ttachment 1.
FACE SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 15. 9. 92.

rincipal Investigator Dr Nigar S. Shahid

pplication No. 92-025

Trainee Investigator (if any)

itle of Study : Maternal Immunization with pneumocal Polysaccharide Vaccines

Supporting Agency (if Non-ICDDR,B)

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

rcle 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 NA
(c) Physical risks — Yes No NA
(d) Sensitive questions — Yes No NA
(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)
✓ 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.

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

r. S. Shahid
Principal Investigator

Trainee

A-032068

APPLICATION FOR PROJECT GRANT

1.	PRINCIPAL INVESTIGATORS:		Dr. Nigar Shahid , ICDDR,B Dr. Mark C. Steinhoff, Johns Hopkins Univ
2.	Co-INVESTIGATORS	:	Dr. F. Qadri ICDDR,B Dr. S.S. Hoque " Dr. Abu Eusof " Dr. M. Hossain " Prof. R.B. Sack " Prof. TA Chowdhury, IPGMR Prof. Kohinoor Begum, DMCH
3.	TITLE OF THE PROJECT	:	Maternal Immunization with Pneumococcal Polysaccharide Vaccines
4.	STARTING DATE	:	December, 1992
5.	COMPLETION DATE	:	November, 1993
6.	TOTAL BUDGET REQUIRED	:	US$111,667
7.	FUNDING SOURCE	:	CGVD/USAID
8.	HEAD OF PROGRAM	:	Professor R.B. Sack Associate Director Community Health Division

MPH TD for Dr. Sack

9. Aims of the Project

a. General Aim

To determine the antibody response of pregnant women to immunization with Streptococcal pneumoniae (Spn) or Meningococcal meningitidis polysaccharide vaccine (Nm), and to determine the levels and proportion of each antibody isotype and of IgG subtype which are transferred to the newborn infant.

b. Specific Aims

This proposal will develop needed data regarding the immune response to to bacterial polysaccharide vaccines in a developing country population, including:

1) the isotype and subtype composition of the antibody response to pneumococcus and M. meningitidis (Nm) polysaccharide vaccine in pregnancy;

2) the level and proportion of each subtype of antibacterial IgG antibody which is transferred to the infant;

3) the kinetics in infants of maternally acquired pneumococcal antibody.

4) The levels of pneumococcal and (Nm) antibody levels in breast milk in immunized and unimmunized mothers;

5) the effect of elevated breast milk pneumococcal antibody on oropharyngeal colonization rates of pneumococci in infants.

6) The effect of vaccination on maternal health and pregnancy outcomes will also be assessed.

c. Significance

Bacterial pneumonia accounts for 1.5 million deaths annually in infants and children worldwide being highest in the first few months of life. A high percentage of this is caused by S. pneumoniae (Spn). Although antibiotic treatment for pneumococcal disease is available, its diagnosis is difficult and the organism is fast developing multiple resistance to common antimicrobials. The National Academy of Sciences (USA) has listed S. pneumoniae vaccine as the highest priority candidate in their vaccine priority listing for developing countries. Since this newly developed Spn polysaccharide vaccine has been shown not to be immunogenic to infants it is thought that maternal immunization with this Spn vaccine may have the potential of reducing infant mortality by providing passive immunization during the vulnerable first few months of life.

10. Ethical Implication

The drawing of blood samples is associated with temporary discomfort and occasional bruising at the site. Both vaccines have been licensed by the FDA and given to adults with no reports of serious systemic reactions and a low rate of local symptoms. (see Data Review.) Biological samples will be collected by appropriately trained personnel.

Participants will likely have higher antibody titers to Spn or (Nm) after vaccination. Mothers who receive Spn vaccines will thus be protected against invasive Spn disease. Severe (Nm) disease in adults is rare; therefore the presence of an increased antibody titre will probably be of little benefit to the mother. However, vaccination of mothers with either of these vaccines has the potential to protect their infants from neonatal (Nm) or Spn disease.

