IgM M-IgG Vit A Zinc	Lab assays Germany (LAG)	M-IgM M-IgG Vit A Zinc	LAG	M-IgM M-IgG Vit A Zinc LAG	M-IgM M-IgG Vit A Zinc LAG	---	---	---	---
	M-IgM M-IgG Vit A Zinc Anti-HBsAg	LAG Skin tests	M-IgM M-IgG Vit A Zinc Anti-HBsAg	LAG Skin tests	M-IgM M-IgG Vit A Zinc LAG Skin tests	M-IgM M-IgG Vit A Zinc LAG Skin tests	M-IgM M-IgG Vit A Zinc Anti-HBsAg	LAG Skin tests	M-IgM M-IgG Vit A Zinc Anti-HBsAg	LAG Skin tests
	Anti-HBsAg	LAG	Anti-HBsAg	LAG	LAG	LAG	Anti-HBsAg	LAG	Anti-HBsAg	LAG

ıple size for laboratory assays in Germany (25 at start) is a subsample of the total .0).

RNAase protection assays: As the sensitivity of the PCR technique usually is limited, critical or unclear cases can be reinvestigated using highly sensitive nuclease protection assays that have been established and successfully conducted for PBMCs in the laboratory at Wurzburg. Although the complete method is now commercially available as a kit rendering the handling rather easy, the application of this method will be confined to exceptional cases.

Immunofluorescent staining: Staining for surface molecules as well as staining for internal antigens of MV structural proteins with permeabilised cells will be performed following standard procedures. Monoclonal and polyclonal antibodies against MV structural proteins as well as against the MV receptor structures which are not commercially available are present in the laboratory in Wurzburg. In some cases, double staining techniques will be applied for the analysis of surface and internal antigen expression at the same time. This will be important for the expression of MV receptor molecules in relation to MV specific antigens and for tracing the pathway of MV in different sub-populations of PBMCs following infection or vaccination. The expression of the individual antigens will be analyzed by flow cytometry. Immunofluorescence staining has been found to be a highly sensitive test in this laboratory. However, *in situ* PCR will also be developed in addition to immunofluorescence staining.

Cytokine profile: Generally, Il-1α, Il-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, TGF-β, TNF-α and type I IFN will be of interest. As far as possible, cytokine levels can be assayed directly in serum samples by commercially available ELISAS (as is the case for most interleukins). In addition, cytokine levels present in the supernatant of cultured PBMCs can be determined. Using sets of specific primers, levels of mRNAs specific for these cytokines can be assessed following reverse transcription by PCR as described above. The synthesis of type I IFN will be detected indirectly by staining for the internal IFN-inducible MxA protein.

9. **Specimen collection and processing** (See above the "Summary table for laboratory assays" for timing of blood samples):
Two ml of blood samples will be obtained by venepuncture from one of the forearm veins of all children in cohorts 1, 2 (measles cases), 5 and 6 (vaccinees) at recruitment and 6 weeks after recruitment. The same amount of blood samples will be obtained from cohorts 3 and 4 (controls) at 6 weeks after recruitment (before hepatitis B vaccine). One ml of blood will be collected by venepuncture from cohorts 1,2,3 and 4 at 6 months after recruitment (after administration of three doses of hepatitis B vaccines at 4 weeks intervals).

The first 25 children aged 6-11 months in each cohort and the groups of vaccinees will be enrolled in the study of surface molecule and cytokine changes. From these children in cohorts 1,2,5 and 6 (measles cases and vaccinees) additional 5 ml (a

minimum of 3 ml) of heparinized blood will be obtained by venipuncture at recruitment, 6 weeks and 6 months after recruitment and in cohorts 3 and 4 (controls) the same amount of blood will be obtained at 6 weeks and 6 months after recruitment. PBMCs will be isolated on Ficoll-hypaque gradients. Cells (resuspended in freezing medium containing 10% DMSO) and serum fractions will be frozen immediately in liquid nitrogen for shipment to the laboratory at the Institute of Virology and Immunology, University of Wurzburg, Germany for detailed virological and immunological studies. A minimum of 3 ml of blood is needed (corresponding to 4 million cells), as the minimal requirement for cDNA synthesis and subsequent PCR amplification is 2.5µg of total RNA (corresponding to 2.5 million PBMCs), and the remainder of the cells would be necessary for the immunofluorescent staining experiments (minimum $1-2*10^5$ cells per assay). In view of these calculations, 5 ml of blood will be collected where possible. For all cohorts, all blood samples will be obtained in the hospital so that samples can be processed and stored immediately for further assay. Parents will be requested to bring their child to the hospital on scheduled dates during follow-up for obtaining blood samples. Parents will be paid for the conveyance and for compensation of loss of their wage. If parents fail to bring their child to the hospital on the scheduled date, a Health Assistant will try to bring the child from his/her residence on the following day. Nasopharyngeal secretions and urine samples will be obtained from subsamples of measles cases and vaccinees in the first week after recruitment for detection of measles virus and cytokines.

9. Skin tests:
The Multitest CMI (Institut Merieux, Lyon, France) for delayed cutaneous hypersensitivity will be conducted by multipuncture on subjects who will have detailed tests of cellular and subcellular molecular changes at the 6 week follow-up visits, following the manufacturer's specifications. The Multitest CMI applicator has 8 heads with 9 tines on each head, loaded with 7 different antigens (tetanus, diphtheria, streptococcus, tuberculin, proteus, trichophyton, and candida). The Multitest CMI is applied firmly to the stretched skin of the forearm near the elbow for 5 seconds. Induration is measured in mm after 48 hours, by means of a caliper. Reactions >=2 mm are considered positive.

10. Autopsies:
Whenever possible, autopsies will be conducted on children who die, to obtain more precise information on the immediate cause of death and to look for persistence of measles virus infection. Amongst the tissues that are available and are of particular interest, lymph nodes, spleen and gut are the most important. Tissue procession usually will be done by either deep freezing the tissue immediately after autopsy and sending it to Germany, or by immediately mincing and lysing the tissue in guanidiniumisothiocyanate buffer for further preparation in Germany.

11. Management of cases and controls:
All cases and hospital-controls will be appropriately treated free of cost either on an outpatient or an inpatient basis according to the routine practice of the ICDDR, B. The community cases will be treated free of cost from the community clinic. All the cases and controls will be provided necessary treatment whenever they are found to be ill during the period of follow-up. The treatment will include administration of vitamin A, dietary management for malnutrition and treatment of infections. Parents of all unvaccinated study children will be encouraged to get their children vaccinated with appropriate EPI vaccines.

12. Data analysis:
The main areas of analysis are as follows:
(1). Measles virus (wild or vaccine) is the exposure of interest and immunological changes the outcome of interest. Immunological changes, including cytokine profile and surface molecules on sub-populations of PBMCs will be compared among (a) acute measles cases vs healthy controls, (b) complicated measles cases vs children with other illnesses (c) acute measles cases from the community and complicated measles cases in hospital (d) primary versus secondary measles cases and (e) acute measles cases versus recipients of measles vaccine at 6 or 9 months of age. For the presence of MV in PBMC samples and subpopulations, children with acute measles will be compared to vaccinees at different time points following exposure. The role nutritional status (measured by anthropometry and serum levels of zinc and retinol) will be assessed for confounding the effect of measles infection on cellular immune functions.

(2). Measles virus is the exposure of interest, and morbidity after recruitment (hospitalization, days of illness, secondary infections), and humoral response to hepatitis B vaccine the outcomes of interest. Age, sex, nutritional status, and micronutrient levels will be assessed as possible confounders.

(3). A subsidiary analysis will be conducted on assessment of early and delayed case fatality rates and factors associated with fatality among measles cases.

13. Quality control:
During planing phase of the study a "Manual" will be developed for the Research Physicians and the field workers. It will contain standard definitions and selection criterias of measles cases, healthy controls and hospital controls. There will be instructions on administration of the questionnaire and on taking anthropometric measurements. It will include guidance on what checks are to be conducted on completed questionnaires and details on when and how samples will be collected and processed for laboratory analysis.

Clinical diagnosis of all measles cases and other diseases will be confirmed by the Principal Investigator or the trained Research Fellow (Physician). Clinical diagnosis of measles will be confirmed in all measles cases and will be ruled out in all

controls by serology. Diagnosis of other diseases will be confirmed, whenever possible, by cultures of blood, stool, urine or other laboratory tests available at the ICDDR,B.

The follow-up rate will be monitored closely. Parents will requested to notify new addresses in case of migration. At least three different addresses of relatives or friends will be recorded to trace the cases or controls in case of unnoticed migration. Apart from migration, other reasons for children being lost to follow-up will be ascertained and remedial measures will undertaken promptly whenever possible.

All records will be checked by the Principal Investigator for completeness and logical consistency prior to computer entry. A data set will be entered twice in two data files by two data entry technicians and these files will be compared by computer programs for typing errors. In addition, interactive and batch checking will be performed to ensure that data are within allowable range and that they are consistent from one question to the another. Errors and queries will be referred back to the relevant worker or the investigator immediately. In addition, interim tabulations of some important variables will be performed on a monthly basis to monitor any marked shift in the pattern of data. This will give a signal to check on particular aspect of the study. All these procedures will also help to monitor performance of field workers. To monitor performance of laboratory procedures blind coded duplicate samples will be introduced regularly into the workload.

During the period of the study regular meetings will be held with all members involved in the study to discuss progress and queries. When new problems arise the solution will be incorporated into the manual so that there is consistency in dealing with the problem in future.

14. Confidentiality:
All existing and new information will be held strictly confidential. Study subjects will be identified by only numerical codes in all data sets used for analysis.

D. STUDY SCHEDULE

The study has four general phases (see bellow the work programme):

1.Planning and setting up field work:
Dr Cutts and Dr ter Meulen will visit Bangladesh to help set up the study, including preparation of a detailed plan of field work and supervisory activities, discussion of the study with the community concerned, arrangement of laboratory procedures including storage and shipping of specimens, pre-test of questionnaires, training of interviewers, selection of criteria for investigation of illness episodes among study children, and arrangement of data management procedures.

2. Field data collection:
Recruitment of measles cases and controls is expected to take 15 months. The first 25 children aged 6-11 months, whose parents consent, will be recruited for assays of cellular immune function, thus follow-up of this subgroup should be completed and all specimens shipped to the University of Wurzburg by the third quarter of the second year of the study.

3. Laboratory analyses:
Analyses will be ongoing in both the collaborating laboratories. It is envisaged that specimens will be carried to Germany by Dr Cutts or Dr Akramuzzaman as accompanied baggage on 3-4 occasions during the study. Laboratory analyses should be completed by the end of the third quarter of the second year of the study.

4. Data analysis and report generation:
An interim analysis of clinical data and laboratory data from Bangladesh will be conducted by Dr Cutts and Dr Akramuzzaman, as part of the monitoring of quality control, at the end of the first year. Preliminary analysis of data from the Wurzburg laboratory will be conducted in Wurzburg as data become available. Joint analysis of clinical and laboratory data will be conducted in London in the last quarter of the second year, with full-time support from a statistician at LSHTM (3 month's salary for the statistician is included in the funding requested).

