Attachment 1. 96 . 1 Date 4/8/97

(FACE SHEET) ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator J.P. VAUGHAN Trainee Investigator (if any)

Application No. 97-014 Supporting Agency (if Non-ICDDR,B) CDC ATLANTA USA

Title of Study Tetravalent Rhesus Rotavirus Vaccine: Proposal for an immunogenicity trial in infants in Matlab

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

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

1. Source of Population:
   (a) Ill subjects — Yes No
   (b) Non-ill subjects — (Yes) No
   (c) Minors or persons under guardianship — (Yes) No
2. 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)
3. 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
4. Are subjects clearly informed about:
   (a) Nature and purposes of study — (Yes) No
   (b) Procedures to be followed including alternatives used — (Yes) No
   (c) Physical risks — (Yes) No
   (d) Sensitive questions — (Yes) No
   (e) Benefits to be derived — (Yes) No
   (f) Right to refuse to participate or to withdraw from study — (Yes) No
   (g) Confidential handling of data — (Yes) No
   (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — (Yes) No
5. Will signed consent form be required:
   (a) From subjects — (Yes) No
   (b) From parent or guardian (if subjects are minors) — (Yes) No
6. Will precautions be taken to protect anonymity of subjects — (Yes) No
7. Check documents being submitted herewith to Committee:
   ___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   ✓ Protocol (Required)
   ✓ Abstract Summary (Required)
   ✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
   X Informed consent form for subjects
   ✓ Informed consent form for parent or guardian
   ✓ Procedure for maintaining confidentiality
   ✓ Questionnaire or interview schedule *

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

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

J Patrick Vaughan
Principal Investigator

Trainee

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH,
BANGLADESH

CENTERS FOR DISEASE CONTROL AND PREVENTION
ATLANTA, USA

TETRAVALENT RHESUS ROTAVIRUS VACCINE:

PROPOSAL FOR A RANDOMIZED, PLACEBO CONTROLLED TRIAL TO EVALUATE IMMUNOGENICITY, REACTOGENICITY AND ACCEPTABILITY IN INFANTS IN MATLAB, BANGLADESH

MARCH 1997

INTERNATIONAL CENTRE FOR DIARRHOEA DISEASE RESEARCH, BANGLADESH (ICDDR,B).

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Tetravalent Rhesus Rotavirus Vaccine:

Proposal for a Randomized, Placebo Controlled Trial to Evaluate Immunogenicity, Reactogenicity and Acceptability in Infants in Matlab, Bangladesh.

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SUMMARY OF PROPOSAL

The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), together with scientific and technical support from the Centers for Disease Control and Prevention (CDC) USA, proposes to organize and conduct an immunogenicity study of tetravalent rhesus rotavirus vaccine (RRV-TV), potentially to be followed by a large efficacy/effectiveness trial if the vaccine proves to be immunogenic.

The proposed immunogenicity trial will be a randomized, double-blind, placebo controlled trial in Matlab, which is the Centre's main site for demographic surveillance and large scale epidemiological research studies. This proposal is in response to the recent WHO report which found that rotavirus vaccine development for developing countries represents a priority for WHO, and because Asia has been thus far under represented in rotavirus vaccine trials. This proposal seeks funding of US$ 82,000 for the immunogenicity trial, but an outline for a subsequent efficacy/effectiveness trial to study to determine the potential for herd protection associated with a rotavirus vaccination program is also provided.

Matlab is a densely populated, rural area with 210,000 people who are under demographic surveillance and who are served by primary health care services and community health workers (CHWs). It is situated in the main river delta area to the southeast of Dhaka city. Matlab has a high incidence of diarrhea in young children, a community-based control program for acute diarrheal diseases, a referral hospital and subcentres to which all children in the area who require medical treatment for diarrhea are referred, and excellent laboratory facilities. In addition, the area has high rates of vaccine coverage for EPI vaccines because of intensive effort by the CHWs. Evidence from recent studies indicates that more than 80% of circulating rotavirus are serotypes G1-G4, i.e. those covered by RRV-TV.

The main objectives of the immunogenicity trial will be to assess the immunogenicity, reactogenicity and acceptability of the vaccine in a small population of Bangladeshi children aged 6 to 18 weeks old. In the immunogenicity trial, 80 children at aged 6-8 weeks will be randomized to receive three doses of RRV-TV or placebo 6, 10 and 14 weeks old, and then followed for a further one month. Blood specimens will be examined for seroconversion and faecal specimens for viral shedding. Children will be enrolled over a period of two months and the study will be completed within 9 months.

If the vaccine is immunogenic in this group of infants, a large population based trial will be conducted to assess the efficacy of the vaccine and the effectiveness of a large rotavirus vaccine program in reducing the incidence of rotavirus hospitalizations in this population. The design of the study will allow for comparison of rates of disease by levels of vaccine coverage, thus evaluating the potential for additional reduction of disease as a result of high vaccine coverage rates (herd protection). Infants would be enrolled over 2 consecutive years and each child will be followed up until they are 2 years of age. The infants will be given the RRV-TV vaccine as part of their routine EPI immunization schedule. In this study, children will be randomized either individually or by groups (villages) to receive RRV-TV or placebo, and passively followed for

hospitalization due to rotavirus diarrhoea.

The Principal Investigator (PI) for these trials will spend most of their time in Matlab during the trials and be a member of the Centre's Public Health Sciences Division, which has direct responsibility for all research in the field site. The scientific management of the trials in Bangladesh will be the responsibility of the Division's Director (Professor Patrick Vaughan, ICDDR,B). Scientific support for the trials will be given by the Viral Gastroenteritis Section of the CDC in Atlanta, Georgia, USA (Drs. Joseph Bresee, Roger Glass, and Jon Gentsch).

1 Phase Two Immunogenicity Trial: Introduction and Background

1.1 Introduction and background

The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), together with scientific and technical support from the Centers for Disease Control and Prevention (CDC) USA, proposes to organize and conduct an immunogenicity study of tetravalent-rhesus rotavirus vaccine (RRV-TV), potentially to be followed by a large efficacy/effectiveness trial if the vaccine proves to be immunogenic in this population.

The proposed immunogenicity trial will be a randomized, double-blind, placebo controlled trial in Matlab, which is the Centre's main site for demographic surveillance and large scale epidemiological research studies. This proposal is in response to the recent WHO report which found that rotavirus vaccine development for developing countries represents a priority for WHO, and because Asia has been thus far under represented in rotavirus vaccine trials. This proposal seeks funding of US$ 82,000 for the immunogenicity trial. However, also provided is an outline proposal for a subsequent efficacy/effectiveness trial to determine the potential for additional herd protection that might be associated with rotavirus vaccination program.

Matlab is a densely populated, rural area with 210,000 people where there is a high incidence of diarrhea in young children and a community-based control program for acute diarrheal diseases. In addition, the area has high rates of vaccine coverage for EPI vaccines because of intensive effort by the CHWs. Evidence from recent studies indicates that more than 80% of circulating rotavirus are serotypes G1-G4, i.e. those covered by RRV-TV.

1.2 Epidemiology of rotavirus infections

Rotavirus is the most common cause of severe diarrhea worldwide. In a review in 1986, the Institute of Medicine (IOM) estimated that 130 million children (nearly equivalent to the world's birth cohort of children) develop diarrhea due to rotavirus each year, 18 million of whom experience moderate or severe dehydration resulting in 873,000 deaths (1). Most children in developing countries develop rotavirus diarrhea in their first 2 years of life (2). In a World Health Organization-sponsored review of the rotavirus literature, rotavirus was estimated to be responsible for 20% of diarrheal deaths and 6% of all diarrheal episodes in children under 5 years (3). Because of the magnitude of disease associated with rotavirus infections, and because public health interventions to provide clean water and improved sanitation are unlikely to decrease the incidence of disease, vaccines are being developed as the first strategy for prevention. The first rotavirus vaccine is expected to be licensed for use in some countries during 1997, and other vaccines may follow in the near future.

Although vaccines have been consistently found to be quite effective in developed countries, studies in developing countries have yielded variable results. In fact, the leading vaccine has been evaluated in only a few developing country settings. None of

these sites were in Asia, where mortality associated with rotavirus is high and which would stand to benefit most from an effective vaccine. Several potential differences in the epidemiology of rotavirus between developing and developed countries may explain the variability of results of vaccine trials between settings, including: earlier age of rotavirus infection in less developed countries; potential different modes of transmission; the presence of other gut flora/infections; factors, such as poor nutrition, that may limit the immunogenicity of vaccines; and the circulation of atypical rotavirus strains. Consequently, plans for further evaluation and assessment of rotavirus vaccines in developing countries, where they would have the greatest impact, are still needed.

1.2.1 Rotavirus epidemiology in Bangladesh

The epidemiology of diarrhea and of rotavirus has been well studied in Bangladesh. Prospective studies have demonstrated that children in Matlab have 0.5 episodes of rotavirus diarrhea per year in the first two years of life (4), after which few episodes of rotavirus diarrhea were observed. This is supported by serologic studies in which 67% of children <5 years had 4-fold rises to antibody during a one year period of observation, but rises in antibody were associated with rotavirus positive diarrhea primarily in children less than 24 months of age (5). In hospital-based studies, more than 90% of cases occur among children less than 24 months, and the majority of these are among children in their first year of life (6-8). Although neonatal rotavirus infections appear to be common in some developing countries (9), and have been detected in high rates in Dhaka hospitals, infection appears to be uncommon in Matlab where most children are born outside of the hospital (10,11). Rotavirus infections appear to occur all year-round in Bangladesh, with a slight increase during the cool, winter months (6-8).

While rotavirus accounts for <5% of all episodes of diarrhea in the community, (4,12) it accounts for 39% of severe diarrhea cases admitted to the hospital (4). In hospital-based studies in Bangladesh, rotavirus is the most common cause of diarrhea among children <24 months of age, being detected in 30-46% of children (6-8,13). In one study, 44% all rotavirus infections detected resulted in dehydration and 30% required medical attention at the treatment center (14). The frequency of diarrhea admissions to Matlab hospital are presented below (Tables 1 and 2).