B. Background

Pneumococcal disease is among the most common severe bacterial diseases of infants and children in developing regions. It is estimated that 20-40% of the 4 million infant and child deaths due to acute lower respiratory infection **(ALRI)** or pneumonia are caused by S. pneumoniae (Spn), with H. influenzae and viral organisms contributing a smaller fraction (1). **ALRI** mortality rates are highest in the post-neonatal period (2), and Spn is known to be an important causative agent in this age group (3). In Bangladesh, 61% of all pneumonia deaths in children under 5 years of age occur in infants under 6 months of age (4). A recent study of pneumonia in infants from the Gambia showed that Spn caused 20% of all cases and at least 13% of the cases where among 0 to 5 month old children (5). In addition, rates of septicemia, meningitis and other severe Spn infections are high during early infancy. A recent surveillance project for bacterial meningitis in the U.S. showed that Spn. was an important cause of meningitis and other severe infections in the first 5 months of life, with annual incidence rates of 38-98/100,000 (6).

Antibiotic treatment of pneumococcal disease is effective; however, in regions with high infant mortality rates, primary care services are often poorly developed or under utilized, especially for newborn infants, and pneumococcal resistance to antimicrobial therapy is an increasing problem (7). For these reasons, the National Academy of Sciences has listed S. pneumoniae vaccine as the highest priority candidate in their vaccine priority listing which considered potential health benefits for developing countries (1). Given the success of immunization programs worldwide in delivering vaccines, this is an opportune time to evaluate the concept of adding an existing vaccine which has the potential for mortality reduction.

Protection against pneumococcal disease is dependent on serum antibody to the bacterial polysaccharide capsule. A vaccine which contains the polysaccharides of the 23 most invasive serotypes of S. pneumoniae has been licensed in the U.S. since 1983 for use in high-risk populations. This product is safe, stable and of proven efficacy against invasive Spn disease in high-risk adults and children older than 2 years of age (8). However, studies of its immunogenicity in infants have shown that some serotypes are poorly immunogenic before the age of two (9-12). Unfortunately, most childhood **ALRI** deaths in developing countries occur before two years of age (2). In Papua New Guinea, for example, 8% of all childhood pneumonia deaths are in newborn infants, and 35% occur in 1 to 5 month old infants (13). Newly developed Spn polysaccharide vaccines which are conjugated to proteins are immunogenic in infants. If proved to be safe and effective, these vaccines will be widely used for infant immunization. However, the proposed conjugate vaccine will be limited to 5-7 serotypes and thus will be limited in its potential efficacy in the highest risk groups. In addition, a conjugate vaccine is unlikely to afford significant protection until after the second dose in received at 2-4 months of age.

Maternal immunization with the licensed 23-valent Spn polysaccharide vaccine has the potential to provide passive protection to infants during the vulnerable first few months of life. A single study has shown that adult women respond to the licensed pneumococcal vaccine and transfer antibody to their infants (14). Maternal immunization to protect both mother and newborn infant against tetanus is an established part of the EPI program. This proposed project will provide the data on placental transmission of pneumococcal antibody which will provide the biological rationale for further studies of the efficacy of maternal immunization.

C. Data Review

Pneumococcal Vaccine

A few studies have investigated placental transfer of Spn antibody in unimmunized women. An assessment of maternal and cord blood levels of antibody to pneumococcal serotype 7F showed that cord levels were 55% of maternal levels in 30 unimmunized women (15). Grunebaum, et al, assessed antibody levels for 12 pneumococcal serotypes in 12 maternal-cord serum pairs from unimmunized subjects. The ratio of cord to maternal antibody levels ranged from 9% to 97% for the 12 serotypes , with a mean of 56% (16). We are aware of only one previous study of pneumococcal immunization of pregnant women. Vincent-Ballereau et al. immunized 37 women in Burkina Faso with pneumococcal vaccine and tetanus toxoid and 17 women with tetanus toxoid alone during their eight month of pregnancy (14). The pneumococcal antibody was determined by a non-standard **ELISA** assay, and the statistical analysis was incomplete. Women who received pneumococcal vaccine had slightly higher titers of pneumococcal antibody than those who had not received the vaccine. Anti-pneumococcal antibody levels in cord blood were similar to those in maternal blood for all maternal-infant pairs. Cord levels of pneumococcal antibody were 20-90% higher in the infants born to immunized women than in those born to women in the control group. An evaluation of immunization with pneumococcal vaccine has been planned, and will be initiated soon on the Navaho reservation in the U.S. (Santosham, personal communication).