C. SIGNIFICANCE

Most of the recent work on the effect of measles and measles vaccines on cellular immune function and cytokine profile have been of a laboratory nature, and the effects of the postulated bias in helper T cell response on clinical outcome has not been fully assessed. Our study will obtain precise data on the clinical progress of measles to assist in understanding the clinical implications of changes detected at the molecular level. The effect of HT vaccines on increased mortality was only observed in countries with high background rates of infant mortality, suggesting that there is an interaction with other factors which cause child mortality. Our study will extend this observation to Bangladesh, examining the possible interaction of different socioeconomic, nutritional and environmental conditions with measles associated immune disruption on incidence and severity of secondary infections.

The recent definition of the surface molecules involved in the MV receptor has opened up a new and exciting experimental approach to relate MV infection with impairments of leucocyte functions as encountered during MV induced immunosuppression. Our study will determine the consequences of downregulating CD46 and moesin on the surface of primary or persistently infected tissue culture cells on cell function. Differences in the expression and regulation of these molecules will be assessed in an acute or complicated measles infection, and in 6 or 9 month old recipients of standard titre measles vaccine, thus investigating the hypothesis that downregulation of these molecules is one of the determinants of the degree of immune suppression induced by wild or vaccine virus. The study will also verify whether macrophages are the main target cells for MV infection, extend this observation to vaccinated children, and investigate the implications for the establishment of persistent infection.

The advancement of knowledge of the effects of wild and vaccine virus on cellular immune function will be a vital contribution to improving the health status of populations in developing countries by opening the way for the design and evaluation of new vaccines and vaccination strategies against measles, a major cause of child mortality in the world. Studies in Bangladesh have shown the importance of measles among children under 9 months of age.[69] A study has recently been conducted a ICDDR,B to investigate waning of maternal antibody in infants, with a view to earlier vaccination. Our study, which will assist in understanding the mechanisms of action and of potential adverse events of measles vaccines in young infants is therefore extremely relevant to Bangladesh for development of it's immunization policy.

E. FACILITIES REQUIRED

No new facilities will be required for this study. Uncomplicated measles cases and healthy controls will be recruited from a peri-urban poor community of Dhaka city, which is served by a weekly clinic of the ICDDR,B. Complicated measles cases and diseased controls will be recruited from the ICDDR,B hospital, which receives approximately 100,000 patients a year, 60% of whom are children, suffering from diarrhoea, ARI and other infectious diseases including measles. Measles vaccinees will be recruited from among the children who obtain the third dose of DPT and polio from the immunization clinic of the hospital, which vaccinates approximately 2000 children with the third dose of DPT and polio vaccines and 3000 children with measles vaccine a year.

The ICDDR,B has an excellent research laboratory which is located in the hospital building. All the laboratory tests planned for this study are already being performed for other ongoing researches. The Institute of Virology and Immunobiology of the University of Wurzburg has long experience of performing all assays planned for this study. Monoconal and polyclonal antibodies against measles virus structural protein as well as receptor structures which are not commercially available are present in the laboratory in Wurzburg. Therefore, no new laboratory set up will be required for this study.

F. COLLABORATIVE ARRANGEMENT (please see the Work Program bellow on page 21):

This will be a collaborative study among the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), the London School of Hygiene and Tropical Medicine (LSHTM) and the University of Wurzburg, Germany.

The ICDDR,B has a long history of research on measles epidemiology and the impact of measles vaccines. However, the ICDDR,B has not conducted any in-depth clinical and immunological studies on measles or measles vaccination although it has the potential to conduct such research. Dr Akramuzzaman has shown his abilities in clinical studies at the ICDDR,B and he has also shown his excellent capacities for research on epidemiology of measles in connection to his MSc course at the University of London. He is the principal investigator of the ongoing research on nosocomial transmission of measles. He will be responsible for conducting the clinical studies on measles for this study.

Prof v ter Meulen is leading a group of scientists at the University of Wurzburg, who are at present analyzing the replication of measles virus in peripheral blood mononuclear cells and the mechanism by which measles virus is inducing immunosuppression. They have identified on lymphocytes and monocytes in addition CD46 moesin as another receptor for measles virus. This receptor is down-regulated after viral infection. Prof ter Meulen will conduct and supervise the laboratory assays for this study at the University of Wurzburg. The University will also welcome training of a post-doctoral fellow in those laboratory assays which are relevant and feasible to conduct in developing countries. Ideally, a candidate will be sought in Bangladesh.

Dr Felicity Cutts of the Communicable Disease Epidemiology Unit of the LSHTM has worked in measles control and measles vaccine trial in developing countries for over 10 years and is currently working on the development of criteria to evaluate the safety and efficacy of current and future measles vaccine. She has ongoing projects to monitor the use of medium titre EZ vaccine at 6 months of age in Kinshasa, Zaire, and conducted two trials of high titre EZ vaccine at Kinshasa. The LSHTM has several scientists involved in measles research. Two colleagues of Dr Cutt's Unit (Prof. Paul Fine and Dr. Jonathan Sterne) conducted an independent review of the data on high titre measles vaccine and mortality for WHO, which led to the global policy change in the use of this vaccine. Dr Cutts will use her expertise in measles research to help set up and supervise the study in Bangladesh, and she will coordinate activities of the study at three collaborating institutions. She will also supervise Dr. Akramuzzaman, who will enrol as an external student for a PhD degree at the University of London and use this project in part fulfillment of the requirements of the PhD.

In year 1, Dr Cutts and Dr ter Meulen will visit Bangladesh to help set up the study, and Dr. Cutts will make a second visit for supervision of field work 6 months later. Dr Cutts will make a third visit at the beginning of year 2 to conduct a interim analysis of clinical and local laboratory data with Dr Akramuzzaman. Dr Cutts and Dr Akramuzzaman will each visit the Wurzburg laboratory once per year, to observe laboratory procedures and discuss progress. Specimens will be transported as accompanied luggage from Bangladesh to Germany on these occasions. Dr ter Meulen and Dr Akramuzzaman will visit London at the end of the study for final data analysis, interpretation and report generation. This collaborative project would help to develop research capacity for ICDDR,B on measles by transfer of clinical, epidemiological and virological expertise from the London School of Hygiene and the University of Wurzburg.

Program:

ases, research tasks and personnel : Dr Akramuzzaman; TM:Dr ter Meulen; : Dr Felicity Cutts	Year 1 *				Year 2 *			
	1	2	3	4	1	2	3	4
Pretest questionnaires (AZ, FC)	-							
Discuss study with community under surveillance (AZ)	-							
Arrange laboratory procedures including cell separation and storage (AZ, TM)	-							
Train interviewers (AZ, FC)	-							
Arrange data entry procedures (AZ, FC)	-							
Enrol measles cases in hospital and community and take blood samples (interviewers and study physicians)	-	-	-	-	-			
Select appropriate controls and enrol them (AZ)	-	-	-	-	-			
Conduct twice weekly visits for 6 weeks post-enrolment (interviewers)	-	-	-	-	-			
Begin hepatitis B vaccine series 6 weeks after enrolment, and take blood samples from cases and controls (interviewers and study physicians)	-	-	-	-	-			
Conduct weekly visits until 6 months post-enrolment (interviewers).	-	-	-	-	-	-	-	
Investigate episodes of illness in study children (study physicians)	-	-	-	-	-	-	-	
Recruit groups of vaccinees and randomize to receive one or two doses of measles vaccine (AZ and study physicians)	-	-						
Administer measles vaccine and take blood samples on the day of vaccination, 6 weeks and 6 months after enrolment (interviewers and study physicians)	-	-	-	-				
Supervise interviewers (AZ, community supervisors, FC)	-	-	-	-	-	-	-	
Process blood samples, separating sera and cells (laboratory technicians)	-	-	-	-	-	-	-	
Conduct laboratory assays in Bangladesh		-	-	-	-	-	-	
Transport cells to Germany (FC, AZ)			-		-		-	
Conduct laboratory assays in Germany (TM)			-	-	-	-	-	
Monitor data entry with quality controls (AZ, FC)	-	-	-	-	-	-	-	
Conduct interim data analyses in Bangladesh (AZ, FC)				-				
Analyze data from laboratory assays in Germany (TM)				-			-	
Conduct final data analysis in London (FC, statistician of LSHTM, AZ, TM)								-
Produce report (FC, AZ, TM)								-

nbers refer to quarters, beginning as soon as possible after funding is obtained for the study.

REFERENCES

1. Cutts FT, Henderson RH, Clements CJ, Chen RT, Patriarca PA. Principles of measles control. Bull WHO 1991;69:1-7.

2. Gordon JE, Jansen AAJ, Ascoli W. Measles in rural Guatemala. Pediatrics 1965;66:779-786.

3. McGregor IA. Measles and child mortality in the Gambia. West Afr Med J 1964;13:251-7.

4. Aaby P, Bukh J, Leerhoy J, Lisse IM, Mordhorst CH, Pederson IR. Vaccinated children get milder measles infection: a community study from Guinea-Bissau. J Infect Dis 1986;154:858-63.

5. Aaby P, Bukh J, Lisse IM, Smits AJ. Overcrowding and intensive exposure as determinants of measles mortality. Am J Epidemiol 1984;120:49-63.

6. Bhuiya A, Wojtyniak B, D'Souza S, Nahar L, Shaikh K. Measles case fatality among the under-fives: a multivariate analysis of risk factors in a rural area of Bangladesh. Soc Sci Med 1987;24:439-43.

7. Aaby P, Lwuewenburg J. Patterns of transmission and severity of measles infection: a reanalysis of data from the Machakos area, Kenya. J Infect Dis 1990;161:171-4.

8. Garenne M, Aaby P. Pattern of exposure and measles mortality in Senegal. J Infect Dis 1990;161:1088-94.

9. John TJ, Joseph A, George TI, Radha Krishnan J, Dayal R, Singh PD, George K. Epidemiology and prevention of measles in rural South India. Ind J Med Res 1980;72:153-8.

10. Scrimshaw NS, Salomon JB, Bruch HA, Gordon JE. Studies of diarrhoea disease in Central America. VIII. Measles, diarrhoea and nutritional deficiency in rural Guatemala. Am J Trop Med Hyg 1966;15:625-31.

11. Barclay AJG, Foster A, Sommer A. Vitamin A supplements and mortality related to measles: a randomized clinical trial. Br Med J 1987;294:294-6.

12. Coutsodis A, Broughton M, Coovadia M. Vitamin A supplementation reduces measles morbidity in young African children: a randomized, placebo-controlled, double blind trial. Am J Clin Nutr 1991;54:890-5.

13. Hussey GD, Klein M. A randomized, controlled trial of vitamin A in children with severe measles. N Engl J Med 1990;323:160-4.

14. Diaz T, Nunez JC, Rullan JV, Markowitz LE, Barker ND, Horan J. Risk factors associated with severe measles in Puerto Rico. Pediatr Infect Dis J 1992;11:836-40.