The current mortality in Matlab associated with rotavirus is unknown. In the late 1970s, the case-fatality rate associated with rotavirus diarrhea was 0.2%, based on small numbers (6). Of 898 patients <2 years old admitted to Matlab hospital between 1987 and 1989, the case fatality rate was 1.1% for rotavirus diarrhea (7). Currently, in the intervention and the control areas of Matlab, the mortality associated with diarrhea among 0-11 month-olds is 9.5 and 10.2 respectively, and among 12-47 month olds is 1.4 and 2.9 per 1000 respectively. By extrapolating the proportion of severe diarrhea accounted for by rotavirus in this population to known diarrheal mortality, rotavirus may result in mortality of approximately 3/1000 children 0-11 months old and 0.7/1000 children 12-47 months.

The importance of the prevention of diarrheal disease in Bangladesh is illustrated by the fact that diarrhea is responsible for almost 26% of all childhood mortality in Bangladesh (15). The total burden of rotavirus diarrhea in Bangladesh has been

estimated at 2.4 million cases each year, of which 290,000 are severe and 21,000 result in death (Unicomb, unpublished data).

Although at present there is no ongoing surveillance specifically for rotavirus diarrhea is performed in Matlab, past hospital- and community-based studies indicate that 4% of mild diarrhea (4) and 30% of severe diarrhea (6,7,13) are attributed to rotavirus among children under 2 years.

Table 1: Number of admissions to Matlab hospital for episodes of diarrhea in children aged 0-23 months living in the Matlab MCH-FP intervention area during 1994-96.

Age (months)	1994	1995	1996	Total	Percent
0- 2	126	100	129	355	14.0
3- 5	66	80	114	260	10.2
6- 8	127	130	160	417	16.4
9-11	149	169	168	486	19.2
12-14	125	128	138	391	15.4
15-17	79	80	97	256	10.1
18-20	73	73	67	213	8.4
21-23	53	51	55	159	6.3
Total	798	811	928	2537	100.0

Table 2: Incidence of admissions for diarrhea to the ICDDR,B Matlab hospital from the Matlab MCH-FP Programme among children between 1 to 23 months of age, 1994-1996 (based on mid-year populations and rates per 1000).

Age months	1994		1995		1996	
	no.	rate	no.	rate	no.	rate
0-11	468	181.1	479	182.8	571	216.7
12-23	330	131.1	332	131.7	357	141.5

1.2.2 Rotavirus strain prevalence in Bangladesh

Like other countries, both developed and less developed, 4 strains of rotavirus predominate in Bangladesh, P[8]G1, P[4]G2, P[8]G3, P[8]G4, and these account for more than 80% of strains detected (Unicomb, unpublished data). There appears to be

some annual variation in predominant serotype, and some unusual strains have been detected; i.e. 10% of strains were found to be either natural reassortants (P[4]G1 or P[4]G4) or unusual strains (P[6]G1) (13), and 10% remained nontypeable (16). These data are consistent with data from most other developing countries, and indicate that current polyvalent vaccines which cover the four common serotypes should protect against most naturally occurring rotavirus infections.

1.3 Tetravalent-Rhesus Rotavirus Vaccine (RRV-TV)

Tetravalent-rhesus rotavirus vaccine is a polyvalent rhesus-human reassortant vaccine which contains strains with VP7 specificities for the 4 commonly circulating human rotavirus strains. In addition to RRV (with serotype 3 specificity), 3 reassortants are present, each representing a prevalent human serotype (D x RRV (serotype 1), DS-1 x RRV (serotype 2), and ST3 x RRV (serotype 4)). Following monovalent vaccine trials which were found to result in highly variable efficacy, polyvalent reassortant vaccines were produced to provide a broader coverage and increased human rotavirus specificity. This vaccine has been subsequently studied in more than 10,000 children, and has proven safe and immunogenic.

The vaccine has been found to induce rises of serum IgA in vaccinated infants in approximately 55-84% (17-23) and neutralizing antibodies against RRV in ~80% of children (17,19,20,22). Lower rates of seroconversions in neutralizing antibody titers against human strains are generally observed (<50%) (17,18,20,22). In studies incorporating the current dose of vaccine ($4x10^5$ pfu), >90% of vaccinees have demonstrated a response to the vaccine by at least one assay.

Six large efficacy trials have been completed in industrialized and less developed countries using RRV-TV. Data from 4 studies are quite similar, demonstrating a 48-68% efficacy against any rotavirus diarrhea, >70% efficacy against severe disease, and a significantly reduced duration of diarrhea in the vaccinated group compared with unvaccinated controls (17,19-21). In 2 of these studies for which sufficient data are available, a 70-100% efficacy against rotavirus disease requiring hospitalization was observed (17, 21). Additionally, in the two US studies, protection from non-serotype 1 disease was found among the RRV-TV recipients; whereas no protection occurred among the monovalent reassortant RRV recipients.

Three of the studies were conducted in less developed countries, all were performed in South America, and only one used the current dosing regimen of the vaccine. An early study in Peru in which 1, 2, or 3 doses of RRV-TV (low dose, $4x10^4$ pfu) were administered to children 2-4 months of age found little or no overall efficacy against rotavirus diarrhea, and limited efficacy against severe, dehydrating diarrhea caused by serotype 1 viruses (18). The lower efficacy rates may be related the lower dose used in the trial or to the age of vaccination relative to the age of first infection in the study group. In this study, 40% of children enrolled had detectable IgA prior to vaccination, compared with 18% of children in an earlier Venezuelan trial in which vaccine was 70% effective in preventing severe disease (24). Similarly, a trial with low dose RRV-TV in Belem Brazil (25) found an efficacy of only 35% over 2 years (50% in the first year, and no efficacy in the second year), although there was a trend towards

greater protection against more severe disease. In this trial only 58% of patients developed IgA response to vaccine (lower than in U.S. trials and the Venezuelan trial where the vaccine was effective).

The only trial thus far conducted in a developing country using 3 doses of $4x10^5$ pfu of RRV-TV was recently completed in Venezuela (17). In this trial, the immunogenicity of the vaccine was similar to that observed in previous studies, with 84% of the vaccinees showing a 4-fold rise of IgA, and more than two-thirds developing neutralizing antibody to RRV. As in other studies, the efficacy against rotavirus diarrhea was 48%, and against severe disease was >70%. During all 4 years of the study, G1 was the predominant strain, and therefore, only efficacy against G1 was demonstrated. This study demonstrated that rotavirus vaccines can be effective in less developed countries as in industrialized countries.

1.4 International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B)

The Centre is affiliated to the United Nations system as an international and independent research institute. It has its own Board of Trustees, made up from members of the international scientific community, together with representatives from the World Health Organisation and UNICEF, as well as the Government of Bangladesh. The Centre is supported by over thirty different donors, including the aid agencies of the Governments of Australia, Bangladesh, Belgium, China, Canada, Denmark, Japan, the Netherlands, Norway, Saudi Arabia, Sri Lanka, Sweden, Switzerland, United Kingdom, and the United States of America; by international organizations including UNICEF, United Nations Development Programme (UNDP), United Nations Population Fund (UNFPA) and the World Health Organisation (WHO); and by private foundations, including the Ford and Sasakawa Foundations. The Centre has excellent relations with the Government of Bangladesh, which is one of the major financial contributors. The total annual budget was approximately US$12.5 million in 1996.

The ICDDR,B has an impressive record as an international health and population research institute, particularly for trials and intervention studies in the areas of family planning, reproductive health and child survival, including diarrhoea and acute respiratory illnesses (See ICDDR,B Annual Report for 1995). It has also implemented six controlled trials for cholera vaccines in Matlab since the Centre was originally established as the Cholera Research Laboratories in 1960. The Centre has its own independent scientific research and ethical review procedures and committees, which are recognized by the Government of Bangladesh.

The Centre has extensive demographic, health and disease surveillance systems in both rural and urban populations, together with its own community based health services and referral hospitals. It has a large multidisciplinary scientific research staff, comprising about 35 international and 140 national members, as well as administrative, support and field staff. The total staff is nearly 1300 people, including 160 community health workers (CHWs). The Centre has a well established and sophisticated research infrastructure, including inpatient hospital facilities, microbiological laboratories and computerized data handling capacity.

Since the early 1960s the Matlab population has been monitored by a demographic surveillance system and since 1978 a half of the population has been included in an area where maternal and child health and family planning (MCH-FP) intervention studies have been carried out. The other half of the Matlab population, which resides in the comparison area for demographic purposes, receives the health services provided by the Government of Bangladesh.

1.4.1 Matlab field study area

Matlab town is situated about two and a half hours travel by road and river transport from Dhaka. It is in an area that is representative of the river delta system formed by the joining of the large Meghna and Padma rivers (See Maps 1 and 2 in Section 6). The area is subject to large seasonal fluctuations in water level of several metres and the agricultural subsistence economy is based on rice cultivation and river fishing. Travel within the study area is mainly by local roads, on foot, and by speed and country boats, particularly during the rainy months of June to September.

An indication of the socioeconomic status of the population is given by the following information. In the Matlab area about 88% of the population are Muslims and most of the remainder are Hindu. Literacy is about 55% for males and 25% for females and the commonest occupation is as 'wage labourers' on farms or for other manual work. The majority of households possess two rooms in houses built of traditional materials, but with brick, concrete and metal roofs being commonly used as well. Most households use tube-well water for drinking in both dry and monsoon seasons but the majority also use surface water for cooking and washing. However, less than 10% use a sanitary latrine. Malnutrition is widely recognized to be common and more frequent in girls than boys, with about two thirds of children having weight-for-age less than minus two Z scores.

Map 1: Bangladesh, showing Dhaka and Matlab.
Map 2: Matlab demographic surveillance area.

The Matlab is one of the larger thanas in Bangladesh, with approximately 500,000 people, and the GoB health services are organised from the thana health complex in Matlab town. The nearest government referral hospital is in the district town of Chandpur, about 45 minutes by road from Matlab. Besides the government and ICDDR,B services there are a wide variety of allopathic practitioners, both formally and informally qualified, together with private pharmacists, traditional healers and drug sellers. However, the Matlab ICDDR,B hospital is widely recognized for treating diarrhoeas and many patients come from long distances.