As part of an evaluation of the 14-valent Spn vaccine in adults in Papua New Guinea, 354 pregnant women received vaccine or placebo (17). A retrospective analysis of the experience of the mothers and their infants showed no evidence of an adverse effect on pregnancy outcome; the frequency of abortions, stillbirths and congenital defects was similar in the two groups of women. There was a 35% reduction in pneumonia episodes among the infants of the vaccinated women, when compared to infants of placebo recipients. In addition, a reduction of 32% in pneumonia episodes was noted in children younger than 17 months at the time of their mother's immunization. The authors suggest this latter group was protected through breast feeding. Other vaccines consisting of bacterial capsular polysaccharide have been evaluated in pregnant women (18,19,20,21,22,23).

Group B Streptococcus Vaccine

Immunization of 40 pregnant women with the polysaccharide of Type III group B streptococcus has been shown to be safe. Two thirds of the mothers had a significant increase in antibody, and 80% of the antibody was transferred to their infants. Of the infants born to women who responded to the vaccine, 64% had protective levels of antibody up to 3 months of age (18).

H. Influenzae Type b Vaccine

Studies of a capsular polysaccharide vaccine for H. influenzae type b ((Hib)) in over 200 pregnant women have indicated that immunization is safe and effective in increasing the level of the mother's serum antibody, at least 30% of which is transferred to the infant (26,27). Infants of immunized mothers had titers of (Hib) antibody 100 times higher than infants born to unimmunized mothers (26). In addition, breast milk IgA antibody against (Hib) was increased 34-fold in immunized women (28). Preliminary results of maternal immunization with an (Hib) protein conjugate vaccine indicated that higher levels of (Hib) antibody were seen compared to that reported with (Hib) polysaccharide vaccine (22).

Meningococcal Vaccine

An evaluation of 51 pregnant Brazilian women immunized with the N. meningitidis type A and C polysaccharide vaccine (Nm) during an epidemic of meningococcal disease showed no adverse effects on the fetus or on pregnancy outcome (23). Moreover, immunization of the 51 infants with meningococcal vaccine at 6 months of age resulted in a normal antibody response, indicating that immune tolerance in the infants was not a consequence of maternal immunization (23).

A recent symposium reviewed the status of maternal immunization for protection of infants and concluded that immunization with polysaccharide vaccines was likely to result in infant protection (24-25). If maternal immunization provides protective antibody levels in early infancy, the effect on infant mortality of the addition of a licensed pneumococcal vaccine to an established EPI program of maternal immunization with tetanus toxoid will be evaluated. If proven effective, the policy implications of maternal immunization are significant, even after protein conjugate Spn vaccines become available for routine use in infants, because maternal immunization has the potential to close the infant's window of vulnerability before the age when active immunization results in protective levels of antibody.

D. Experimental Design and Methods

Study design

The study will be a prospective randomized double-blind controlled trial comparing Spn polysaccharide vaccine with N meningitidis type A and C vaccine ((Nm)) in healthy pregnant women. No placebo immunization will be used; all participants will receive a vaccine known to be immunogenic and safe. These vaccines have also been used in pregnant women without evidence of toxicity to mother or child.

Approximately 60 healthy pregnant women in the third trimester of pregnancy will receive either Spn vaccine or (Nm) simultaneously with routine tetanus toxoid during prenatal immunization at 32-33 weeks of gestation. The Expanded Program of Immunization (EPI) in Bangladesh recommends that tetanus toxoid (TT) be administered between 16-32 weeks. The interval between the two doses is 4-6 weeks and the second dose must have been administered 4 weeks prior to the expected date of delivery. Women will be recruited at 32-33 weeks with informed consent. Dates of last menstrual period will be noted and corroboration will be made by physical examination.

Study Population and recruitment of Participants

The study will be a prospective randomized double-masked controlled trial comparing Spn polysaccharide vaccine with Nm type A and C in healthy pregnant women. Study participants will be recruited from mothers in Dhaka who are planning or registered to deliver in maternity units of our co-investigators. Vaccination will occur in the third trimester of pregnancy as per schedule noted above. Women who have had Spn or Nm immunization or known pneumococcal or meningeal disease within the past 5 years, who are <15 or >40 years of age, who are known to have had complicated pregnancies, or who are at risk for premature labor will be excluded. After giving witnessed written consent, women will be randomized to receive one of the vaccines (Spn or Nm) using the permuted table of randomization. The code will be maintained with a disinterested third party and will be broken when the protocol is completed.