15. Krasinski K, Borkowsky W. Measles and measles immunity in children infected with human immunodeficiency virus. JAMA 1989;261:2512-6.

16. Bhaskaram P, Reddy V, Raj S, Bhatnagar RC. Effect of measles on the nutritional status of preschool children. J Trop Med Hyg 1984;87:21-5.

17. Reddy V, Bhaskaram P, Rahuramulu N et al. Relationship between measles, malnutrition, and blindness: a prospective study in Indian children. Am J Clin Nutr 1986;44:924-30.

18. Hull HF, Williams PJ, Oldfield F. Measles mortality and vaccine efficacy in rural West Africa. Lancet 1983;i:972-5.

19. Osagie HF. Delayed mortality and morbidity 12 months after measles in young children in Nigeria. London: Institute of Child Health, University of London, 1986 (MSc Thesis).

20. Williams PJ, Hull HF. Status of measles in The Gambia, 1981. Rev Infect Dis 1983;5:391-394.

21. Aaby P, Bukh J, Kronborg D, Lisse IM, da Silva MC. Delayed excess mortality after exposure to measles during the first six months of life. Am J Epidemiol 1990;132:211-9.

22. Aaby P, Andersen M, Knudsen K. Excess mortality after early exposure to measles. Int J Epidemiol 1993;22(1):156-62.

23. Kim-Farley R. Global immunization. Expanded Programme on Immunization team. Annu Rev Public Health 1992;13:223-37.

24. The World Bank. World Development Report 1993. Investing in Health. Oxford University Press, Oxford, 1993.

25. Expanded Programme on Immunization. Measles immunization. Wkly Epidemiol Rec 1979;54:337-9.

26. Halsey NA, Boulos R, Mode F et al. Response to measles vaccine in Haitian infants 6 to 12 months old. N Engl J Med 1985;313:544-9.

27. Dabis F, Sow A, Waldman R et al. The epidemiology of measles in a partially vaccinated population in an African city: implications for immunization programs. Am J Epidemiol 1988; 127:171-8.

28. Taylor WR, KaLisa R, Ma-Disu M, Weinman JM. Measles control in urban Africa complicated by high incidence of measles in the first year of life. Am J Epidemiol 1988;127:788-94.

29. Markowitz LE, Sepulveda J, Diaz-Ortega JL et al. Immunization of six-month old infants with different doses of Edmonston-Zagreb and Schwarz measles vaccines. N Engl J Med 1990;322:580-7.

30. Whittle HC, Hanlon P, O'Neill K et al. Trial of high-dose Edmonston Zagreb measles vaccine in the Gambia: antibody response and side effects. Lancet 1988;i:963-6.

31. Job JS, Halsey NA, Boulos R et al. Successful immunization of infants at 6 months of age with high dose Edmonston-Zagreb measles vaccine. Pediatr Infect Dis 1991;10:303-11.

32. Berry S, Hernandez H, Kanashiro R et al. Comparison of high titer Edmonston-Zagreb, Biken-CAM and Schwarz measles vaccines in Peruvian infants. Pediatr Infect Dis J 1992;11:822-7.

33. Expanded Programme on Immunization. Measles immunization before 9 months of age. Wkly Epidemiol Rec 1990;2:8

34. Garenne M, Leroy O, Beau JP, Sene I. Child mortality after high-titer measles vaccines: prospective study in Senegal. Lancet 1991;338(ii):903-7.

35. Halsey NA. Increased mortality after high titre measles vaccines: too much of a good thing. Pediatr Infect Dis J 1993;12:462-5.

36. Expanded Programme on Immunization. Safety of high-titre measles vaccines. Wkly Epidemiol Rec 1992;67:357-61.

37. Aaby P, Bukh J, Lisse IM, et al. Determinants of measles mortality in a rural area of Guinea-Bissau: crowding, age, and malnutrition. J Trop Pediatr 1984;30:164-8.

38. Lamb WH. Epidemic measles in a highly immunized rural West African (Gambian) village. Rev Infect Dis 1988;10:457-62.

39. Koster FT, Curlin GC, Aziz KMA, Haque A. Synergistic impact of measles and diarrhoea on nutrition and mortality in Bangladesh. Bull WHO 1981;59:901-8.

40. Garenne M. Variations in the age pattern of infant and child mortality with special reference to a case study in Ngayokheme (rural Senegal). PhD dissertation. Philadelphia: University of Pennsylvania, 1982.

41. Narain JP, Khara S, Rana SRS, Banerjee KB. Epidemic measles in an isolated unvaccinated population, India. Int J Epidemiol 1989;18:952-8.

42. Aaby P. Influence of cross-sex transmission on measles mortality in rural Senegal. Lancet 1992;340:388-91.

43. Aaby P, Leeuwenburg J. Gender and the pattern of transmission of measles infection. A reanalysis of data from the Machakos area, Kenya. Ann Trop Pediatr 1991;11:397-402.

44. Van den Broeck J. Influence of cross-sex transmission on measles mortality in rural Senegal (letter). Lancet 1992;340:734.

45. Griffin DE, Ward BJ. Differential CD4 T cell activation in measles. J Infect Dis 1993;168:275-81.

46. Ward BJ, Leon ME, Halsey NA, Griffin DE. Possible mechanisms of immunologic changes after high titre measles vaccination. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory (in press).

47. Van Binnendijk RS, Poelen MCM, Kuijpers KC, Osterhaus ADME, Uytdehaag FGCM. The predominance of CD8+ T cells after infection with measles virus suggests a role for CD8+ class 1 MHC-restricted cytotoxic T lymphocytes (CTL) in recovery from measles. J Immunol 1990;144:2394-9.

48. Kimura A, Tosaka K, Nakao T. Measles rash. I. Light and electron microscopic study of skin eruptions. Arch Virol 1975;47:295-307.

49. Nelson JD, Sandusky G, Peck FB. Measles skin test and serologic response to intradermal measles antigen. JAMA 1966;198:185-6.

50. Tamashiro VG, Perez HH, Griffin DE. Prospective study of the magnitude and duration of changes in tuberculin reactivity during complicated and uncomplicated measles. Pediatr Infect Dis J 1987;6:451-4.

51. Whittle HC, Bradley-Moore A, Fleming A, Greenwood BM. Effects of measles on the immune response of Nigerian children. Arch Dis Child 1973;48:753-6.

52. Hirsch RL, Griffin DE, Johnson RT et al. Cellular immune responses during complicated and uncomplicated measles virus infections of man. Clin Immunol Immunopathol 1984;31:1-12.

53. Arneborn P, Biberfeld G, Forsgren M, von Stedingk LV. Clin Exp Immunol 1983;51:165.

54. Griffin DE, Cooper SJ, Hirsch RL et al. Changes in plasma IgE levels during complicated and uncomplicated measles virus infections. J Allergy Clin Immunol 1985;76:206-13.

55. Esolen LM, Ward BJ, Moench TE, Griffin DE. Infection of monocytes during measles. J Infect Dis 1993;168:47-52.

56. Schnorr JJ, Schneider-Schaulies S, Simon-Jodicke A, Pavlovic J, Horisberger MA, ter Meulen V. MxA dependent inhibition of measles virus glycoprotein synthesis in a stably transfected human monocytic cell line. J Virol 1993;67:4760-8.

57. Ingendahl C, Schneider-Schaulies S, Rima BK, ter Meulen V. Delayed replication of measles virus in human monocytes is linked to a specific truncation of the nucleocapsid protein. Submitted for publication.

58. Schneider-Schaulies S, Schneider-Schaulies J, Loffler S et al. Host cell dependent transcriptional regulation of measles virus in human brain cells constitutively expressing MxA. Submitted for publication.

59. Attibele N, Wyde PR, Trial J, Smole SC, Smith CW, Rossen RD. Measles virus induced changed in leucocyte function antigen 1 expression and leucocyte aggregation: possible role in measles virus pathogenesis. J Virol 1993;67:1075-9.

60. Naniche D, Varior-Kishnan G, Cervoni F et al. Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus. J Virol 1993;67:6025-32.

61. Dunster LM, Schneider-Schaulies J, Loffler S et al. Moesin: A cell membrane protein linked with susceptibility to measles virus infection. Virology 1993; in press.

62. Liszewski MK, Post TW, Atkinson JP. Membrane cofactor protein (MCP or CD46): newest member of the regulators of complement activation gene cluster. Ann Rev Immunol 1991;9:431-55.

63. Furthmayer HW, Lankes W, Amieva A. Moesin, a new cytoskeletal protein linked with susceptibility to measles virus infection. Virology 1993; in press.

64. Expanded Programme on Immunization. Measles control in the 1990s: Plan of action for global measles control. World Health Organization. WHO/EPI/GEN/92.3.

65. Ozzane G, D'Halewyn M. Performance and reliability of the Enzygnost measles enzyme-linked immuno-sorbent assay for detection of measles virus-specific immunoglobulin M antibody during a large epidemic. J Clin Microbiol 1992;30(3):564-9.

66. De Ruyter MGM, De Leenheer AP. Determination of serum retinol (vitamin A) by high speed liquid chromatography. clin Chem 1976;22:1593.

67. Prasad AS, Oberleas D, Halsted JA. Determination of zinc in biological fluids by atomic absorption spectrophotometry in normal and cirrhotic subjects. J Lab clin Med 1965;66:508.

68. Vyas GN et al. Hemagglutination assay for antigen and antibody associated with viral hepatitis. Science 1970;170:332.

69. Fauveau V, Chakraborty J, Sarder AM, Khan MA, Koenig MA. Measles among under-9-months-olds in rural Bangladesh: its significance for age at immunization. Bull WHO 1991;69(1):67-72.