1.4.2 Matlab demographic and geographical surveillance systems

Matlab offers excellent facilities for conducting rotavirus vaccine trials. The study population of 210,000 people, who are resident in 142 villages, have been included in the Centre's demographic surveillance system (DSS) since 1966. The bari is a local demographic unit formed by some 6-7 households that share a common courtyard and there were nearly 7,200 in 1994 in the full DSS area. Each individual has a unique identification number which locates them to a specific village, bari and household. The present DSS has

used male health assistants (HAs) and the female CHWs to collect data every two weeks through household visits and, based on the unique individual identification number, records are kept on all marriages, births, deaths, in- and out-migration, and internal movements.

In 1977 the DSS area was divided into two equal populations. The first was where the Maternal, Child Health and Family Planning (MCH-FP) Programme has carried out health and population intervention studies, while the other has served as a demographic comparison area. The MCH-FP Programme has developed its own computerized household information on all women aged 15-44 years and children aged 0-4 years in its area, called the Record Keeping System (RKS). This is considered in more detail below.

In addition to the DSS and RKS there is a geographical information system (GIS) which shows the delineation of 142 villages and the location of the total Matlab population of 210,000 by their bari. Both demographic and epidemiological data, such as that for diarrheal mortality, can be demonstrated spatially by the GIS (See Map 3 below). The GIS can also display the population covered by each of the CHWs individually and by health facilities (See Map 4). Thus the demographic and epidemiological information can be located and displayed by the GIS at various levels of aggregation.

Map 3: GIS distribution of diarrhea deaths by bari.
Map 4: Population covered by individual CHWs for contraceptive acceptance.

As the DSS is linked at the individual level, accurate denominator population estimates are available for calculating age and sex specific mortality rates, including for acute respiratory infections, for both the intervention and comparison area populations. Trends over time and space-time clustering can also be demonstrated. Combining the DSS and GIS data with the RKS data from the MCH-FP Programme in the intervention area enables diarrheal incidence rates to be derived. In addition, access to and geographical coverage of the MCH-FP services can be estimated by level of facility, such as CHWs and subcentres, and down to bari level.

1.4.3 Demographic characteristics of the Matlab population

Some relevant demographic information for the 1995 population is given in Table 4 below (See also the Centre's publication: Demographic Surveillance System, Matlab Early Indicators, 1995). Analysis of the 1995 DSS data shows that the male:female ratio is approximately equal at all ages and that more than 10% of the population is aged 0-9 years. In the intervention and comparison areas the total fertility rates have fallen to 3.0 and 3.7 respectively and the average expectation of life at birth is now more than 62 years for both males and females in both study areas.

The mean age of first marriage in both areas is now about 26 and 19 years for men and women respectively. Contraceptive prevalence in the MCH-FP area is nearly 70%, compared to the national average of about 45%, and the preferred methods are injectable contraceptives (53%), the oral pill (24%) and female sterilization (13%). Prenatal care in the MCH-FP area is mainly from the programme midwives (81%) whereas in the comparison it is from government facilities (15%) and for deliveries in these areas the main birth attendants are traditional birth attendants (TABS) and

midwives (82% and 9% for the MCH-FP area compared to 97% and 1% for the comparison area respectively). Stillbirths have been estimated to be 2.7% and 3.7% of all births in these areas and the miscarriage rates are 6.4% and 10.0% respectively. In 1994 these two populations had crude birth rates of 25.4 and 29.1 per 1000 live births/year respectively, resulting an estimated 2,730 and 3,030 live births per year, or 5,760 for the whole DSS area.

Table 3: Matlab DSS study population 1995 (Rates per 1000 population).

Study Populations	MCH-FP Intervention	Comparison
Total number	106,477	102,917
Crude birth rate	25.2	27.8
Total fertility rate	3.0	3.7
Infant mortality rate	51.1	78.6
Child mortality rate (0-4 years)	75.6	105.7

1.4.4 Matlab maternal, child health and family planning programme

Since 1977 the Centre's MCH-FP Programme has utilized more than 80 CHWs to provide a range of health and family planning services to mothers and their young children in their own baris (26). Many of these baris are separated by water, particularly during the rainy season of June to September. The CHWs currently visit each household in their area every two weeks in order to collect population based incidence data on common childhood illnesses, record information on their case management and to deliver preventive health services. A referral system ensures that severely ill children and mothers are referred on to health subcentres staffed with nurse-midwives and medical assistants and, if necessary, patients are then again referred to the Centre's Matlab hospital. The Centre provides emergency speed-boat transport for severely ill patients needing referral.

The data collected by all the CHWs is maintained in record books as a part of the record keeping system (RKS), from where it is collated and computerized monthly. The computerized RKS data is complete since 1986. Close monitoring of HAs and CHWs ensures that household coverage is high and that there are good quality data available. The RKS includes information on dates of vaccinations and vitamin A supplementation, diarrhoea and ALRI episodes and their case management, patient referrals, nutritional status and temporary absenteeism. The diarrhea-specific information collected by the MCH-FP Programme includes the type of detection (active or passive), severity, case management, referral and follow up measures.

Based on this information the CHWs in the MCH-FP area are supplied monthly with EPI vaccines from Matlab hospital and they are responsible for vaccinating all eligible children in their area, according to pre-prepared and printed lists based on government EPI schedules. These procedures ensure that vaccinations are given close to the child's home and at the correct age, thus substantially reducing delayed and missed vaccinations. The EPI programme includes antenatal maternal tetanus toxoid, and for infants BCG at birth or soon afterwards, DPT and oral polio vaccines at 6, 10 and 14 weeks, and measles vaccine at 9 months of age. Immunization coverage for 3 doses of DPT were estimated for four different dates in 1995 to be between 86% and 90% in the MCH-FP area and the Government of Bangladesh estimates that it is 70% in the comparison area.

1.4.5 Matlab hospital and laboratory services

At the central Matlab research facility there are separate wards for treatment of diarrhoea and for ALRI, where all patients with severe illness are admitted for intensive investigation and treatment. For the five years 1991 to 1995 the average number of diarrhea patients admitted was 15,000 per annum. The childhood diarrhea ward currently has 70 beds, but has capacity for at least 150 patients. It is staffed with specially trained physicians and nurses. The Matlab hospital has mains electricity and a standby generator, well organized water and road transport facilities, and good storage for drugs and vaccines. There is also a 10 room hostel for investigators.

The laboratory at Matlab undertakes microbiological investigations as would be required for the case management of diarrhoea and ALRI patients. Stool and rectal swabs are cultured for bacterial pathogens, and tested for parasitic agents. All positive bacterial cultures are tested for antibiotic susceptibility. In the past, when studies have involved rotavirus testing of specimens, they have been sent to the main ICDDR,B laboratory in Dhaka for testing. The transport of specimens is logistically simple and well-practiced.

1.5 Conclusion: Advantages of Matlab for a rotavirus vaccine trial

The huge of burden of disease caused by rotavirus infections among young children in developing countries was a main impetus for vaccine development in the 1980s and 1990s. Now that effective oral live vaccines have been developed, and are near licensure in some developed countries, there is a need to test the new RRV-TV vaccines in populations that could stand to benefit the most. Bangladesh is one of the poorest and most densely populated countries in the world. Like other developing countries, childhood mortality and morbidity remains high, due in large part to infectious diarrhea.

The presence of ICDDR,B research facilities in Dhaka and Matlab for the past 30 years has resulted in Matlab becoming one the best sites for field research in the world. Not only is the population well described, which makes population-based research possible, but decades of research in diarrheal diseases has led to accurate estimates of the rates and causes of rotavirus diarrhea in this population. Additionally, researchers and staff at the field station have been involved in 6 large placebo-controlled field trials

for cholera vaccines. In this rural setting children live in distinct villages and isolated baris, which provides a unique opportunity to determine whether there is additional protection due to herd immunity. It is also possible to maintain a high level of surveillance in both vaccinated and control populations throughout the four years required for such a trial. Finally, these trials in Bangladesh would enable the RRV-TV vaccine to be tested in Asia for the first time.

2. Immunogenicity, Reactogenicity and Acceptability of Tetravalent Rhesus Rotavirus Vaccine in Infants in Matlab, Bangladesh

2.1 Study objectives

1. Determine the immunogenicity of RRV-TV in this population of infants when given with EPI vaccines, including oral poliovirus vaccine.

2. Determine factors associated with immune response to the vaccine.

3. Determine the rate of and types of adverse reactions to the vaccine among this population of children.

4. Determine the acceptability of the RRV-TV vaccine to the population.

Based on the findings from this trial a decision will be made regarding the appropriateness of a subsequent efficacy/effectiveness trial.

2.2 Summary of study design

The immunogenicity trial will be a randomized, double-blind, placebo-controlled trial comparing immune responses, adverse reactions, and acceptability between recipients of RRV-TV and a placebo. Children will be randomized to receive either 3 doses of RRV-TV or of an identically tasting and appearing placebo. They will receive vaccine or placebo at 6-8, 10-12, and 14-16 weeks of age together with their regularly scheduled EPI vaccines. Participants will be asked to donate serum specimens immediately prior to each vaccination and 4 weeks following the third vaccination. In addition, stool specimens will be obtained at regular intervals following each vaccination to test for shedding of vaccine virus. Information regarding demographic characteristics, health status, recent or intercurrent illness, immunizations, medication history and other information will be gathered as part of the regular surveillance of the population.

2.3 Study methods

2.3.1 Study subjects and enrollment

In the Matlab MCH-FP area population, approximately 2700 births occur annually, or about 225 per month. When children are born, one of 80 Community Health Workers (CHWs) will enroll them into the DSS program where they will be given a unique DSS identification number (usually within 2-4 weeks of birth). Assuming that all children born into the cohort will be identified by a CHW, be given an ID number by the time of their first EPI vaccination, and that 20% will refuse to participate, it is possible for as many as 180 children to be enrolled within 1 month of beginning the study. Even with a dropout rate of 20%, more than 140 of these children could be available for the study.