Vaccines

The vaccines will be supplied in pre-filled syringes identified by code only. The Spn vaccine is commercially available and licensed by the U.S.F.D.A. It consists of 25ug of the purified capsular polysaccharide of 23 serotypes of Spn, and is among the safest vaccines in current use with <1% rate of severe local reaction or fever in adult recipients (8). We will use an (Nm) polysaccharide-protein vaccine simiolar to the one used in pregnant women (23). The licenced vaccine contain 50 ug of purified capsular polysaccharide of each of the serotypes. Both are safe and immunogenic products (16-28).

Post-Immunization Monitoring

Each participant will be given a questionnaire regarding possible local and systemic side effects at 24, 48 & 72 hours after immunization. Data on pregnancy outcome will be obtained on all women in both groups. Information regarding illness during the subsequent pregnancy and delivery, estimated gestational age (as described below) of the infant at delivery, complications of delivery, birth weight and neonatal illnesses will be recorded. We will arrange for follow-up of the mothers and infants to ensure collection of the specimens. Routine medical care routine immunizations will be provided for the infants during the period of the study.

The gestational age of the infants will be estimated by two methods. We will ask the mothers the date of the first day of the last menstrual period and calculate the duration of gestation from that date to the date of birth. In addition, each child will be examined within 48 hours of age by a physician who will use the method of Ballard to assess the neuromuscular and physical maturity of the infant. The Ballard technique assigns a standard score for neuromuscular and physical maturity and allows estimation of gestational age (29).

The dates of the last menstrual period, the dates of immunization of the mother and of delivery of the infant will be recorded and the gestational age and post-immunization interval will be calculated for each child. This will allow us to assess the effect of gestational age and interval from immunization to delivery on cord antibody levels.

Serum, Breast Milk and Culture Specimens

All mothers will have serum collected immediately before and 1 month after immunization, and at the time of delivery. Infant cord blood will also be collected at the time of delivery. Colostrum will be collected within a day of birth, and breast milk will be obtained at 3,7,15,28 days after delivery and then at 3 and 5 months. The infants will have serum samples collected at the end of 1,3 and 5 months. Nasopharyngeal cultures for Spn will be obtained every fortnight through the age of five months. The maternal pre-and post-immunization sera will be used to assess the immunoglobulin isotype and subtype of the maternal antibody response. The maternal-cord serum pairs will provide data on the transplacental transfer of antibody. The infants' sera will be used to determine the half-life of the passively acquired antibody. The results of the nasopharyngeal Spn cultures of the infants will be correlated with the presence and level of specific breast milk antibody and with infant serum antibody level.

Antibody Assays and Cultures

Serum will be separated and aliquoted; IgG antibodies to 6 Spn serotypes (3 and 6 and to 4 other types common serotypes in Bangladesh) and to Nm antigen will be measured in pre-and post-immunization samples simultaneously by enzyme-linked immunosorbent assay (ELISA) (see appendix I, ref. 30). The assay for pneumococcal antibody incorporates a C polysaccharide absorption step, to improve specificity of the assay. Pneumococcal types 6 and 3 have been selected for initial analysis because they are poorly and highly immunogenic, respectively in infants and adults. There is as yet no information on the serotypes of pneumococci producing morbidity in Bangladesh; as such ICDDR,B is currently stocking all pneumococcal strains for identification of serotype. ICDDR,B will also assay the antibody levels to 4 additional locally common Spn serotypes, after they have been determined. Dr. Siber's laboratory has extensive experience with the Spn ELISA assay, and will be analyzing the sera from the Navaho maternal immunization study. The analysis of both sets of sera in the same laboratory allows us to compare the responses of Navaho and Bangladeshi women. Colostrum and breast milk samples will be centrifuged, separated and aliquoted, then analyzed by ELISA for IgA antibody specific for Spn and (Nm) using a

modification of a previously reported methodology (30). Nasopharyngeal cultures for isolation and identification of Spn will be processed by the microbiology laboratory of ICDDR,B in Dhaka, which has previous experience in these standard procedures (31).