Study of the immune disruption caused by measles and its associatio
clinical progress in Dhaka, Bangladesh

BUDGET FOR TWO YEARS
[amount in US $]

PERSONNEL COST	% OF SALARY	FIRST YEAR	2ND YEAR	TOTAL
PRESENT STAFF				
Dr SM Akramuzzaman	50%	5,466	6,012	11,478
Mr MA Wahed	10%	1,483	1,631	3,114
Dr Anowar Hossain	05%	606	666	1,272
Mr DK Barua	100%	4,872	5,359	10,231
Ms Rekha Chanda	100%	3,948	4,343	8,291
Ms Jyotsna Biswas	100%	3,948	4,343	8,291
				0
NEW RECRUITMENT				0
Res Fellow (1)	100%	2,154	2,369	4,523
Res Assistant (1)	100%	2,154	2,369	4,523
Data Proc Asstt (1)	100%	3,300	3,630	6,930
Field Workers (4)	100%	4,062	4,468	8,530
SUB-TOTAL		31,993	35,190	67,183
				0
LOCAL TRAVEL		22,625	24,887	47,512
[For follow-up home				0
vists/clinic visits]				0
INT'L TRAVEL		3,000	3,300	6,300
[Travel to LSHTM for				0
data analysis etc.]				0
				0
SUPPLIES & MATERIALS				0
Hospital supplies		500	550	1,050
Office supplies		500	550	1,050
Laboratory supplies				
Hepatitis B vaccine		3,000	3,300	6,300
ELISA-kit:measles-specific IgM		2,100	2,300	4,400
ELISA-kit:measles-specific IgG		5,000	5,000	10,000
CMI kit		5,000	5,000	10,000
Glassware (pippets etc.)		1,500	1,650	3,150
Chemicals		500	550	1,050
Drugs [for wkly clinic]		2,000	2,200	4,200
Non-stock drugs		100	110	210
Nonstock others		100	100	200
Clearing & forwarding		100	110	210
SUB-TOTAL		20,400	21,420	41,820

	First Year	2nd Year	Total
Carried Forward	20,400	21,420	41,820
			0
OTHER CONTRACTUAL			0
Rent, commun. & utilities	100	110	210
Printing & Publication	100	110	210
Repairs & Maintenance	1,000	1,100	2,100
Staff development	2,000	2,000	4,000
SUB-TOTAL	3,200	3,320	6,520
			0
INTERDEPARTMENTAL SERVICES			0
Land Transport-Dhaka	220	220	440
Xerox & mimeograph	220	220	440
Pathological test	5,500	6,050	11,550
Microbiological test	3,000	3,300	6,300
Biochemistry test	7,203	7,923	15,126
X-ray	300	330	630
Medical illustration	100	110	210
Fax/Telex	100	110	210
Library charges	100	110	210
Staff clinic - Dhaka	200	220	420
SUB-TOTAL	16,943	18,593	35,536
			0
CAPITAL EXPENDITURES			0
Weighing scales	300		300
Length boards	200		200
Microcomputer & printer	3,500		3,500
SUB-TOTAL:	4,000	0	4,000
TOTAL DIRECT COST	102,161	106,710	208,871
			0
INDIRECT COST (31% of direct cost)	31,670	33,080	64,750
			0
TOTAL PROJECT COST	133,831	139,790	273,621

Shamima Hai 8/11/93
Head, Budgeting Accounts

IMMUNE DISRUPTION CAUSED BY MEASLES AND IT'S ASSOCIATION WITH CLINICAL PROGRESS IN DHAKA, BANGLADESH

Two years summary budget for three collaborating institutions (amount in US $)

Expense category	ICDDR,B	LSHTM	UW
Personnel cost	67,183	51,207	161,700
Local travel	47,512		
International travel	6,300	16,500	12,705
Supplies and materials	41,820	1,515	57,750
Other contractual	6,520		
Interdepartmental services	35,536		
Capital expenditure	4,000		
Direct cost	208,871	69,222	232,155
Indirect cost	64,750	27,690	
Project cost	273,621	96,912	232,155

TOTAL DIRECT COST: 510,248

TOTAL PROJECT COST (INCLUDING OVERHEAD): 602,688

Abstract summary for the Ethical Review Committee

This study will investigate different hypotheses for the biological mechanisms underlying the excess delayed mortality after measles or high titre measles vaccines. Morbidity will be monitored after mild measles (among children recruited through community-based surveillance) or complicated measles (recruited at hospital), and compared to controls (a cohort of healthy children and a cohort of children hospitalized with other illnesses). One hundred and ten children will be recruited in each group, and will be examined twice weekly in the first 6 weeks then weekly until 6 months after recruitment. Appropriate laboratory and radiological investigations of episodes of illness will be conducted to identify the causative agent where possible. Measles infection will be confirmed by serology. To investigate the hypothesis that measles virus infection causes a prolonged bias to type 2 helper T cell response (which is predicted to increase the antibody response to infectious agents), the response to vaccination with hepatitis B vaccines 6 weeks after recruitment will be compared between cases and controls. In each analysis, age, sex, socioeconomic status, nutritional status, and levels of serum retinol and zinc will be assessed as possible confounders.

On a subsample of subjects aged 6-11 months, (n=25 per group), the study will measure the lymphoproliferative response to stimulation with measles virus and measles virus purified membrane proteins, cytokine profiles, and changes in surface molecules, particularly those involved in the measles virus receptor complex, on subpopulations of peripheral blood mononuclear cells (PBMCs). Two groups of measles vaccinees (n=25 per group) will also be studied, one group receiving two doses of standard titre measles vaccines at ages 6 and 9 months, the other a single dose at 9 months, to allow comparison of the effects of wild versus vaccine virus on cellular function. The presence of measles virus in PBMC samples and subpopulations in children with acute measles will be compared to vaccinees at different time points following exposure.

We are addressing the following items for necessary information for the committe:

1. Children between 6 months and 12 years of age will be selected for this study because measles is common in this age group. WHO reccomends the use of currently used normal titre vaccines at 6 and 9 months of age who are hospitalized or are at increased risk of acquiring measles. Children between 6 and 11 months will be selected for investigation of cellular immune functions so that we are able to compare them with those of measles vaccinees, who will be vaccinated with measles vaccine either at 6 and 9 months or only at 9 months of age. The results of immune function tests in children of 6-11 months of age will also help in the development and evaluatiation of new a measles vaccine that could be used in children of this age group.

2. The study will not cause any risk to the study children.

3. All cases and hospital controls will be appropriately treated free of cost according to the routine practice of the ICDDR,B. The community cases will be treated free of cost from the community clinic. All the cases and controls will be provided necessary

treatment whenever they are found ill during the period of follow-up. The treatment will include administration of vitamin A, dietary management for malnutrition and treatment of infections. Parents of all unvaccinated study children will be encouraged to get their children vaccinated with appropriated EPI vaccines.

4. Confidentiality of children's records will be maintained. Unique study numbers will be used during analysis.

5. Informed consent in a consent form will be obtained from the legal guardian or parents before inclusion in the study.

6. A short interview of the mothers or legal guardians of all cases, controls and measles vaccinees will be taken by the health assistants on their children's present health, past illnesses, socio-economic and environmental conditions and history contact with measles cases. The interview will last for about ten 10-15 minutes. Information on illnesses and anthropometric measurements will be obtained twice weekly during the first 6 weeks and then at weekly intervals upto 6 months after recruitment.

7. The short-term benifits of the study includes appropriate treatment of all study children during hospitalization and during the period of 6 months follow-up. All cases and controls will also be protected from hepatitis B infection and it's consequences by vaccination with hepatitis B vaccine. The long-term benefit of the study includes the advancement of knowledge of the effects of wild and vaccine virus on cellular immune function, which will be a vital contribution to improving the health status of populations in developing countries by opening the way for the design and evaluation of new vaccines and vaccination strategies against measles, a major cause of child mortality in the world.

8. Information obtained from the questionniare, anthropometric measurements, laboratory tests and hospital records will be used for data analysis. One to two ml of blood will be obtained from forearm veins of all cases on three occassions - once at recruitment, six weeks later and then at six months after recruitment. The same amount of blood will be collected in controls at 6 weeks and 6 months after recruitment. These samples of blood will be used to measure serum vitamin A and zinc, measles specific IgG and IgM and anti-HBsAg.

   In addition, five to six ml of blood will be obtained from forearm veins of subsets of 25 cases and 25 controls, and of two groups measles vaccinees on three occassions - once at recruitment, six weeks later and then at six months after recruitment. The same amount of blood will be collected in controls at 6 weeks and 6 months after recruitment. These samples of blood will be used for cellular immune function tests. A skin test will be performed on these subsets of cases and controls, and on measles vaccinees at 6 weeks after recruitment for assessing cell mediated immunity. These tests will cause some discomfort to study children, but will not cause any harm and these are currently being performed for other studies in this hospital.

CONSENT FORM - 1

STUDY OF IMMUNE DISRUPTION CAUSED BY MEASLES AND IT'S ASSOCIATION WITH CLINICAL PROGRESS IN DHAKA, BANGLADESH

(For measles/post-measles cases for studying cellular immune functions)

A large number of children in Bangladesh suffer from measles each year and many of them die from it's complications. A large proportion of these children are bellow 9 months of age, who are not eligible to receive currently used measles vaccine. Several high titre measles vaccines were tried to prevent measles in these young infants, but these were associated with higher death rate in vaccinated children. The ICDDR,B is carrying out research to find out underlying cause of this high death rate. There are many similarities and parallels between the causes of deaths following measles and the causes of deaths following vaccination with high titre measles vaccines. Since your child is suffering from measles/complications of measles, we will be able to find out the cause of increased death rate in young infants due to measles or vaccination with high titre measles vaccines by comparing illnesses and immune status of your child with children receiving currently used measles vaccines, healthy children and children suffering from other diseases. This study will also help to develop new measles vaccines that could prevent measles in young infants and to evaluate safety and efficacy of novel measles vaccines. If you agree to enrol your child in this study, the following procedures will be performed.

A Health Assistant will interview you on your child's present and past health, socio-economic and environmental conditions, history of contact with other measles cases etc and a physician will examine her/him thoroughly. The interview will last for about 10 to 15 minutes. The Heath Assistant will also obtain information on your child's illness and measure height, weight and mid-upper-arm circumference at recruitment and twice a week during first 6 weeks and then at weekly intervals up to 6 months. We will vaccinate your child with hepatitis B vaccine after 6 weeks. Six to seven ml (a little more that a teaspoonful) of blood will be obtained from one forearm vein of your child at recruitment, and at 6 weeks and at 6 months after recruitment to evaluate serum vitamin A and zinc levels, immune status and protection offered by hepatitis B vaccine. We will also do a skin test a 6 weeks to further evaluate immune status of your child. These are all simple procedures and are currently being performed on other study children of this hospital. However, these procedures will cause some discomfort, but will not cause any harm to your child. During hospitalization and throughout the follow-up period your child will receive standard treatment available from this hospital/clinic free of cost.

If at any time if you wish to withdraw your child from the study, you are free to do so and even then she/he will receive standard treatment available from this hospital/clinic free of cost. If you are interested we will inform you the results of the laboratory investigations when they become available. We will compensate for the loss of your wage for bringing your child to this hospital/clinic for follow-up.

If you are willing to include your child in this study, please sign or put your left thumb impression bellow.

Thank you.