The mothers of all newborn children identified by the DSS in Matlab beginning August 1st 1997, and who live in the MCH-FP intervention area, will be contacted for

possible recruitment into this trial. Only MCH-FP-born infants will be included in this phase of the study because of their proximity to Matlab hospital and the greater availability of resources for enrollment and follow-up for this population. Once a child is given a DSS identification number (usually by 2-4 weeks of age), the child's parents will be contacted by a study assistant who will explain the study and ask them to enroll their child. Participation will be strictly voluntary. Refusal to participate will in no way change the care received by the child or the family, and will not affect participation in other future studies. Informed consent will be signed by at least one parent or recognized guardian of all children prior to enrollment (Appendix A).

2.3.2 Exclusion criteria

Children will be excluded from enrollment if they:

1. Die prior to enrollment or are no longer living in the study area at the time of the initial vaccine visit;
2. Are older than 8 weeks of age at the time DSS ID number assignment;
3. Have a known chronic disease for which live vaccine administration would be contraindicated (such as an immunodeficiency disorder or diseases for which the child is receiving immunosuppressive chemotherapy) or
4. Have a household member who is or may be immune suppressed.

There will be no exclusion of children based on timing of EPI vaccinations relative to timing of vaccination for this study.

2.3.3 Study outcomes and sample size

The main study outcomes will be a 4-fold rise in anti-rotavirus IgA titer or neutralizing antibody levels against rhesus rotavirus strains. In the RRV-TV trial performed in Venezuela using the current dose (17), 88% of vaccinees developed a rise in serum antibodies to at least one test compared to 29% of placebo recipients (17). Individually, 84% of children demonstrated a 4-fold or greater rise in IgA to rotavirus compared to 22% of placebo recipients; 10-45% of vaccinees developed neutralizing antibodies to human rotavirus strains compared to 2-22% of placebo recipients; and 77% developed neutralizing antibodies to RRV compared to 2% of placebo recipients.

Given a IgA seroconversion rate of 30% in the placebo group, a study with 72 children would allow for the detection of 35% difference in the groups (a seroconversion rate in the vaccinated children of 65%)(with 80% power and 0.05 level of significance). If the rates of IgA seroconversion were equal to those in the Venezuelan trial, a study with 72 children would have a power of >99% to detect this difference (given a rate in the placebo group of 30%). The table below shows ranges of expected results and power given the expected sample size.

To ensure that the results on at least 72 children are available for analysis, more than 80 children will be enrolled to allow for a 10% drop-out rate. Given the size of the monthly birth cohort in this population, we would expect that 80 children could be enrolled within 2-3 weeks.

Table 4. **Minimum rate of seroconversion among vaccinees given range of expected seroconversion rates among placebo recipients by test (assuming a sample size of 36 children in each group, a power of .80 and a 0.05 level of significance).**

Test	Expected seroconversion rate in placebo recipients	Minimum necessary seroconversion rate in vaccinees
IgA ELISA	20	54
	30	65
	40	75
Neutralizing antibodies to human strains	5	33
	10	41
	20	54

2.3.4 Randomization procedure

Once enrolled children are assigned their unique DSS identification number and they agree to participate in the study, they will be assigned a Immunogenicity Study ID number, which will signify whether the child will receive vaccine or placebo. The number will be assigned by sequentially proceeding down a preprinted list of numbers (Enrollment Log, Appendix B), so that the first subject will receive the first number on the list, followed by the second subject enrolled, and so on. The study numbers will be randomized in blocks of 4 to assure an equal number of subjects in each study group. The study numbers will be range from 001 to 080. The ID numbers will be assigned by an assistant who is not involved in the care of the subject or the collection of outcome measures. When an infant presents to the hospital for a dose, a vial with matching number and appropriate dose number will be removed from storage, reconstituted, and administered as below. On subsequent visits, study subjects will be identified and matched to the list to determine the appropriate dose. At each administration, the date and time of administration will be documented on the Study Record (Appendix C), so that the presence of a date and time will indicate successful administration of the vaccine.

2.3.5 Study vaccine and placebo

Lyophilized tetravalent rhesus rotavirus vaccine (RRV-TV) will be supplied by Wyeth-Ayerst Research Laboratories. The vaccine will be constituted to contain 4 x 10^5 plaque forming units/ml of RRV-TV (1 x 10^5 each of the 4 vaccine strains, RRVxD, RRVxDS-1, RRV, and RRVxST-3). Both vaccine and placebo will be provided as

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prelabelled, identically appearing vials. Diluent-buffer for reconstitution of placebo and vaccine will be supplied by Wyeth-Ayerst Research Laboratories, and consists of 25.6 mg/ml sodium bicarbonate and 9.6 mg/ml sodium citrate.

Vials of study vaccine/placebo and diluent-buffer will be shipped at controlled temperature (2-30°C) to the participating research centers, and stored at these temperatures until administration. At the time of administration, vaccine and placebo will be resuspended in 3 ml of buffer at room temperature for oral administration to subjects as described below. Vials will be packaged individually and prelabelled and sorted by Wyeth-Ayerst. The labels will contain a unique identification number for each vial, a study subject number (3 vials would share each subject ID number), and a dose number (1, 2, or 3). The randomization code will be kept at CDC by study collaborators not involved in enrollment or follow-up of patients, and study personnel will not have access to this information.

2.3.6 Procedure for administering vaccine/placebo

Prior to receipt of any dose of study drug, each child's temperature and current weight will be measured, and documented on the Study Record in the appropriate spaces. Lyophilized vaccine or placebo will be resuspended in 3cc sodium bicarbonate/sodium citrate buffer diluent. 2.5 ml of the solution will be placed at the back of the mouth (in 1cc aliquots as tolerated) using a small syringe without the needle.

Three doses of the study drug will be given at greater than 3 week intervals to coincide with a child's routinely scheduled health care visits at age 6, 10, and 14 weeks of age. The vaccine will be administered with other regularly scheduled EPI vaccines, including diphtheria/pertussis/tetanus (DPT), and oral poliovirus (OPV). Oral poliovirus has been shown to diminish the immunogenicity of RRV-TV, however this decrease is overcome by administration of 3 doses of the RRV-TV (27,28). Conversely, there appears to be no interference with OPV immunogenicity by coadminstration with RRV-TV (27,28). All non-rotavirus vaccines administered will be recorded on the Study Record.

If on physical exam prior to any of the 3 vaccine/placebo administrations a subject is found to have an illness involving fever of >38.0C, diarrhea, abdominal pain or vomiting, the vaccination will be delayed until these symptoms have resolved. Vaccination will also be delayed if a child has received a dose of vaccine or placebo within 21 days prior to the present visit.

Children and parents will be brought to the hospital by boat or truck at no charge to ensure completion of the study.

2.3.7 Withdrawal from study

A subject's parents may refuse to participate at any point after consenting to be in the study. Refusal to participate will in no way affects the subjects' or families' medical care or standing at the health care facility.

2.3.8 Follow-up of study subjects and specimen collection

Blood samples will be collected immediately prior to each of the 3 doses of study drug and 4 weeks following the final dose. 3-4cc of blood will be collected by venipuncture from each enrollee at each scheduled blood draw using a butterfly needle with syringe. Stool specimens will be collected 4 and 7 days following each of the 3 doses. During the vaccination visits to the hospital, parents will be provided stool collection materials and reminded that a study assistant will come to the house to collect the specimens on the appropriate days. Parents will be encouraged to collect samples from soiled diapers during the morning of collection.

All blood samples will be transported to Matlab Hospital where sera will be removed and stored in 2cc Nalgene cryovials at -20°C. Specimens will be transported at least once weekly to ICDDR,B Virology Laboratory for storage until testing. Serum will be tested for anti-rotavirus IgA by ELISA and neutralizing antibodies to each of 4 common human serotypes as well as to RRV. Stool samples collected will be placed in a sterile screw-top container and refrigerated prior to transport. These will be transported to ICDDR,B 3 times weekly, where they will be tested for rotavirus antigen using a rotavirus EIA and stored at 4°C until testing. At the end of the study, stools positive by EIA for rotavirus will further characterized using monoclonal antibodies for G-typing and reverse transcriptase-polymerase chain reaction (RT-PCR) for P typing. Positive samples which can not be characterized will be further evaluated by Dr. Jon Gentsch's laboratory at CDC. An aliquot of a subset of sera will be sent to CDC for testing as well as a quality control measure.

2.4 Laboratory studies

a. Serum anti-rotavirus IgA ELISA

Microtitration plates (Nunc Maxisorb Immunoplates, Nunc, Glostrup, Denmark) will be coated with hyperimmmune rabbit anti-rotavirus antisera for 2 hours at 37°C. Plates will be washed 3 times with phosphate-buffered saline (PBS) with 0.05% tween 20 (Sigma, St. Louis, Missouri, USA) (PBST); then cell lysates of MA104 cells inoculated with RRV or cell culture maintenance medium will be added, and plates will be incubated overnight at 4°C. Following washing, sera will be diluted in 2% (w/v) skim milk powder in PBST to 1/100, serial 3 fold dilutions will be made (SMP), and plates will be incubated at 37°C for 1 hour. Plates will be washed and optimally diluted; HRP-conjugated anti-human IgA in SMP will be added and plates will be incubated at 37°C for 1 hour. Plates will be washed, substrate containing 3,3,s,s' tetramethylbenzidine will be added, and plates will be incubated at room temperature for 10 minutes. The reaction will be stopped with 2M H_2SO_4, the OD_{450} will be read in an ELISA reader and titers will be determined using a computer-based program "MULTI" ((Data Tree Inc., Walthams, MA, USA).

b. Plaque reduction neutralization

Sera will be tested for neutralizing antibodies to the 4 major rotavirus G serotypes: Wa (P1A[8]G1), DS-1 (P1B[4]G2), P (P1A[8]G3), and ST3 (P2A[6]G4), and

to RRV using an immunoperoxidase focus neutralization test. Serum will be tested at a starting dilution of 1:200, according to previously described method (29), with viruses at a concentration of 4 x 10^3 to 10^4 foci/ml (except that horseradish peroxidase conjugated anti-rabbit immunoglobulin, F(ab')2 fragment (Sigma, Missouri, USA) and substrate containing 3-amino-9-ethyl carbazole (according to the manufacturer's instructions) will be used). A positive titer is equivalent to 50% reduction of peroxidase foci compared to wells inoculated with rotavirus diluted to give approximately 100 foci per well.