The ICDDR,B laboratories have experience in ELISA assays, and it will be possible to provide reagents and protocols for the pneumococcal antibody ELISA. This technology transfers will also be a first step toward additional studies of pneumococcal vaccines in Bangladesh.

Bacteriologic methods

Nasopharyngeal Swabs will be collected every 2 weeks. Two swabs will be obtained each time. One will be inoculated on sheep blood agar (with gentamycin) to identify S. pneumoniae using standard methods (32).

The second throat swab specimen will be used for the detection of pneumococci using the mouse virulence test(33) (as in Appendix V).

Sample Size

(See Appendix II for formulae and calculations used to determine sample size.) Nineteen subjects are required in each of two groups for an 80% power to detect a difference of 45% or greater in the proportion of women who respond to the vaccine with protective levels or $\geq$ 4 fold increase in titer. We will recruit at least 30 women in each group to allow for dropouts, delivery outside of the hospital, premature delivery and other losses.

In developing countries, carriage of Spn is much more common than in developed countries. Studies of newborn acquisition of Spn in Papua New Guinea showed that the mean age of acquisition was 17.1 days (range 1-80 days), with a mean duration of 96 days (range 5-290 days); 96% of the infants studied were colonized by 40 days of age and 100% by 80 days of age. Given our sample size of 19 Spn vaccinated subjects and 25 (Nm) vaccinated subjects, and assuming 90% colonization with a vaccine serotype, this study has an 80% power to detect a true difference in colonization rates of 45% (alpha = 0.05, beta = 0.20). In other words, if (Nm) immunized infants have a Spn colonization rate of 90%, and the Spn immunized infants have a colonization rate of less than 55%, this study has 80% power to detect this difference. Thus the study design has adequate power to detect a substantial reduction in carriage rate.

Data Analysis

Because antibody titers are not normally distributed, we will log-transform this data before analysis. Antibody concentrations below the lower limit of the assay will be assigned a value of one half the lower limit. The geometric mean titers of antibodies to selected serotypes of Spn and (Nm) will be compared between maternal and infant groups using the two-sided t-test and appropriate non-parametric tests. The mean proportion of total antibody and of each Ig subtype which is transferred across the placenta will be calculated for each vaccine. The proportion of infants in each group whose anti-Spn antibody level exceeds 300ug/ml of antibody nitrogen or whose anti-(Nm) antibody exceeds 0,15 and 1.0 ug/ml, (the levels associated with long-term protection respectively), will be compared using the Chi-square and two-sided Fisher's exact probability tests. The mean levels, fold-increase an antibody levels and the proportion of the isotypes and IgG subtypes of Spn and (Nm) antibody will also be compared. The relationship of gestational age at immunization, gestational age at delivery and the interval between the two on antibody titre and isotype and subtype composition will be determined.

We will compare the proportions of infants who are colonized with Spn vaccine serotype between Spn and (Nm) vaccinees, and assess correlation between breast milk and serum antibody and carriage of specific serotypes.

Time Schedule

It is estimated that a full year will be required to complete recruitment of mothers, follow-up of infants for 5 months and laboratory studies.

Local approvals, hiring of field staff	Dec 1992 - Jan 1993
Recruiting of subjects	Jan - May 1993
Follow-up of infants	Jan - Sept.1993
Laboratory analysis	June- Sept.1993
Report and publication writing	Oct.- Nov. 1993

Rationale for Bangladesh Site

Because the International Center for Diarrheal Disease Research, Bangladesh (ICDDR,B) has extensive experience in vaccine evaluations, it is proposed as the site for this study. Dr. R.B. Sack is resident in Dhaka serving as the Associate Director of the Divisions of Community Health and Laboratory Sciences at the ICDDR,B providing supervision and a communications role. ICDDR,B has greatly encouraged collaboration with National scientists and institutes in Bangladesh; this protocol will strengthened this effort. If the vaccines are shown to produce protective levels of antibody in infants, it would be possible to consider a large scale field efficacy trial in the Matlab field area of the ICDDR,B. Matlab was the site for the recent large oral cholera vaccine efficacy study, and clearly has the capacity for a maternal immunization study.