Signature of the investigator

Signature/left thumb impression of legal guardian

Signature of the witness

Date_____

সম্মতি পত্র ১

হাম দ্বারা রোগ প্রতিরোধ ক্ষমতা ব্যবস্থাপনা
ও তৎসম্পর্কে পরবর্তী অসুখ বিসুখের সম্পর্ক।

হাম এবং হামের জটিলতার রোগীদের মাঝে অনুমান ইমিউন ফাংশন
পরীক্ষা করার জন্য)

প্রতি বছর বাংলাদেশের অসংখ্য শিশু হামে ভোগে এবং
তাদের অনেকেই এ রোগের কারনে মারা যায়। এই শিশুদের অনেকেই
বয়স ৯ মাসের নীচে এবং তারা প্রচলিত হামের টীকা নেবার জন্য
উপযোগী নয়। এ সব শিশুর হাম প্রতিরোধে যে সব উচ্চ মাত্রা সম্পন্ন
টীকা দেয়া হয়েছে, দেখা গেছে তাতে মৃত্যুর হার অনেক বেশী।
তাই ডব্লিউ এইচ ও এবং ইউ এস সি ডি সি আর বি এই উচ্চ মৃত্যু হারের কারন নির্ণয়ের জন্য
গবেষনা করছে। হামের এবং উচ্চ ক্ষমতাসম্পন্ন টীকার জন্য মৃত্যুর
মাঝে বহু দিন দেখা যায়। যেহেতু আপনার শিশু হাম/হামের
জটিলতায় ভুগছে, আমরা আপনার শিশু ও অন্য শিশুরা যারা প্রচলিত
হামের টীকা নিচ্ছে তাদের মধ্যে এবং কিছু যক্ষ্মা ও অন্য রোগের
শিশুদের মধ্যে রোগ প্রতিরোধ ক্ষমতা এবং অন্ত্রস্থ তুলনা করে
উচ্চ মাত্রা সম্পন্ন হামের টীকা/হামের জন্য মৃত্যুর কারন বের করতে পারব।
এই গবেষনা হামের নতুন টীকা উদ্ভাবনে এবং তার নিরাপত্তা ও কার্যকারিতা
নির্ণয়েও সাহায্য করবে।

আপনি যদি আপনার শিশুকে এ গবেষনায় অংশগ্রহণ করতে
চান তাহলে, একজন স্বাস্থ্যকর্মী আপনার শিশুর বর্তমান ও অতীত
স্বাস্থ্য, আপনার পারিবারিক ও আর্থিক অবস্থা, পরিবেশ এবং আপনার
শিশু অন্য হাম রোগীর কাছে গিয়েছিল কি না ইত্যাদি বিষয়ে কিছু
কথা বলবে। এ কারনে আপনার ১০-১৫ মিনিট সময় নষ্ট হতে পারে।
একজন স্বাস্থ্যকর্মী আপনার শিশুর ওজন, উচ্চতা ও হাতের মাপ নিবেন
একজন ডাক্তার তাকে খুব ভালভাবে পরীক্ষা করবে। রক্তের নিয়োগের
সময় এবং প্রথম ১ সপ্তাহে প্রতি সপ্তাহে দু দিন। তার পর ছয় মাস

র্যন্ত সপ্তাহে বক দিন ৫ বার রক্ত নিবে। ছয় সপ্তাহ পর আমরা
াপনার শিশুকে হেপাটাইটিস বি টীকা দেব। গবেষনায় বিয়োগের
ময়, ছয় সপ্তাহ, ও ছয় মাস পর রক্তবার করে রক্তে
টো মিন এ, জিঙ্ক(দস্তা), রোগ প্রতিরোধ ক্ষমতা ও হেপাটাইটিস
ি টীকার প্রতিরোধ ক্ষমতা পরীক্ষার জন্য ১/৭ বয়বন (১ চা চামচের
কটু বেশী) রক্ত হাত থেকে নেয়া হবে। ৬ সপ্তাহ পর, রোগ প্রতিরোধ
ক্ষমতা দেখার জন্য আপনার শিশুর হাতের চামড়ায় পরীক্ষা করা
হবে। রক্তের পরীক্ষা অতি সহজ ও সাধারন পদ্ধতি রক্ত বই
াসপাতালে গবেষনার জন্য অন্য রোগীদের থেকে নেয়া হয়। রক্ত
াপনার শিশুর কিছু অস্বস্থি করলেও কোন ক্ষতি করবে না। বই
বেষনাকালীন সময় হাসপাতাল রক্ত ক্লিনিকের সমস্ত চিকিৎসা বিনামূল্যে
বা হবে।

আপনি যে কোন সময় বই গবেষনা থেকে আপনার শিশুকে
ত্যাহার করে নিতে পারবেন। প্রত্যাহার করার পরও আপনার শিশু
হাসপাতালের প্রচলিত চিকিৎসা বিনা মূল্যে পাবে। আপনি
ইলে সমস্ত পরীক্ষার ফলাফল আমরা আপনাকে জানাব। ব
াসপাতালে আসা-যাওয়ার জন্য আপনার যে কাজের ক্ষতি ও
র্থ ব্যয় হবে তা আমরা পূরন করব।

এই গবেষনায় আপনার শিশুকে অংশগ্রহন করাতে চাইলে
ীচে স্বাক্ষর করুন/বাম হাতের বুড়ে আঙ্গুলের ছাপ দিন।

বেষনাকারীর স্বাক্ষর
ারিখঃ
াক্ষীর স্বাক্ষর
ারিখ :

অভিভাবকের স্বাক্ষর/বাম হাতের বুড়ে
আঙ্গুলের ছাপ।
তারিখ :

CONSENT FORM - 2

STUDY OF IMMUNE DISRUPTION CAUSED BY MEASLES AND IT'S ASSOCIATION WITH CLINICAL PROGRESS IN DHAKA, BANGLADESH

(For measles vaccinees for studying cellular immune functions)

A large number of children in Bangladesh suffer from measles each year and many of them die from it's complications. A large proportion of these children are bellow 9 months of age, who are not eligible to receive currently used measles vaccine. Several high titre measles vaccines were tried to prevent measles in these young infants, but these were associated with higher death rate in vaccinated children. The ICDDR,B is carrying out research to find out underlying cause of this high death rate. There are many similarities and parallels between the causes of deaths following measles and causes of deaths following vaccination with high titre measles vaccines. Since your child will be receiving measles vaccine, we will be able to find out the cause of increased death rate in young infants due to measles or vaccination with high titre measles vaccines by comparing immune status of your child with children suffering from measles/complications of measles, healthy children and children suffering from other diseases. This study will also help to develop new measles vaccines that could prevent measles in young infants and to evaluate safety and efficacy of novel measles vaccines. If you agree to enrol your child in this study, the following procedures will be performed.

Your child will be randomly selected to receive currently used measles vaccine either now and again at 9 months, or three months later at 9 months only. At 9-month's dose of measles vaccine a Health Assistant will interview you on your child's present and past health, socio-economic and environmental conditions, history of contact with other measles cases etc and a physician will examine her/him thoroughly. The interview will last for about 10 to 15 minutes. The Heath Assistant will also obtain information on your child's illness and measure height, weight and mid-upper-arm circumference at 9-month's dose of measles vaccine and then twice a week during first 6 weeks and at weekly intervals up to 6 months. Six to seven ml (a little more that a teaspoonful) of blood will be obtained from one forearm vein of your child just before the 9-month's dose of measles vaccine, then at 6 weeks and 6 months after completing measles vaccination to evaluate serum vitamin A and zinc levels, and immune status. We will also do a skin test a 6 weeks after the 9-month's dose of measles vaccine to further evaluate immune status of your child. These are all simple procedures and are currently being performed on other study children of this hospital. However, these procedures will cause some discomfort, but will not cause any harm to your child. During hospitalization and throughout the follow-up period your child will receive standard treatment available from this hospital/clinic free of cost.

If at any time if you wish to withdraw your child from the study, you are free to do so and even then she/he will receive standard treatment available from this hospital/clinic free of cost. If you are interested we will inform you the results of the laboratory investigations when they become available. We will compensate for the loss of your wage for bringing your child to this hospital/clinic for follow-up.

If you are willing to include your child in this study, please sign or put your left thumb impression bellow.

Thank you.

Signature of the investigator

Signature of the witness

Signature/left thumb impression of legal guardian

Date_____

সম্মতি পত্র ২

হাম দ্বারা রোগ প্রতিরোধ ক্ষমতা বৃদ্ধিকরন
ও তৎপরে পরবর্তী অসুখ বিসুখের সম্পর্ক।

যাদের হামের টীকা দেয়া হবে তাদের জেনুমার ইমিউন ফাংশন পরীক্ষা করার জন্য)

প্রতি বছর অসংখ্য শিশু হামে ভোগে এবং এদের অনেকেই এ রোগের কারণে মারা যায়। এই শিশুদের অনেকেরই বয়স ৯ মাসের নীচে এবং তারা প্রচলিত হামের টীকা নেবার উপযোগী নয়। এই সব শিশুর হাম প্রতিরোধে যে সব উচ্চমাত্রা সম্পন্ন টীকা দেয়া হয়েছে দেখা গেছে তাতে মৃত্যুর হার অনেক বেশী। তাই সি ডি ডি আর, বি এই উচ্চ মৃত্যু হারের কারন নির্নয়ের জন্য গবেষনা করছে। হামের এবং উচ্চ ক্ষমতা সম্পন্ন টীকার জন্য মৃত্যুর মাঝে বহু দিন দেখা গেছে। যেহেতু আপনার শিশু হামের টীকা পাবে আমরা আপনার শিশু ও অন্য শিশুরা যারা হাম/ হামের জটিলতায় ভুগছে তাদের মধ্যে এবং কিছু সুস্থ্য ও অন্য রোগের শিশুদের মধ্যে রোগ প্রতিরোধ ক্ষমতা এবং অসুস্থ্যতা তুলনা করে উচ্চমাত্রা সম্পন্ন হামের টীকা/ হামের জন্য মৃত্যুর কারন বের করতে পারবো। এই গবেষনা হামের নতুন টীকা উদ্ভাবনে এবং তার নিরাপত্তা ও কার্যকারিতা নির্নয়ে ও সাহায্য করবে।

আপনি যদি আপনার শিশুকে এ গবেষনায় অংশগ্রহন করাতে চান তাহলে আপনার শিশুকে প্রচলিত হামের টীকা দেয়া হবে। হয় এখনই এবং ৯ মাস পর নতুবা তিনমাস পর শুধুমাত্র ৯ মাস বয়সের সময়। ৯ মাস এর টীকা দেবার সময় একজন স্বাস্থ্যকর্মী আপনার শিশুর বর্তমান ও অতীত স্বাস্থ্য, আপনার পারিবারিক ও আর্থিক অবস্থা, পরিবেশ এবং আপনার শিশু অন্য হাম রোগীর কাছে গিয়েছিল কিনা ইত্যাদি বিষয়ে কিছু কথা বলবে। এ কারনে আপনার ১০-১৫ মিনিট সময় নষ্ট

হতে পারে। একজন স্বাস্থ্যকর্মী আপনার শিশুর ওজন, উচ্চতা ও হাতের মাপ নিবে এবং একজন ডাক্তার তাকে খুব অল্পভাবে পরীক্ষা করবে। একবার ৯ মাসের টীকা দেবার সময় এবং প্রথম ৬ সপ্তাহে প্রতি সপ্তাহে দুইদিন তার পর ৬ মাস পর্যন্ত সপ্তাহে একদিন ২ মাপ সংগ্রহ নিবে। ৯ মাসের টীকা দেবার সময়, তার পর ছয় সপ্তাহ ও ছয় মাস পর একবার করে রক্তের হিমোগ্লোবিন, জিঙ্ক (দস্তা) ও রোগ প্রতিরোধ ক্ষমতা পরীক্ষার জন্য ১/৭ বয় বয় (১ চা চামচের একটু বেশী) রক্ত হাতের শিরা থেকে নেয়া হবে। ৬ সপ্তাহ পর, রোগ প্রতিরোধ ক্ষমতা দেখার জন্য আপনার শিশুর হাতের চামড়ায় পরীক্ষা করা হবে। এসব পরীক্ষা অতি সহজ ও সাধারন পদ্ধতি এবং এই হাসপাতালে গবেষনার জন্য অন্য রোগীদের থেকে নেয়া হয়। এটি আপনার শিশুর কিছু অস্বস্থি করলেও কোন ক্ষতি করবে না। এই গবেষনাকালীন সময় হাসপাতাল এবং ক্লিনিকের সমস্ত চিকিৎসা বিনা মূল্যে করা হবে।