c. Detection of rotavirus in stool by EIA

An enzyme immunoassay (EIA) will be used to detected rotavirus from a 10% (w/v) stool extract in phosphate buffered saline (PBS), pH 7.2, based on DAKOPATTS commercial kit (30).

d. Rotavirus G serotyping by ELISA

Microtiter plates (Nunc Maxisorb Immunoplates, Nunc, Glostrup, Denmark) will be coated with MAb specific for VP7 serotypes 1-4 and MAbs that recognize common epitopes on VP7 (Silenus Laboratories, Melbourne, Australia) for 3 hours at 37°C, blocked with 2% SMP for 1 hour at 37°C. Test samples are added and allowed to incubate overnight at 4°C. Rabbit anti-rotavirus antiserum is then added and incubated for 1 hour, washed, and replaced by HRP-conjugated anti-rabbit immunoglobulin for 1 hour at 37°C. TMB substrate is then added.

e. Rotavirus P typing by reverse transcriptase-polymerase chain reaction (RT-PCR)

P typing will be performed using RT-PCR on glass powder extracted RNA as described previously (31). Primers will be provided by Dr. Jon Gentsch, CDC, Atlanta, Georgia, USA. Samples negative for G-type by ELISA will also be tested using RT-PCR.

2.5 Surveillance for adverse reactions

Parents of all children will be asked about adverse events or reactions that occur in the 7 days following each vaccination using a standard reporting form. At 4 and 7 days following each vaccination (coinciding with follow-up visits for stool collection), CHWs will administer the Adverse Reaction questionnaire to at least one parent or caretaker of each subject (Appendix D). In addition, the CHW will take the child's rectal temperature and record it on the adverse reaction questionnaire. Specific questions will be asked about signs and symptoms previously found to be associated with rotavirus vaccines, and general questions will be included to assess any other potential adverse effects.

2.6 Parental acceptability and community perceptions

Beliefs, attitudes and practices towards the vaccinations will be studied amongst the families living in a bari from which a trial infant has been recruited, using appropriate anthropological methods and rapid ethnographic assessment techniques similar to those

already developed by the World Health Organisation (32,33). Particular attention will be given to any symptoms or illnesses that families attribute to the vaccinations and to any perceived positive benefits (or negative effects) that might be used (or avoided) in order to enhance the success of the main trial. Special attention will also be given to how community relations and staff cooperation can be enhanced and sustained, since these will be critically important for the success of the possible subsequent eficacy/effectiveness trial.

2.7 Study outcome measures and statistical analysis

The primary comparisons of interest are the differences in immunologic response or shedding between vaccine and placebo recipients (i.e. $\geq$ 4-fold rise or geometric mean titers (GMTs) of serum antibody titer, or proportions of subjects that shed rotavirus at each time point). The rates of reports of adverse reactions will also be compared by dose for each of the first 7 days following vaccination.

Proportional outcomes will be compared between vaccine and placebo recipients using chi-square tests. In addition, GMTs will be calculated for each group and compared using nonparametric tests (Wilcoxon rank-sum test). Stratified analyses will be performed to assess risk factors for response using Cochrane-Mantel-Hanszel tests and multivariate regression analyses.

2.8 Timetable

Enrollment of all subjects will be completed within 2 months from the beginning of the study. Given that 3 doses of vaccine will be admininstered, starting with the first dose at aged 6-10 weeks and that the final blood collection is 4 weeks following the third dose, all sample collection will be complete within 18 weeks of enrollment of individual infants. It is expected that preliminary laboratory testing will be complete within 2 months of the end of the study data collection, and a preliminary report of results will be prepared within 1 month following the conclusion of laboratory testing. Thus, the entire study should be completed well within 9 months from the start of the study.

Month/Year	Activity
April/May 1997	Submit protocol to research and ethical review committees.
July 1997	Start of Study. Hire and train study staff.
August 1997	Begin enrollment and followup of study subjects.
September 1997	Complete enrollment.
January 1998	Complete followup of subjects and specimen collection.
February 1998	Complete IgA testing and laboratory analyses.
March 1998	Preliminary analysis of results. Panel convened to evaluate results and determine appropriateness of subsequent efficacy/effectiveness trial. Apply for funding for subsequent trial.
April 1998	Complete all analyses. Final report prepared.
July 1998	Start efficacy/effectiveness trial.

3. Outline Proposal For A RRV-TV Vaccine Efficacy/Effectiveness Trial In Matlab, Bangladesh.

3.1 Introduction

At the conclusion of the immunogenicity trial, a monitoring group will evaluate the data and findings and then make recommendations regarding the potential for further studies of rotavirus vaccines in this population. If the RRV-TV vaccine is found to be immunogenic, we intend to submit a proposal for funding to conduct a large vaccine trial to determine the efficacy and effectiveness of RRV-TV in this population. The proposed trial design will also allow the determination of the effect of herd protection. An outline for this study is provided below and a detailed protocol will be forwarded at a later date.

3.2 Study objectives

1. To determine the efficacy of RRV-TV in preventing hospitalizations due to rotavirus diarrhoea and/or reducing the length of hospitalizations in this population of Bangladeshi infants.

2. To determine the effectiveness of the RRV-TV in reducing the incidence of hospitalizations for rotavirus diarrhoea when it is included with the three doses of of the routine EPI immunizations.

3. To determine if high vaccination coverage rates result in protection above the expected efficacy rates predicted by the vaccine efficacy trial in this population.

4. Determine factors associated with efficacy and effectiveness in this population, such as season, serotype of prevalent strain, and other patient characteristics.

3.3 Outline of efficacy/effectiveness study design

This study will be conducted in the Demographic Surveillance area in Matlab over a 3 year period. All villages in Matlab DSS will randomly assigned to one of two main groups, after stratification by whether they are within the MCH-FP or Comparison areas, and by population size. All children born in the full DSS surveillance area during the study period will be eligible. The study subjects will be then be divided into two distinct study populations, based on whether the subjects are randomly allocated individually or by villages to receive the 3 doses of RRV-TV vaccine or the identically tasting placebo.

In Group A villages the infants will be individually randomized, as would be the design for an efficacy trial. In these villages the population coverage for RRV-TV vaccine would be about 45% in the ICDDR,B's MCH-FP area. In Group B villages, however, the infants will be randomly assigned to receive the RRV-TV vaccine or placebo according to the village in which they live. Infants living in Group B villages that receive the Centre's MCH-FP services from CHWs the RRV-TV vaccine coverage would be expected to be about 90% and 0% in the those villages receiving only placebo.

For the Group B villages in the Comparison Area the population coverage achieved by the Government's health services would be about 60-70% in those villages that received the RRV-TV vaccine. This design thus allows for the comparison of population coverages of RRV-TV of 45%, 60-70% and 90%.

Infants will be enrolled shortly after birth and randomly assigned to receive RRV-TV or an identically tasting and appearing placebo. The study will be double-blinded and placebo-controlled, and infants in both groups will be vaccinated at 6-8, 10-12, and 14-16 weeks of age with routine EPI vaccinations. Children will be passively followed for rotavirus hospitalizations at 3 sites which provide all diarrhoeal treatment for the Matlab DSS area, ensuring 100% surveillance coverage of the study population. Children will be enrolled over a period of two years and followed up until aged 18 or 24 months, depending on the sample sizes required. Participation in the study will not affect a subject's receipt of other routine childhood immunizations or other medical care in any way.

Vaccine efficacy will be assessed in the Group A children be comparing rates of hospitalization for rotavirus diarrhoea. For this calculation, the analysis could be performed on an intention to treat basis, as well as only outcomes that occur among children receiving a full course of vaccinations at appropriate ages.

Vaccine effectiveness will be assessed in Group B villages by comparing the hospitalization rates among children in villages that received the RRV-TV vaccine compared to those that received the placebo. In addition, the potential additional reduction of illness due to high vaccine coverage rates (herd protection) will be assessed by comparing rates of disease among unvaccinated children in villages receiving vaccine and villages receiving placebo, and by comparing the relative vaccine efficacy calculated from outcome rates among infants in Group A and Group B.

We estimate that during 2 years of enrollment, nearly 10,000 infants will be enrolled in the efficacy/effectiveness study, and the expected rates of rotavirus hospitalizations for the control population are 0.03 hospitalization/child/year during the first 2 years of life. This study would have a 80% power to demonstrate a 44% efficacy rate in the Group A children (with a significance of 0.05). This is well below the observed efficacy of the vaccine against severe disease.

3.4 Advantages of such an efficacy/effectiveness trial

The advantages of conducting such a trial would include:

1. Efficacy of RRV-TV would be determined in Asia for the first time, where poor environmental conditions may favour higher rates of rotavirus transmission.
2. Herd protection due to RRV-TV can be estimated when high population vaccination coverage is achieved.
3. The possible effects of such high coverage can be demonstrated over time on transmission, including incidence, seasonality and case severity.
4. The methodological advantages of conducting efficacy and effectiveness trials in the same population will be examined.

4. Additional Information

4.1 Vaccine side effects and precautions

Recent studies using RRV-TV in the same doses and schedule have indicated the following potential side effects associated with vaccine administration.

1. Fever - Fever of >38°C has been documented in 21% of vaccine recipients compared to 6% of placebo recipients, while temperature greater than 39°C has been observed in 2% of vaccinees versus 1% of placebo recipients. The fever usually occurs on the 3rd or 4th day following vaccination and is more common following the first dose of vaccine. Acetaminophen administered as part of routine childhood vaccinations should further decrease the proportion of children with clinically apparent fevers.

2. Vomiting and diarrhea - There does not appear to be any diarrhea or vomiting associated with the vaccine.

3. Excessive irritability and decreased activity - Slightly higher rates of irritability and decreased activity have been reported in some trials, again primarily following the first does of vaccine.