Confidentiality

All participants will be assigned a study number at the time of recruitment, which will be used for all data analysis. Forms and records with personal identifiers will be kept in a secure file cabinet in Dhaka.

G. Collaborators

Obstetricians in charge of units where the deliveries will take place will be collaborators. Dr. Nigar Shahid is the P.I. at ICDDR,B, Dhaka and Dr. M. Steinhoff at Johns Hopkins University, Baltimore. Dr. Shahid will be responsible for all steps in the protocol. Which includes recruitments of women follow-up, immunization, collection and processing of samples at ICDDR,B. Dr. R.B. Sack will supervise all stages of the project including the laboratory assays at Dhaka. The Department of International Health at Johns Hopkins School of Public Health has a long history of successful collaboration with scientists at ICDDR,B.

H. Consultants, Contracts

The DANA FABER Cancer Institute (laboratory of Dr George Siber) will be our reference laboratory for antibody assays for Spn and (Nm). His laboratory has long experience with these assays and has collaborated with Johns Hopkins University in vaccine evaluation for many years.

I. Duties of Co-investigators

Dr. Ferdausi Qadri will supervise the performance of the ELISA antibody assays. Drs. A. Eusof and S.S. Hoque will help in the follow-up and clinical assessment of subjects.

Dr. M. Hossain will perform the mouse inoculation test for pneumococci. Professors T.A. Chowdhury and Kohinoor Begum will help recruit mothers for enrolling into the study.

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PROJECT TITLE: Maternal Immunization with Spn and (Nm) vaccines
PROJECT DATES: December 1, 1992 to November 30, 1993

PERSONNEL	% EFFORT Inst	Base Salary	FRINGE Benefits	TOTALS
Mark Steinhoff,MD	10%	$7,899	$2,182	$10,081
R Bradley Sack	10%	$0	$0	$0
TBN Secretary	5%	$1,005	$278	$1,283
SUBTOTAL		$8,904	$2,460	$11,364
CONSULTANT				$0
EQUIPMENT				$0
SUPPLIES				
Office supplies			$500	
Immunization Supplies @$16x60 patients			$960	
Lab supplies			$500	
Pneumococcal typing sera			$3,763	
SUBTOTAL SUPPLIES				$5,723
TRAVEL				
FOREIGN Per Diem @$135 per day x 20 days			$2,700	
2 trips/Balto-Dhaka $4,612 per trip			$9,224	
SUBTOTAL TRAVEL				$11,924
OTHER EXPENSES				
Photocpying			$400	
Shipping Costs			$1,500	
Telephone/FAX			$1,720	
Federal Express/DHL			$720	
SUBTOTAL OTHER EXPENSES				$4,340
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD				$33,351
CONSORTIUM/CONTRACTUAL/SUBCONTRACT COSTS				
ICDDR,B:				
DIRECT COSTS		$42,837		
INDIRECT COSTS		$11,572		
TOTAL ICDDR,B		$54,409		
Dana Farber Cancer Institute:		$16,111		
SUBTOTAL CONSORTIUM/CONTRACUAL/SUBCONTRACT COSTS				$70,520
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD				$103,871
INDIRECT COSTS @23.375% (MTDC)				$7,796
TOTAL COSTS FOR INITIAL BUDGET PERIOD				$111,667

Personnel (ICDDR,B)

			US$
Dr. Nigar Shahid - PI	25%	NO-C	3359
Dr. Ferdousi Qadri - C.I	5%	NO-C	No cost
Dr. Syed Samiul Hoque - C.I	5%	NO-A	No cost
Dr. Abu Eusof - C.I	10%	NO-C	No cost
Dr. M. Hossain -C.I (6M)	10%	NO-A	381
Prof. TA Chowdhury-C.I	-	-	No cost
Prof. Kohinoor Begum - C.I	-	-	No cost
Mr. M. Nurur Rahman - C.I	5%	GS-6	210
Field Supervisor (1)	40%	GS-5	2375
Health Assistant (2)	100%	GS-3	4740
Field Worker (5)	100%	-	4500
Research Officer (1)	100%	GS-V	3790
Senior Technician Vet (1) 6m	20%	GS-V	420
Total:			$19,775