আপনি যে কোন সময় এই গবেষনা থেকে আপনার শিশুকে প্রত্যাহার করে নিতে পারবেন। প্রত্যাহার করার পর ও আপনার শিশু এ হাসপাতালের প্রচলিত চিকিৎসা বিনামূল্যে পাবে। আপনি চাইলে সমস্ত পরীক্ষার ফলাফল আমরা আপনাকে জানাবো। এ হাসপাতালে আসা যাওয়ার জন্য আপনার কাজের যে ক্ষতি ও অর্থ ব্যয় হবে আমরা তা পূরন করবো।

এই গবেষনায় আপনার শিশুকে অংশগ্রহন করাতে চাইলে নীচে স্বাক্ষর করুন/বাম হাতের বুড়ো আঙ্গুলের ছাপ দিন।

গবেষনাকারীর স্বাক্ষর
তারিখ:

স্বাক্ষীর স্বাক্ষর
তারিখ:

অভিভাবকের স্বাক্ষর/বাম হাতের বুড়ো আঙ্গুলের ছাপ।
তারিখ:

CONSENT FORM - 3

STUDY OF IMMUNE DISRUPTION CAUSED BY MEASLES AND IT'S ASSOCIATION WITH CLINICAL PROGRESS IN DHAKA, BANGLADESH

(For controls for studying cellular immune functions)

A large number of children in Bangladesh suffer from measles each year and many of them die from it's complications. A large proportion of these children are bellow 9 months of age, who are not eligible to receive currently used measles vaccine. Several high titre measles vaccines were tried to prevent measles in these young infants, but these were associated with higher death rate in vaccinated children. The ICDDR,B is carrying out research to find out underlying cause of this high death rate. There are many similarities and parallels between the causes of deaths following measles and the causes of deaths following vaccination with high titre measles vaccines. Although your child is not suffering from measles or complications of measles, we will be able to find out the cause of increased death rate in young infants due to measles or vaccination with high titre measles vaccines by comparing illnesses and immune status of your child with children suffering from measles/complications of measles and children receiving currently used measles vaccines. This study will also help to develop new measles vaccines that could prevent measles in young infants and to evaluate safety and efficacy of novel measles vaccines. If you agree to enrol your child in this study, the following procedures will be performed.

A Health Assistant will interview you on your child's present and past health, socio-economic and environmental conditions, history of contact with other measles cases etc and a physician will examine her/him thoroughly. The interview will last for about 10 to 15 minutes. The Heath Assistant will also obtain information on your child's illness and measure height, weight and mid-upper-arm circumference at recruitment and twice a week during first 6 weeks and then at weekly intervals for 6 months. We will vaccinate your child with hepatitis B vaccine after 6 weeks. Six to seven ml (a little more that a teaspoonful) of blood will be obtained from one forearm vein of your child at 6 weeks and 6 months after recruitment to evaluate serum vitamin A and zinc levels, immune status and protection offered by hepatitis B vaccine. We will also do a skin test a 6 weeks to further evaluate immune status of your child. These are all simple procedures and are currently being performed on other study children of this hospital. However, these procedures will cause some discomfort, but will not cause any harm to your child. During hospitalization and throughout the follow-up period your child will receive standard treatment available from this hospital/clinic free of cost.

If at any time if you wish to withdraw your child from the study, you are free to do so and even then she/he will receive standard treatment available from this hospital/clinic free of cost. If you are interested we will inform you the results of the laboratory investigations when they become available. We will compensate for the loss of your wage for bringing your child to this hospital/clinic for follow-up.

If you are willing to include your child in this study, please sign or put your left thumb impression bellow.

Thank you.

Signature of the investigator

Signature/left thumb impression of legal guardian

Signature of the witness

Date_____

030779

সম্মতিপত্র ৩

হাম দ্বারা রোগ প্রতিরোধ ক্ষমতা ব্যহতকরন
ও তৎসঙ্গে পরবর্তী অন্যান্য বিষয়ের সম্পর্ক।

(ঘুষ্যু ও অন্য রোগের শিশুদের মেনুমার ইমিউন ফাংশন পরীক্ষা করার জন্য)

প্রতিবছর অসংখ্য শিশু হামে ভোগে এবং তাদের অনেকেই এ রোগের কারনে মারা যায়। এ শিশুদের অনেকেরই বয়স ৯ মাসের নীচে এবং তারা প্রচলিত হামের টীকা নেবার উপযোগী নয়। এইসব শিশুর হাম প্রতিরোধে যে সব উচ্চমাত্রা সম্পন্ন টীকা দেয়া হয়েছে দেখা গেছে তাতে মৃত্যুর হার অনেক বেশী। আই ঘি ডি ডি আর বি এই উচ্চ মৃত্যুর হার এর কারন নির্নয়ের জন্য গবেষনা করছে। হামের এবং উচ্চ ক্ষমতাসম্পন্ন টীকার জন্য মৃত্যুর মাঝে বহু মিল দেখা গেছে। যদিও আপনার শিশু হাম/হামের জটিলতায় ভুগছে না তবু আমরা আমরা আপনার শিশু ও অন্য শিশুরা যারা হাম/হামের জটিলতায় ভুগছে তাদের মধ্যে এবং যারা প্রচলিত হামের টীকা নিয়েছে তাদের মাঝে রোগ প্রতিরোধ ক্ষমতা এবং অন্যান্য তুলনা করে উচ্চমাত্রা সম্পন্ন হামের টীকা/হামের জন্য মৃত্যুর কারন বের করতে পারব। এই গবেষনা হামের নতুন টীকা উদ্ভাবনে এবং তার নিরাপত্তা ও কার্যকারিতা নির্নয়ে সাহায্য করবে।

আপনি যদি আপনার শিশুকে এ গবেষনায় অংশগ্রহন করাতে চান তাহলে একজন স্বাস্থ্যকর্মী আপনার শিশুর বর্তমান ও অতীত স্বাস্থ্য, আপনার পারিবারিক ও আর্থিক অবস্থা, পরিবেশ আপনার শিশু অন্য হাম রোগীর কাছে গিয়েছিল কিনা ইত্যাদি বিষয়ে কিছু কথা বলবে। এ কারনে আপনার ১০-১৫ মিনিট সময় নষ্ট হতে পারে। একজন স্বাস্থ্যকর্মী আপনার শিশুর ওজন, উচ্চতা ও হাতের মাপ নেবে এবং একজন ডাক্তার তাকে খুব ভালভাবে পরীক্ষা করবে। একবার নিয়োগের সময় এবং

রপর প্রথম ৬ সপ্তাহ প্রতি সপ্তাহে দুইদিন তারপর ছয় মাস
র্যন্ত সপ্তাহে একদিন ৯ মাপ গ্রহণ নিবে। ছয় সপ্তাহ পর আমরা
াপনার শিশুকে হেপাটাইটিস বি টীকা দিব। গবেষনায় নিয়োগের
য় সপ্তাহে ও ছয় মাস পর ব্যবহার করে রক্তে ডিটোমিন ব,
জিঙ্ক (দস্তা), বোগ প্রতিরোধ ক্ষমতা ও হেপাটাইটিস বি টীকার
তিরোধ ক্ষমতা পরীক্ষার জন্য ৬/৭ মি.লি (এক চা চামচের কম
শী) রক্ত হাতের শিরা থেকে নেয়া হবে। ৬ সপ্তাহ পর, রোগ প্রতিরোধ
মতা দেখার জন্য আপনার শিশুর হাতের চামড়ায় পরীক্ষা করা হবে। সকল
রীক্ষা অতি যত্নে ও আধুনিক পদ্ধতি এবং এই হাসপাতালে গবেষনার জন্য অন্য
োগীদের থেকে নেয়া হয়। এটি আপনার শিশুর কিছু অসুবিধা করলেও কোন ক্ষতি
রবে না। এই গবেষনাকালীন সময় হাসপাতাল এবং ক্লিনিকের যাবতীয়
চিকিৎসা বিনা মূল্যে করা হবে।

আপনি যে কোন সময় এই গবেষনা থেকে আপনার শিশুকে
ত্যাহার করে নিতে পারবেন। প্রত্যাহার করার পরও আপনার শিশু
হাসপাতালের প্রচলিত চিকিৎসা বিনামূল্যে পাবে। আপনি চাইলে
মগ্র পরীক্ষার ফলাফল আমরা আপনাকে জানাবো। এ হাসপাতালে
াসা যাওয়ার জন্য আপনার কাজের যে ক্ষতি ও অর্থ ব্যয় হবে
ামরা তা পূরন করবো।

এই গবেষনায় আপনার শিশুকে অংশগ্রহন করাতে চাইলে
ীচে স্বাক্ষর করুন / বাম হাতের বুড়ো আঙ্গুলের ছাপ দিন।

বেষনাকারীর স্বাক্ষর
রিখ
াক্ষীর স্বাক্ষর
রিখ

অভিভাবকের স্বাক্ষর / বাম হাতের
বুড়ো আঙ্গুলের ছাপ।
তারিখ

CONSENT FORM - 4

STUDY OF IMMUNE DISRUPTION CAUSED BY MEASLES AND IT'S ASSOCIATION WITH CLINICAL PROGRESS IN DHAKA, BANGLADESH

(For measles/post-measles cases)

large number of children in Bangladesh suffer from measles each year and many of them die
om it's complications. A large proportion of these children are bellow 9 months of age; who are
t eligible to receive currently used measles vaccine. Several high titre measles vaccines were
ed to prevent measles in these young infants, but these were associated with higher death rate in
ccinated children. The ICDDR,B is carrying out research to find out underlying cause of this
gh death rate. There are many similarities and parallels between the causes of deaths following
easles and the causes of deaths following vaccination with high titre measles vaccines. Since your
ild is suffering from measles/complications of measles, we will be able to find out the cause of
creased death rate in young infants due to measles or vaccination with high titre measles vaccines
comparing illnesses and immune status of your child with children receiving currently used
easles vaccines, healthy children and children suffering from other diseases. This study will also
lp to develop new measles vaccines that could prevent measles in young infants and to evaluate
fety and efficacy of novel measles vaccines. If you agree to enrol your child in this study, the
lowing procedures will be performed.