4.2 Ethical issues

This vaccine has been used in more than 7,000 children in the current dosing regimen. It has been associated with protection from disease and with no serious adverse effects. Currently there is no commercially available rotavirus vaccine, so that children enrolled in this study will either receive the current standard of care or a potentially beneficial vaccine. Parents of all children will provide informed consent before enrollment, and they may withdraw their child at any time during the study with no adverse consequences. Refusal to participate or withdrawal from the study will in no way affect the patient's or family's care.

4.2.1 Research and ethical review committees and boards

This study will be submitted for approval by the Research and Ethical Review Committees of ICDDR,B and by the Institutional Review Board of CDC Atlanta prior to enrollment of any patients. A copy of the approval letters will be kept by the principal investigator.

4.2.2 Informed consent

Informed consent will be obtained from the parents or guardian's of each subject prior to enrollment in the study (Appendix A). The Food and Drug Administration, USA, has issued final regulations to provide protection for human subjects in clinical investigations. The Code of Federal Regulations (21 CFR Part 50) establishes the general requirements for informed consent, and informed consent for this study conforms

to these requirements.

4.2.3 Subject identification

Study subjects will be identified only by study number in all materials that are used for analysis of study data, including all materials that are sent to CDC or the funding agency. No patient identifiers will be used in the analyses, nor will any identifying information be used in any reports or publications that may arise from this study.

4.3 Monitoring of the study

The study will be monitored by a consulting group and, if requested, by the funding agency. This will involve visits to the site prior to the beginning of the study, and potentially at intervals during the study to assure that adherence to the protocol and to solve problems. Additionally, investigators will communicate regularly, by telephone, fax and email, for updates and as needed for problem solving.

The study consulting group will review the results of the Phase II immunogenicity trial to determine of the vaccine is sufficiently immunogenic and safe to proceed with the Phase III and IV studies. A report of this meeting will be made available to funding agencies. The consulting group will also meet following the first 12 months of enrollment in the Phase III and IV studies to review results of an interim analysis, and again at the end of enrollment.

4.4 Disposition of study materials

4.4.1 Vaccine and placebo inventory

The investigator will maintain a clinical stock record of all medication dispensed during this study.

4.4.2. Unused medication

All investigational drug materials not used in clinical trials will be returned to the sponsor before or at the termination of the study. Two copies of the completed clinical stock record will be returned under separate cover from the unused medication.

4.4.3 Study data

All data will be kept at ICDDR,B by the principal investigator, and shared with co-investigators at his/ her discretion. Any data that is taken away from the study site (Bangladesh) will be stripped of any identifying information, so that study subject confidentiality will be ensured.

4.5 Investigators and other study personnel

The Public Health Sciences Division (PHSD) at ICDDR,B will be responsible for the conduct of the trial and the principal investigator will be Dr Joseph Bresee, who will be seconded from CDC Atlanta and be based in Bangladesh during the study. ICDDR,B collaborators, particularly Dr Md Yunus from PHSD and Drs John Albert and Tasnim Azim and Ms Leanne Unicomb from the Laboratory Sciences Division (LSD), will participate in the conduct of the study and in overseeing the study operations. Drs Glass and Gentsch will act as a study consultants, and Dr Gentsch's laboratory will participate in testing of specimens for the study. The CVs of collaborators are attached below

International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B)

International Staff:

Professor J. Patrick Vaughan	Director, PHSD, Epidemiologist
Dr John Albert	Interim Director, LSD, Microbiologist
Ms Leanne Unicomb	Virologist, LSD

National Staff:

Dr Mohammed Yunus	Head, Matlab Health Services Programme, PHSD
Dr Tasnim Azim	Immunologist, LSD
Dr Goutam Podder	Assistant Scientist, LSD
Mr J. Chakraborty	Manager, Matlab Community Health Services

Centers for Disease Control and Prevention (CDC), Atlanta

Dr Joseph S. Bresee
Dr Roger I. Glass
Dr Jon R. Gentsch

5 Budget.

5.1 Proposed budget for Phase Two immunogenicity trial

The proposed total budget for the Phase 2 trial is US $82,000. The costs of the Principal investigator will be covered separately. The ICDDR,B is not charging any overhead costs for the administration of the trial. The budget is summarised by WHO categories as follows:

ICDDR,B Proposed budget For The RRV-TV Phase 2 Vaccine Trial.

WHO BUDGET CATEGORY	TOTAL BUDGET US dollars
Personnel	21,200
Supplies	8,000
Equipment	2,000
Animals	None
Patient costs	11,000
Travel	16,000
Other expenses	23,800
GRAND TOTAL	82,000
ICDDR,B will not be charging for overhead expenses.	

Budget is ok. S. Hoi 4/8/97

5.2 Budget Justification For Phase Two.

Personnel:

The PI will be a full-time scientific staff member of ICDDR,B from July Ist 1997 and on secondment from CDC Atlanta. The PI and all other Centre's international staff involved in the trial, such as Dr John Albert and Professor Patrick Vaughan, will be closely involved in both Phases 2 and 3 and they will be paid for by the Centre.

The personnel requested in the budget is the minimum number needed to oversee the day-to-day management of the trial and to maintain the intense followup required, particularly if the trial is to be carried out quickly and efficiently. It is also important that all families and their children in the Phase 2 trial should get excellent attention, so that good foundations are laid for the Phase 3 trial. Participating families must be satisfied with the services offered by ICDDR,B in Matlab.

The medical officer (NOA x1, included under Personnel) and field research officer (GS5 x1; All general staff have been included under "Other Expenditures") would be team leader and deputy respectively, and be full-time throughout the trial. The health assistant would mainly be involved in the large number of household visits required for followup of the vaccinees and the nurse-midwife (GS4 x1) and female attendant (daily paid worker x1) will be working at Matlab hospital for the vaccination sessions and they will also attend to any pertinent requests from families and ill subjects. The laboratory technician (GS4 x1) will be dedicated full-time to this study and be on-call at all times for any matters to do with collecting blood and faecal samples. The speed-boat driver (GS2 x1) will be full-time on this project in order to avoid any conflicts with other requirements for water transport.

Supplies:

These cover the cost of the laboratory analysis of the blood and faecal samples. The funds requested also cover the purchase of all necessary chemicals and materials. The total requirement has to cover samples from approximately 80 or more children, allowing for some dropouts.

Equipment:

Only small items of equipment will be required.

Patient Costs:

Families agreeing to participate in the Phase 2 trial will be brought to the Matlab hospital research centre and vaccinated, following which they will be observed for 2 hours on day 0 and then transported back to their bari. They will then have to be visited on days 4 and 7 for followup observation and faecal specimen sampling. Thus for each of the three vaccine doses, each child will need to be brought into Matlab once and visited at home on 2 occasions. This accounts for the local transport costs. An allowance has also been made for food for the family while they are at Matlab during their day time visit.

International Travel:

Travel funds have been requested for collaborators from CDC Atlanta to visit Dhaka so that they be closely involved in training and supervising the Centre's staff. In addition, allowance has been made for one visit by a Bangladeshi based laboratory scientist to CDC Atlanta to be trained in laboratory procedures and quality control methods.

Other Expenditures:

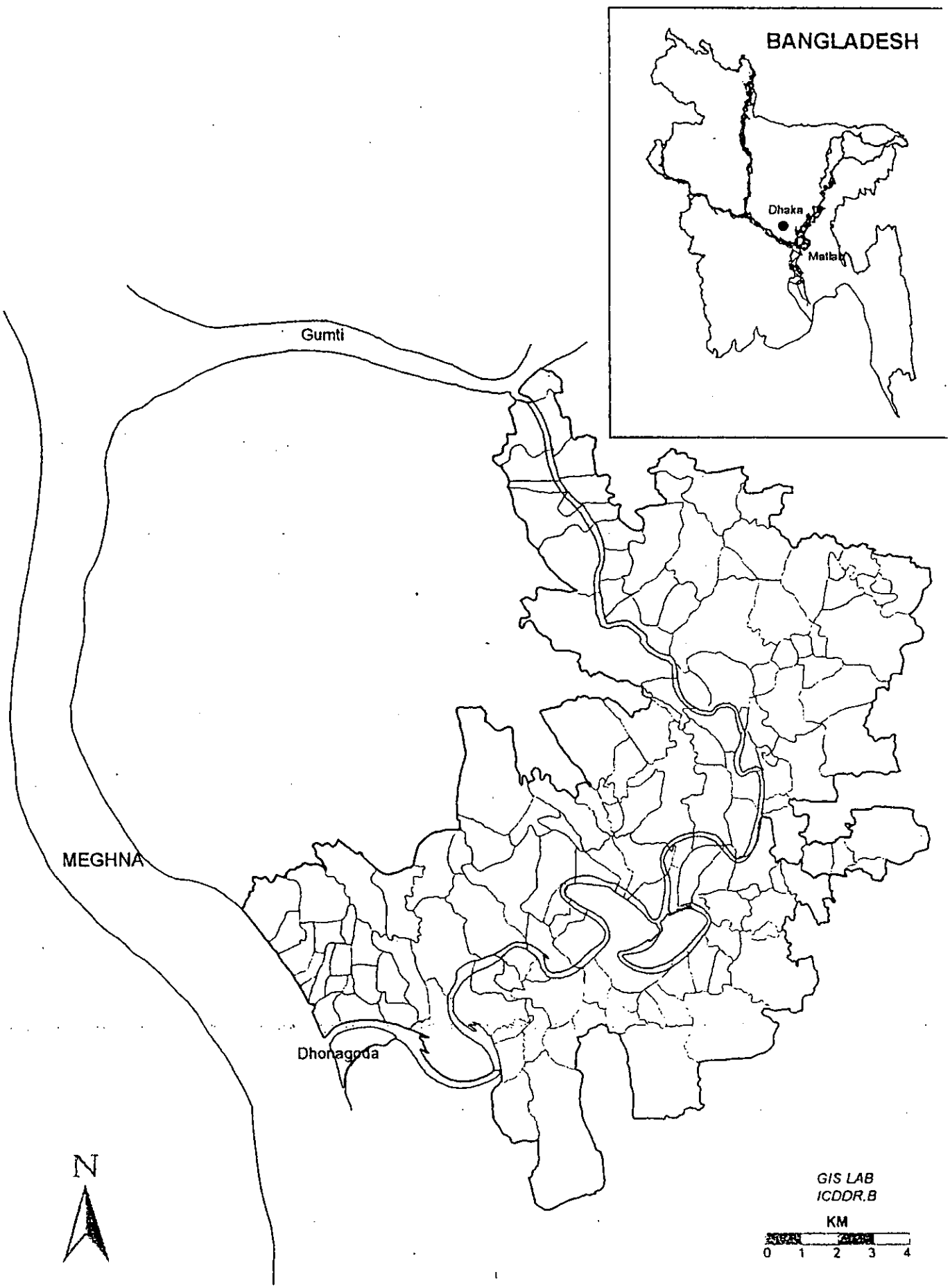
The main item here is for the cost of the field support staff, communications and transport of specimens, including for internationally quality control purposes. International communications will be another considerable expense.