Lab supplies

One dram vials	(15 gross)	700	
10 ml vials	(1 gross)	100	
Pasteur Pipette	(15 gross)	50	
Multi-channel pipette(8)	(2 pcs)	700	
Finne Pipette	(3 pcs)	750	
Micro-titre pipette	(600 pcs)	1,300	
Micro-titre tips	(1500 pcs)	1,300	
Human IgG conjugate	(10 ml)	900	
Human IgA conjugate	(10 ml)	900	
Substrate buffer	-	100	
	Sub-total		$6,800

COST FOR NASOPHARYNGEAL CULTURES AND MOUSE ASSAYS

Spn Culture	$4.00 x 60 x 10 x 2	$4,800		
Mouse assay	$0.77 x 60 x 10	$462		
			$5,262	
Travel				
Local (Tk.1500/week)		$2,000		
			$2,000	
Other cost				
Telephone/ Fax		$500		
Shipping cost		$1,000		
Photocopy		$300		
Cost for collecting cord blood		$200		
Mis.		$500		
			$2,500	
	Sub Total			$36,337
Indirect cost at the rate of 31%				$11,265
		TOTAL		$47,602
Travel International		$3,507		
Dhaka - USA		$3,300		
Per diem	$110x30			
	Sub Total			$6,807
	GRAND TOTAL			$54,409

International Centre for Diarrhoeal Disease Research, Bangladesh
P.O. Box 128, Mohakhali, Dhaka-1212
Bangladesh

Consent form (for pneumonia vaccine protocol)

Pneumonia is a common problem in children throughout the world. Mortality due to pneumonia is highest during the first six months of life. Research has shown that a high proportion of deaths are caused by S. pneumonia (Spn) and Meningococcal meningitidis (Nm). Treatment in many cases may not be effective as the organism is fast gaining resistance to common antibiotics. Diagnosis of pneumonia is also difficult. It is hypothesized that babies born of mothers who are vaccinated at 32 weeks of pregnancy with Spn or Nm vaccine will have immunity to these organisms during the vulnerable first few months of life. This has previously been shown in the instance of tetanus and as such tetanus toxoid is now given routinely to pregnant mothers during the last trimester of pregnancy.

We will greatly appreciate your participation in this study. One of the two vaccines will be administered to you during the end of eighth month of your pregnancy. 2ml blood will be drawn from your vein at the time of vaccination and one month later. You will be requested to give another sample of blood (2ml) when your child is delivered. You will also be requested to provide samples of breast milk soon after birth and when your child is 1,3 and 5 months.

Cord blood will be obtained at delivery in collaboration with the clinic staff. We will perform a thorough physical examination of your child between day 1-2 of birth and collect a throat swab for culture. 1-2ml of blood will be drawn from the baby's vein when he/she is 1 month and 3 month old. These body fluids are required for assessing the extent to which the vaccine may be effective. We will visit you on alternate weeks for 5 months and enquire about the well-being of your new-born. Throat-swab cultures will be collected at each visit. The treatment of diarrhoea and respiratory illness will be provided by us.

If you decide to participate in the study please put your signature/ thumb impressing in the following place.

Signature of Subject : _____

Signature of Husband : _____

Signature of Investigator: _____

Date enrolled : _____

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ
পি ও বক্স ১২৮, মহাখালী, ঢাকা - ১২১২
বাংলাদেশ

সম্মতি পত্র (নিউমোনিয়া টিকা- প্রকল্প)

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ গত তিনদশক ধরে ডাইরিয়া রোগ প্রতিরোধ ও নিরাময়ের জন্যে গবেষণা ও চিকিৎসা প্রদান করে আসছে। তারই ফলশ্রুতিতে মুখে খাওয়ার স্যালাইন আবিষ্কার এবং ব্যবহারের মাধ্যমে বহু শিশুর প্রানরক্ষা হয়েছে। কিন্তু ডাইরিয়ার সাথে নিউমোনিয়ার জটিলতার কারণে অনেক মৃত্যু এড়ানো সম্ভব হয়নি। তাই আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র নিউমোনিয়ার গবেষণা প্রকল্প গ্রহন করতে চলেছে।