Health Assistant will interview you on your child's present and past health, socio-economic and
vironmental conditions, history of contact with other measles cases etc and a physician will
amine her/him thoroughly. The interview will last for about 10 to 15 minutes. The Heath
sistant will also obtain information on your child's illness and measure height, weight and mid-
per-arm circumference at recruitment and twice a week during first 6 weeks and then at weekly
ervals up to 6 months. We will vaccinate your child with hepatitis B vaccine after 6 weeks. One
two ml (a little less than half teaspoonful) of blood will be obtained from one forearm vein of
ur child at recruitment, and at 6 weeks and 6 months after recruitment to evaluate serum vitamin
and zinc levels, immunity against measles and protection offered by hepatitis B vaccine. This is
simple procedure and is currently being performed on other study children of this hospital.
owever, this will cause some discomfort, but will not cause any harm to your child. During
spitalization and throughout the follow-up period your child will receive standard treatment
ailable from this hospital/clinic free of cost.

at any time if you wish to withdraw your child from the study, you are free to do so and even
en she/he will receive standard treatment available from this hospital/clinic free of cost. If you
e interested we will inform you the results of the laboratory investigations when they become
ailable. We will compensate for the loss of your wage for bringing your child to this
spital/clinic for follow-up.

you are willing to include your child in this study, please sign or put your left thumb impression
llow.

ank you.

---

gnature of the investigator

Signature/left thumb impression of legal guardian

---

gnature of the witness

Date_____

সম্মতি পত্র ৪

হামদ্বারা রোগ প্রতিরোধ ক্ষমতা ব্যহতকরন ও
তৎসম্পৃক্ত পরবর্তী অসুখ বিসুখের সম্পর্ক।

(হাম এবং হামের জটিলতার রোগীদের জন্য)

প্রতি বছর বাংলাদেশের অসংখ্য শিশু হামে ভোগে এবং
দের অনেকেই এ রোগের কারনে মারা যায়। এই শিশুদের
অনেকেরই বয়স ৯ মাসের নীচে এবং তারা প্রচলিত হামের টীকা
নেবার জন্য উপযোগী নয়। এ সব শিশুর হাম প্রতিরোধে যে সব
চ্চ মাত্রা সম্পন্ন টীকা দেয়া হয়েছে, দেখা গেছে তাতে মৃত্যুর হার
নেক বেশী। আই ম্যি ডি ডি আর বি এই উচ্চ মৃত্যুর হার এর কারা
র্নয়ের জন্য গবেষনা করছে। হামের এবং উচ্চ ক্ষমতা সম্পন্ন টীকার জন্য
ত্যুর মাঝে বহু মিল দেখা যায়ে। যেহেতু আপনার শিশু হাম/ হামের
টিলতায় ভুগছে আমরা আপনার শিশু ও অন্য শিশুরা যারা প্রচলিত
ামের টীকা নিচ্ছে তাদের মধ্যে এবং কিছু সুস্থ্য ও অন্য রোগের
শিশুদের মধ্যে রোগ প্রতিরোধ ক্ষমতা এবং অসুস্থ্যতা তুলনা করে
চ্চ মাত্রা সম্পন্ন হামের টীকা/ হামের জন্য মৃত্যুর কারন বের করতে
ারব। এই গবেষনা হামের নতুন টীকা উদ্ভাবনে এবং তার নিরাপত্তা
ও কার্যকারিতা নির্নয়েও সাহায্য করবে।

আপনি যদি আপনার শিশুকে এ গবেষনায় অংশগ্রহন করাতে
ন তাহলে একজন স্বাস্থ্যকর্মী আপনার শিশুর বর্তমান ও অতীত
স্বাস্থ্য, আপনার পারিবারিক ও আর্থিক অবস্থা, পরিবেশ এবং
আপনার শিশু অন্য হাম রোগীর কাছে গিয়েছিল কি না ইত্যাদি
বিষয়ে কিছু কথা বলবে। এ কারনে আপনার ১০-১৫ মিনিট
সময় নষ্ট হতে পারে। একজন স্বাস্থ্যকর্মী আপনার শিশুর ওজন,
উচ্চতা ও হাতের মাপ নিবে এবং একজন ডাক্তার তাকে খুব ভালভাবে
পরীক্ষা করবে। এ করবার নিয়োগের সময় এবং প্রথম ৬ মাসাহে
প্রতি মাসাহে দু দিন। তার পর ছয় মাস পর্যন্ত মাসাহে একদিন

মাপ সমূহ নিবে। ৬ সপ্তাহ পর আমরা আপনার শিশুকে
পাটাইটিস বি টীকা দেব। গবেষণায় নিয়োগের সময়, ছয়
প্তাহ ও ছয় মাস পর রক্ত ভিটামিন এ, জিঙ্ক(দস্তা),
াস প্রতিরোধ ক্ষমতা ও হেপাটাইটিস বি টিকার প্রতিরোধ
মতা পরীক্ষার জন্য ১/২ মিঃ লিঃ (১ চা চামচের অর্ধেকের কম)
ও হাত থেকে নেয়া হবে। এটি অতি সহজ ও সাধারণ পদ্ধতি
বং এই হাসপাতালে গবেষনার জন্য অন্য যে সীদের থেকে নেয়া
য়। এটি আপনার শিশুর কিছু অস্বস্তি করলেও কোন ক্ষতি
রবে না। এই গবেষনাকালীন সময় হাসপাতাল এবং ক্লিনিকের
সমস্ত চিকিৎসা বিনা মূল্যে করা হবে।

আপনি যে কোন সময় এই গবেষনা থেকে আপনার শিশুকে
ত্যাহার করে নিতে পারবেন। প্রত্যাহার করার পরও আপনার
শিশু এ হাসপাতালের প্রচলিত চিকিৎসা বিনা মূল্যে পাবে। আপনি
ইলে সমস্ত পরীক্ষার ফলাফল আমরা আপনাকে জানাব। এ হাসপাতালে
াসা যাওয়ার জন্য আপনার যে কাজের ক্ষতি ও অর্থ ব্যয় হবে
া আমরা পূরন করব।

এই গবেষনায় আপনার শিশুকে অংশ গ্রহন করাতে চাইলে
ীচে স্বাক্ষর করুন/বাম হাতের বুড়ো আঙ্গুলের ছাপ দিন।

বেষনাকারীর স্বাক্ষর
ারিখ:
াক্ষীর স্বাক্ষর
ারিখ

অভিভাবকের স্বাক্ষর/বাম হাতের
বুড়ো আঙ্গুলের ছাপ।
তারিখ:

CONSENT FORM - 5

STUDY OF IMMUNE DISRUPTION CAUSED BY MEASLES AND IT'S ASSOCIATION WITH CLINICAL PROGRESS IN DHAKA, BANGLADESH

(For controls)

large number of children in Bangladesh suffer from measles each year and many of them die m it's complications. A large proportion of these children are bellow 9 months of age, who are t eligible to receive currently used measles vaccine. Several high titre measles vaccines were ed to prevent measles in these young infants, but these were associated with higher death rate in ccinated children. The ICDDR,B is carrying out research to find out underlying cause of this gh death rate. There are many similarities and parallels between the causes of deaths following easles and the causes of deaths following vaccination with high titre measles vaccines. Although ur child is not suffering from measles or complications of measles, we will be able to find out e cause of increased death rate in young infants due to measles or vaccination with high titre easles vaccines by comparing illnesses and immune status of your child with children suffering m measles/complications of measles and children receiving currently used measles vaccines. is study will also help to develop new measles vaccines that could prevent measles in young fants and to evaluate safety and efficacy of novel measles vaccines. If you agree to enrol your ild in this study, the following procedures will be performed.

Health Assistant will interview you on your child's present and past health, socio-economic and vironmental conditions, history of contact with other measles cases etc and a physician will amine her/him thoroughly. The interview will last for about 10 to 15 minutes. The Heath sistant will also obtain information on your child's illness and measure height, weight and mid-per-arm circumference at recruitment and twice a week during first 6 weeks and then at weekly tervals for 6 months. We will vaccinate your child with hepatitis B vaccine after 6 weeks. One to o ml (a little less that half teaspoonful) of blood will be obtained from one forearm vein of your ild at 6 weeks and 6 months after recruitment to evaluate serum vitamin A and zinc levels, mmunity against measles and protection offered by hepatitis B vaccine. This is a simple procedure d is currently being performed on other study children of this hospital. However, this will cause me discomfort, but will not cause any harm to your child. During hospitalization and throughout e follow-up period your child will receive standard treatment available from this hospital/clinic ee of cost.

at any time if you wish to withdraw your child from the study, you are free to do so and even en she/he will receive standard treatment available from this hospital/clinic free of cost. If you e interested we will inform you the results of the laboratory investigations when they become vailable. We will compensate for the loss of your wage for bringing your child to this spital/clinic for follow-up.

you are willing to include your child in this study, please sign or put your left thumb impression llow.

hank you.

---

ignature of the investigator

Signature/left thumb impression of legal guardian

---

ignature of the witness

Date_____

সম্মতি পত্র ৫

হাম দ্বারা রোগ প্রতিরোধ ঋরগা ব্যহতকরন ও তৎসঙ্গে পারবর্তী অসুখ বিসুখের সম্পর্ক।

(সুস্থ্য ও অন্য রোগের শিশুদের জন্য)

প্রতি বছর বাংলাদেশের অসংখ্য শিশু হামে ভোগে এবং দের অনেকেই ঐ রোগের কারনে মারা যায়। এই শিশুদের নেকেরই বয়স ৯ মাসের নীচে এবং তারা প্রচলিত হামের টীকা নবার জন্য উপযোগী নয়। ঐ সব শিশুর হাম প্রতিরোধে যে সব চ্চ মাত্রা সম্পন্ন টীকা দেয়া হয়েছে, দেখা গেছে তাতে মৃত্যুর হার নেক বেশী। আই ষি ডি ডি আর বি এই উচ্চ মৃত্যুর হার কর কারন নির্নয়ের জন্য গবেষনা করছে। হামের এবং উচ্চ ঋরগাসম্পন্ন টীকার জন্য মৃত্যুর মাঝে বহু মিল দেখা যায়। যেহেতু আপনার শিশু হাম/ হামের জটিলতায় ভুগছে আমরা আপনার শিশু ও অন্য শিশুরা যারা প্রচলিত হামের টীকা নিচ্ছে তাদের মধ্যে এবং কিছু সুস্থ্য ও অন্য রোগের শিশুদের মধ্যে রোগ প্রতিরোধ ক্ষমতা এবং অসুস্থ্যতা তুলনা করে উচ্চ মাত্রা সম্পন্ন হামের টীকা/হামের জন্য মৃত্যুর কারন বের করতে পারব। এই গবেষনা হামের নতুন টীকা উদ্ভাবনে এবং তার নিরাপত্তা ও কার্যকারিতা নির্নয়েও সাহায্য করবে।