Administrative Costs For The Trial:

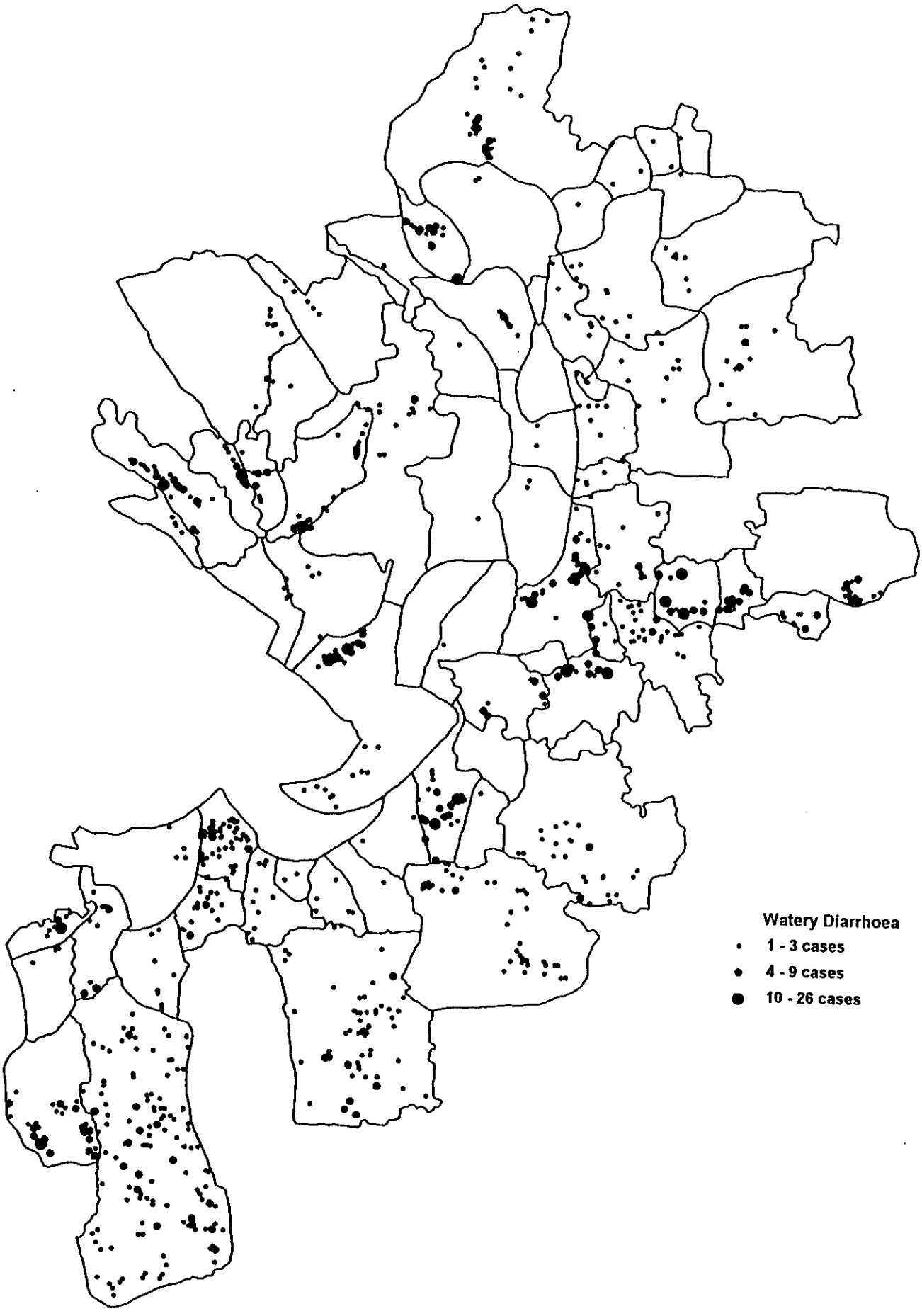
The Centre will waive its normal overhead charge of 31%.

6. Maps of Matlab, Bangladesh.

Map 1: Bangladesh, showing Dhaka and Matlab

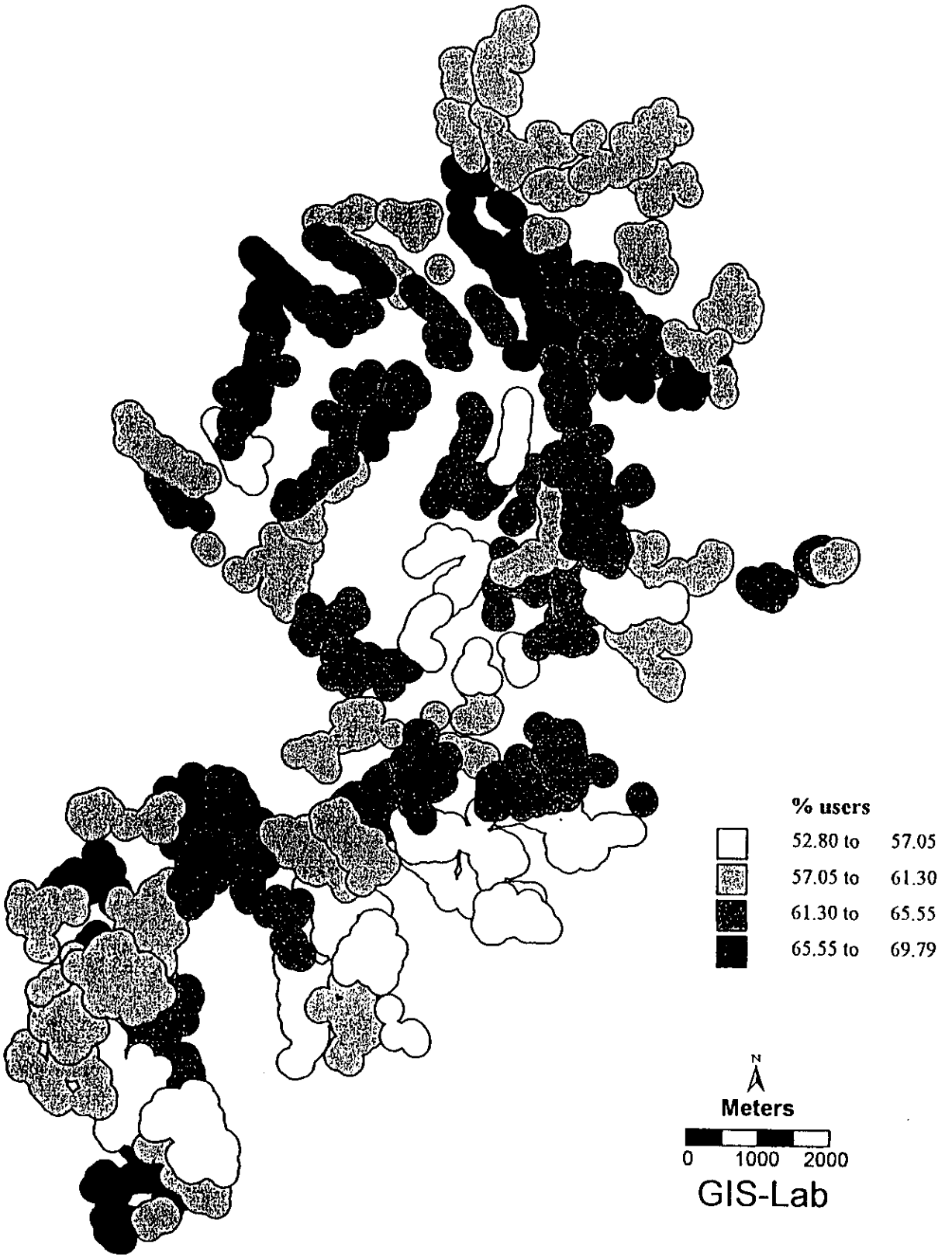


Acute watery Diarrhoea cases 1992



Contraceptive Coverage Rates by CHW areas

Matlab MCH-FP Intervention Area, 1995



7. References

1. Institute of Medicine. The prospects of immunizing against rotavirus. In: **New vaccine development: diseases of importance in developing countries.** Volume 2 ed. Washington, D.C.: National Academy Press, 1986:D-13 to D-13-2.

2. Kapikian AZ, Chanock RM. Rotaviruses. In: Fields BN, Knipe DM, Chanock RM, Hirsch MS, Melnick JL, Monath TP, et al., editors. **Virology.** second ed. v.2. New York: Raven Press, 1990:1353-404.

3. de Zoysal I, Feachem RV. Interventions for the control of diarrhoeal diseases among young children: rotavirus and cholera immunization. **Bull WHO** 1985;63:569-83.

4. Black RE, Brown KH, Becker S, Abdul Alim ARM, Huq I. Longitudinal studies of infectious diseases and physical growth of children in rural Bangladesh. **Am J Epidemiol 1982;115:315-24.**

5. Black RE, Greenberg HB, Kapikian AZ, Brown KH, Becker S. Acquisition of serum antibody to Norwalk virus and rotavirus and relation to diarrhea in a longitudinal study of young children in rural Bangladesh. **J Infect Dis** 1982;145:483-9.