আপনি যদি আপনার শিশুকে এ গবেষনায় অংশগ্রহন করাতে চান তাহলে একজন স্বাস্থ্যকর্মী আপনার শিশুর বর্তমান ও অতীত স্বাস্থ্য, আপনার পারিবারিক ও আর্থিক অবস্থা, পরিবেশ এবং আপনার শিশু অন্য হাম রোগীর কাছে গিয়েছিল কি না ইত্যাদি বিষয়ে কিছু কথা বলবে। এ কারনে আপনার ১০-১৫ মিনিট সময় লাগতে পারে। একজন স্বাস্থ্যকর্মী আপনার শিশুর ওজন, উচ্চতা ও হাতের মাপ নিবে এবং একজন ডাক্তার তাকে খুব ভালভাবে পরীক্ষা করবে। একবার নিয়োগের সময় এবং প্রথম ৬ সপ্তাহে প্রতি সপ্তাহে দুইদিন তারপর ছয় মাস পর্যন্ত মাসে এক দিন ঐ মাপ গ্রহন নিবে।

সপ্তাহ পরে আমরা আপনার শিশুকে হেপাটাইটিস বি টীকা দেব।
বেক্ষায় নিয়োগের সময় ছয় সপ্তাহ বয়ঃ ছয় মাস পর ও বকবার
রে রক্তে হিপাটাইটিস এ, জিক্ক(দস্তা), হার প্রতিরোধ ক্ষমতা ও
পাটাইটিস বি টীকার প্রতিরোধ ক্ষমতা পরীক্ষার জন্য ১/২ চা.চ.
চা চামচের অর্ধেকের কম) রক্ত হাত থেকে নেয়া হবে। এটি অতি
হজ ও সাধারন পদ্ধতি এবং এই হাসপাতালে গবেষনার জন্য
ন্য রোগীদের থেকে নেয়া হয়। আপনার শিশুর কিছু অস্বস্তি
রনেও কোন ক্ষতি করবে না। এই গবেষনাকালীন সময় হাসপাতাল
বং ক্লিনিকের সমস্ত চিকিৎসা বিনামূল্যে করা হবে।

আপনি যে কোন সময় এই গবেষনা থেকে আপনার শিশুকে
ত্যাহার করে নিতে পারবেন। প্রত্যাহার করার পরও আপনার
শু এ হাসপাতালের প্রচলিত চিকিৎসা বিনামূল্যে পাবে।
পনি চাইলে সমস্ত পরীক্ষার ফলাফল আমরা আপনাকে জানাব।
হাসপাতালে আসা যাওয়ার জন্য আপনার যে কাজের ক্ষতি ও
র্থ ব্যয় হবে তা আমরা পূরন করব।

এই গবেষনায় আপনার শিশুকে অংশগ্রহন করাতে চাইলে
িচে স্বাক্ষর করুন/বাম হাতের বুড়ো আপুলের ছাপ দিন।

বেক্ষনাকারীর স্বাক্ষর
রিখ:
ক্ষীর স্বাক্ষর
রিখ:

অভিভাবকের স্বাক্ষর/বাম হাতের বুড়ো
আপুলের ছাপ।
তারিখ:

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH

IMMUNE DISRUPTION STUDY FOLLOWING MEASLES

QUESTIONNAIRE FOR BASELINE INFORMATION ON MEASLES CASES, CONTROLS AND VACCINES

ADDRESS AND DESCRIPTION OF THE LOCATION OF THE CHILD'S HOUSE

Child's full name: _____

Father's name: _____

Head of household's(HH) name (if different from father's name)

_____

Respondent's name: _____

Present address: House no_____ Road_____

Lane_____Locality_____

Thana _____ Telephone no_____

Describe the location of the house in relation to the nearest main road or an important landmark, if necessary draw a map showing the location of the house in the blank space bellow:

_____

_____

_____

ID: |___|___|___|___|

PARTICULARS OF THE CHILD

1. Identification number: {ID} | | | | |

2. Case-control set number: {SET} | | | |

3. *Case: 1=Community case* {CASE} |
   2=Community control 3=Hosp case
   4=Hosp control 5=Measles vaccines (6-9 months)
   6=Measles (9 months)

4. Age (completed months): {AGE} | | |

5. Date of birth: {BIRTHDT} Date | | Month | | Year | |

6. Place of birth: {BIRTHPL} |
   1=Home 2=Hospital

7. Sex: *1=Male 2=Female* {SEX} |

8. Date of recruitment: {RECRDT} Date | | Month | | year | |

DEMOGRAPHIC AND SOCIOECONOMIC INFORMATION

09. Respondent's age: (in years) {AGERESP} | |

10. Respondent's relationship to child: {RESPREL} |
    1=Mother
    *2=Other*_____

11. HH's relationship to child: {HHREL} |
    1=Father
    *2=Other*_____

SOCIOECONOMIC AND DEMOGRAPHIC INFORMATION
(Continued)

12. Occupation of the Head of the household: {OCCHH} ☐☐
1=Unemployed 2=Service
3=Business 4=Farmer
5=Driver 6=Technician
7=Professional
8=Laborer
9=Rickshawpuller
*10=Other*_____

13. Occupation of mother: {OCCMOTH} ☐
1=Housewife 2=Service
3=Laborer
4=Business
*5=Other*_____

14. Education of the HH: ☐☐
(years of schooling)

15. Mother's education: {EDUMOTH} ☐☐
(years of schooling)

16. Total income of the household in taka: {INCOM} ☐☐☐☐☐☐

17. Floor of the house: {FLOR} ☐
1=Pucca 2=Earthen 3=Wooden
*4=Other*_____

18. Wall of the house: {WAL} ☐
1=Pucca 2=tin 3=Wooden
4=bamboo 5=Thatched
6=Earthen
*7=Other*_____

19. Roof of the house: {ROF} ☐
1=Pucca 2=Tin 3=Wooden
4=Thatched
*5=Other*_____

20. Number of rooms in the household: {ROM} ☐☐

21. Number of persons ≥12 years of age in the household: {PERSON} ☐☐

ID: |___|___|___|___|

SOCIOECONOMIC AND DEMOGRAPHIC INFORMATION
(Continued)

22. Number of children <12 years of age in the household: {NOCHIL} ☐☐

23. No of children <12 years of age sleeping in the same room with the child (including this case or this control): {CHILROM} ☐☐

24. For how long the respondent has been living in Dhaka: (Write 0 for <1 year) {LIVDUR} ☐☐

For Community cases, community controls and measles vaccines: Skip items 25 to 39 and start from item 41

25. Distance of this hospital from child's house: (miles) (Write <1 as 0 mile) {DISTAN} ☐☐

26. Time required to travel to this hospital: (in minutes) {TIME} ☐☐☐

Method of transport

27. Bus: *1=Yes 0=No* {BUS} ☐

28. Train: *1=Yes 0=No* {TRAIN} ☐

29. Taxi: *1=Yes 0=No* {TAXI} ☐

30. Rickshaw: *1=Yes 0=No* {RICK} ☐

31. Walking: *1=Yes 0=No* {WALK} ☐

32. Other: *1=Yes 0=No* {OTHTRANS} ☐

Specify_____

ID: |___|___|___|___|

HISTORY OF MEASLES

For hospital controls: Skip items 34 to 40 and start from item 41

		Date	Month	Year
33. Date of onset of rash:	{RASHDT}			

⇓

⇓

34. Has the rash disappeared? ***If YES, write duration in days; if NO, write '99'***	{RASHDUR}	

		Date	Month	Year
35. Date of onset of fever: (preceding or with rash)	{MFEVRDT}			

⇓

36. Has fever with rash subsided? ***If YES, write duration in days; if NO, write '99'***	{MFEVRDUR}	

Does or did the child have one or more of the following symptoms with rash? If YES, write '1'; if NO, write '0'

37. Cough:	{MCOUGH}	
38. Coryza:	{MCORYZA}	
39. Conjunctivitis:	{MCONJTIV}	

ID: |___|___|___|___|

HISTORY OF PRESENT ILLNESS

Does the child have one or more of the following symptoms? If YES, write duration of symptoms in days; if NO write '99'

40. Fever:	{FEVERDUR}	
41. Cough:	{COUGHDUR}	
42. Difficulty in respiration:	{RESPRDUR}	
43. Watery diarrhoea:	{WDIARDUR}	
44. Mucoid diarrhoea:	{MDIARDUR}	
45. Bloody diarrhoea:	{BDIARDUR}	
46. Oliguria/anuria:	{URINDUR}	
47. Any other important symptom:	{OTHDUR}	

Specify_____

SEVERITY OF ILLNESS

48. Respondent's perception about the severity of the child's illness: {SEVRESP}

0=Not assessed
1=Normal
2=Very mildly ill
3=Mildly ill
4=Moderately ill
5=Moderately severe
6=Severely ill

ID: |___|___|___|___|

HISTORY OF EXPOSURE

49. Does the respondent remember the child meeting another with measles? *1=Yes 0=No* {EXP} ☐

Date Month Year

50. If yes, date of exposure: {EXPDT} ☐☐ ☐☐ ☐☐

51. Sex of index case: {SEXIND} ☐
1=Male 2=Female

52. Age of index case: {AGEIND} ☐☐☐
(in months)

53. Does index case attend school? *1=Yes 0=No* {SCHLIND} ☐

54. Place of exposure: {EXPPL} ☐☐
1=Home
2=Neighbor's house
3=Friend's/relative's house
4=School
5=Thana Health Center OPD
6=Thana Health Center IPD
7=Private/NGO clinic OPD
8=Private/NGO clinic IPD
9=Hospital OPD
10=Hospital IPD
11=Vaccination center
*12=Other*_____

ID: |___|___|___|___|

HISTORY OF VACCINATION

Has the child received one or more of the following vaccines? If YES, write '1'; if NO write '0'. Is there any document of vaccination? If YES write, '1'; if NO write '0'.

55. BCG: {BCG} ☐

56. Document: {DOCBCG} ☐

57. DPT 1: {DPT1} ☐

58. Document: {DOCDPT1} ☐

59. Polio 1: {POL1} ☐

60. Document: {DOCPOL1} ☐

61. DPT 2: {DPT2} ☐

62. Document: {DOCDPT2} ☐

63. Polio 2: {POL2} ☐

64. Document: {DOCPOL2} ☐

65. DPT 3: {DPT3} ☐

66. Document: {DOCDPT3} ☐

67. Polio 3: {POL3} ☐

68. Document: {DOCPOL3} ☐

69. Measles: {MVAC} ☐

70. Document: {DOCMVAC} ☐

71. If yes, vaccination date: {MVACDT} ☐☐☐☐☐☐

ID: |___|___|___|___|

ANTHROPOMETRY

For Cases and Hospital Controls: Take all three measurements. For Neighborhood Controls: Measure only MUAC and keep other boxes blank.

72. Stature (in cm): {STATR} ☐☐☐.☐

73. Weight (in kg): {WT} ☐☐.☐☐

74. MUAC (in cm) {MUAC} ☐☐.☐

75. Health Assistant (HA) {HA} ☐

HA's signature_____

NOTES

Signature of the PI:_____ Date: |__|__|__|__|__|__|

ID: |___|___|___|___|