6. Black RE, Merson MH, Mizanur Rahman ASM. A two-year study of bacterial, viral and parasitic agents associated with diarrhea in rural Bangladesh. **J Infect Dis** 1980;142:660-4.

7. Bingnan F, Unicomb L, Rahim Z, Banu NN, Podder G, Clemens J, et al. Rotavirus-associated diarrhea in rural Bangladesh: two-year study of incidence and serotype distribution. **J Clin Microbiol** 1991;29:1359-63.

8. Stoll BJ, Glass RI, Huq MI, Khan MU, Holt JE, Banu H. Surveillance of patients attending a diarrhoeal disease hospital in Bangladesh. **Br Med J** 1982;285:1185-8.

9. Cicirello HG, Das BK, Gupta A, Bhan MK, Gentch JR, Kumar R, et al. High prevalence of rotavirus infection among neonates born at hospitals in Dehli, India: predisposition of newborns for infection with unusual rotavirus. **Pediatr Infect Dis J** 1994;13:720-4.

10. Kilgore PE, Unicomb LE, Gentsch JR, Albert MJ, McElroy CA, Glass RI. Neonatal rotavirus infection in Bangladesh: strain characterization and risk factors for nosocomial infection. **Pediatr Infect Dis J** 1996;15:672-7.

11. Oyejude CO, Fagbami AH. An epidemiological study of rotavirus diarrhoea in a cohort of Nigerian infants: II incidence of diarrhoea in the first two years of life. **Int J Epidemiol** 1988;17:908-12.

12. Stanton B, Silimperi DR, Katun K, Kay B, Ahmed S, Khatun J, et al. Parasitic, bacterial and viral pathogens isolated from diarrhoeal and routine specimens of urban Bangladeshi children. **J Trop Med Hyg** 1989:92-46-55.

13. Bern C, Unicomb L, Gentsch JR, Banul N, Yunus M, Sack RB, et al. Rotavirus diarrhea in Bangladeshi children: correlation of disease severity with serotypes. **J Clin Microbiol** 1992;30:3234-8.

14. Black RE, Merson MH, Huq I, Alim A, Yunus M. Incidence and severity of rotavirus and Escherichia coli diarrhoea in rural Bangladesh. **Lancet** 1981;X:141-3.

15. Salway SM, Nasim SM. Levels, trends and causes of mortality in children below 5 years of age in Bangladesh: findings from a national survey. **J Diarrhoeal Dis Res** 1994;3:187-93.

16. Unicomb LE, Bishop RF. Epidemiology of rotavirus strains infecting children throughout Australia during 1986-1987: a study of serotype and RNA electropherotype. **Arch Virol** 1989;106:23-34.

17. Perez-Schael I et al. RRV-TV efficacy in Venezuela, **World Pediatric Congress, Mexico City, Mexico,** December 1996.

18. Lanata CF, Midthun K, Black RE, Butron B, Huapaya A, Penny ME, et al. Safety, immunogenicity, and protective efficacy of one and three doses of the tetravalent rhesus rotavirus vaccine in infants in Lima, Peru. **J Infect Dis** 1996;174:268-75.

19. Bernstein DI, Glass RI, Rodgers G, Davidson BL, Sack DA, US Rotavirus Vaccine Efficacy Group. Evaluation of rhesus rotavirus monovalent and tetravalent reassortant vaccines in US children. **JAMA** 1995;273:1191-6.

20. Rennels MB, Glass RI, Dennehy PH, Bernstein DI, Pichichero ME, Zito ET, et al. Safety and efficacy of high-dose rhesus-human reassortant rotavirus vaccines-report of the national multicenter trial. **Pediatrics** 1996;97:7-13.

21. Vesikari T et al. Tetravalent-rhesus rotavirus vaccine efficacy in Finland. **Interscience Conference on Antimicrobials and Chemotherapy, New Orleans, LA.**

22. Glass RI, Kilgore PE, Holman RC, Jin S, Smith JC, Woods PA. et al. The epidemiology of rotavirus diarrhea in the United States: surveillance and estimated of disease burden. **J Infect Dis** 1996;174 (suppl):S-5-S11.

23. Migasena S, Simasathien S, Samakoses R, et al. Simultaneous administration of oral rhesus-human reassortant tetravalent (RRV-TV) rotavirus vaccine and oral poliovirus vaccine (OPV) in Thailand. **Vaccine** 1995;13:168-74.

24. Flores J, Perez-Schael I, Blanco M, White L, Garcia D, Vilar M, et al.

Comparison of reactogenicity and antigenicity of M37 rotavirus vaccine and rhesus-rotavirus-based quadrivalent vaccine. **Lancet** 1990;2:330-4.

25. Linhares AC, Gabbay YB, De Freitas RB, Mascarenhas JDP, Oliveira CS, Bellesi N, et al. Immunogenicity, safety and efficacy of rhesus-human reassortant rotavirus (RRV-tetravalent) vaccine in Belem, Brazil. **Presented at the Fifth Rotavirus Vaccine Workshop, Atlanta, Georgia, U.S.A., October 16-17, 1995.**

26. de Francisco A, Chakraborty J. **Matlab maternal and child health and family planning program: briefing information.** ICDDR,B, Dhaka 1995:1-36.

27. Perez-Schael I, Blanco M, Garcia D, White L, Alfonzo E, Crespo I, et at. Evaluation of the antigenicity and reactinogenicity of varying formulations of the rhesus rotavirus-based quadrivalent and the M37 rotavirus vaccine candidates. **J Med Virol** 1994;42:330-7.

28. Rennels MB, Ward RL, Mack ME, Zito ET, US Rotavirus Vaccine Efficacy Group. Concurrent oral poliovirus and rhesus-human reassortant rotavirus vaccination: effects on immune responses to both vaccines and on efficacy of rotavirus vaccines.
J Infect Dis 1996;173:306-13.

29. Coulson BS, Fowler KJ, Bishop RF, Cotton RGH. Neutralizing monoclonal antibodies to human rotavirus and indications of antigenic drift among strains from neonates. **J Virol** 1985;54:14-20.

30. Unicomb LE, Bingnan F, Rahim Z, Banu NN, Gomes JG, Podder G, et al. A one-year survey of rotavirus strains from three locations in Bangladesh. **Arch Virol** 1993;132:201-8.

31. Gentsch JR, Glass RI, Woods P, Gouvea V, Gorziglia M, Flores J, et al. Identification of group A rotavirus gene 4 types of polymerase chain reaction. **J Clin Microbiol** 1992;30:1365-73.

32. Gove S, Pelto GH. Focused ethnographic studies in the WHO programme for the control of acute respiratory infections. **Med Anthropol** 1994;15:409-24.

33. World Health Organization. **Focused ethnographic study of acute respiratory infection.** WHO/CDR 1993.

8. Appendix A

Rotavirus Vaccine Trial Consent Form.

Name of infant:

Date of birth:

DSS Number:

Bari and Village:

Investigators should seek consent from the mother, father or appropriate guardian using the following information, given as an explanation in Bangla, together with a copy of the printed Bangla version.

"Diarrhea is a very serious disease in Matlab, which causes many deaths in young children. These children usually have loose, watery stools and vomiting which may last for several days. As you probably already know, most of these ill children can be treated by the community health workers but some children may die, particularly if they do not receive treatment early with Oral Rehydration Solution (ORS). We are investigating a new vaccine that has a very good chance of preventing some of these illnesses.

The ICDDR,B is working with the World Health Organisation and some scientists in the USA, with the permission of the Government of Bangladesh, to try and find out if this new vaccine will work here in Matlab. To help us do this we are asking if you will allow your child to join this trial. If you agree, your baby will receive the normal 3 injections with vaccines against diphtheria, pertussis and tetanus given by the EPI programme. Your baby will also be given 3 doses of either rotavirus vaccine or solution with no vaccine by mouth as a small amount of liquid. The rotavirus vaccine may cause fever 3-4 days after your child takes it, which is usually mild and lasts less than one day. The vaccine should not cause any other serious problems.

We will also need to follow your child until it reaches about 20 weeks old and we will need to take blood and saliva 4 times to see if the new injections are working well. In addition, we will need to get stool samples 2 times after each dose of vaccine (we can get this from a dirty diaper). If your child does get ill at any time we will look after you well in your village or at Matlab hospital. You do not have to let your child participate in the study. It is completely voluntary."

Do you have any questions?	**Yes**	**No**
Can your baby join in this trial?	**Yes**	**No**

To be completed by trial staff:

I have given the above explanation verbally and given a written version to the mother, father or guardian about enrolling their child in the pneumococcal vaccine trial.

I affirm that they understood my explanation and freely gave their consent.

Signature _____

Name in full _____

Date ___/___/___

Appendix B

Enrollment Log

Study ID#	DSS ID#	Name	Village	Date of birth	Date of enrollment	Informed Consent (✔)
IMM-001				___/___/___	___/___/___	
IMM-002				___/___/___	___/___/___	
IMM-003				___/___/___	___/___/___	
IMM-004				___/___/___	___/___/___	
IMM-005				___/___/___	___/___/___	
IMM-006				___/___/___	___/___/___	
IMM-007				___/___/___	___/___/___	
IMM-008				___/___/___	___/___/___	
IMM-009				___/___/___	___/___/___	
IMM-010				___/___/___	___/___/___	
IMM-011				___/___/___	___/___/___	
IMM-012				___/___/___	___/___/___	
IMM-013				___/___/___	___/___/___	

Appendix C.

Study Record

Study ID Number:

DSS ID Number:

Subject Name:
Birth Date
Enrollment Date

Sample Collection Checklist

Sample	Done? (✔)	Date Collected
Consent Form		
1st serum sample		
1st dose vaccine		
Day 4 stool after 1st dose		
Day 7 stool after 1st dose		
2nd serum sample		
2nd dose vaccine		
Day 4 stool after 2nd dose		
Day 7 stool after 2nd dose		
3rd serum sample		
3rd dose vaccine		
Day 4 stool after 3rd dose		
Day 7 stool after 3rd dose		
4th serum sample		

Date of 1st serum