নিউমোনিয়া বিশ্বের সর্বত্রই শিশুদের একটি সার্বিক সমস্যা। জীবনের প্রথম ছয়মাস বয়সের মধ্যে এ রোগের মৃত্যুর হার সবচেয়ে বেশী। গবেষণায় দেখা গেছে যে, মৃত্যুর একটা বড় অংশ স্ট্রেপটোকক্কাস নিউমোনিয়া (SPn) ও মেনেংগোকক্কাল মেনিনজাইটিডিস (Nm) দ্বারা সংঘটিত হয়। এ রোগের জন্য ব্যবহৃত এন্টিবায়োটিক গুলোর কার্যকরীতা দ্রুত নষ্ট হয়ে যাওয়ার জন্যে অনেক ক্ষেত্রেই চিকিৎসা ফলপ্রসু হয়না। নিউমোনিয়া রোগ নিরুপন করাও অসুবিধাজনক। বহিঃবিশ্বে সম্প্রতি গর্ভাবস্থায় এস পি এন (SPn) ও এনএম (Nm) টিকাগ্রহনকারী মায়ের সন্তান ভূমিষ্ঠ হওয়ার কয়েক মাস পর পর্যন্ত এ রোগের জীবাণুর প্রতিরোধ ক্ষমতা থাকবে বলে ধারনা করা হচ্ছে। এই ভাবে ধনুষ্টংকারের জন্যে মায়ের গর্ভকালের শেষের তৃতীয়াংশে টিটেনাস টক্সয়েড প্রদান করে ফল পাওয়া গেছে।

আমরা আপনাকে এ গবেষণায় অংশ গ্রহনের জন্য সাদর আহ্বান জানাবো। অর্থাৎ আপনার, গর্ভাবস্থায় আপনাকে যেকোন একটি টিকা দেয়া হবে। টিকা দেয়ার সময় এবং টিকা দেয়ার একমাস পর দুই এম এল (2 ml) রক্ত আপনার শিরা থেকে নেয়া হবে। সন্তান প্রসবের সময় আমরা আপনাকে দুই এমএল (2 ml) রক্ত দেয়ার জন্য অনুরোধ জানাবো। শিশুর জন্মের অব্যবহিত পরে এবং ১, ৩ ও ৫ মাস বয়সের সময় আপনাকে বুকের দুধের নমুনা দিতে অনুরোধ করবো।

ক্লিনিকের কর্মচারীদের মাধ্যমে সন্তান প্রসবের সময় নাড়ীর রক্ত সংগ্রহ করা হবে। শিশুর জন্মের এক বা দুই দিনের মধ্যে আমরা শিশুর সম্পূর্ণ শারীরিক পরীক্ষা করবো এবং গলার লালা কালচার করবো। শিশুর এক ও তিনমাস

মাসিক কিস্তির হার হবে ১-২ মিঃ লিঃ (২য়ে) রক্ত সংগ্রহ করা হবে। চিকিৎসা কার্যকারিতা পরীক্ষার জন্য এই রক্ত পরিক্ষা করা করা হবে। এক সপ্তাহ অন্তর অন্তর পাঁচ মাস পর্যন্ত আপনার কিডনিতে আমরা চেষ্টা করে যাবো। প্রতিবারই আপনার কিডনি ভালোর দিকে হচ্ছে কিনা পরিক্ষার জন্যে রক্ত সংগ্রহ করবো। শ্বাস ও ডায়রিয়া হওয়ার জন্যে আমরা চিকিৎসা প্রদান করবো। যদি আপনি এ গবেষনায় অংশ গ্রহনে সিদ্ধান্ত গ্রহন করেন তবে নীচে আপনার দস্তখত / টিপ সই দিন।

অংশগ্রহনকারীর দস্তখত : _____

সাক্ষীর দস্তখত : _____

গবেষকের দস্তখত : _____

অংশগ্রহনের তারিখ : _____

Abstract Summary for ERC:

1. Death rates with pneumonia is highest during the first six months of life and antibiotic resistance to the organisms causing childhood pneumonia is increasing rapidly. Various ways to combat infant deaths is being considered. It was observed that vaccinating very young children with pneumonia vaccine did not produce acceptable levels of serum antibody so that infection to the organisms may be avoided. This project is aimed at examining whether vaccinating mother against pneumonia during pregnancy provides protection to newborns. As in the case of tetanus the thought of providing antibodies to infants from vaccinated mothers is being considered. This project will require the cooperations of pregnant mothers and in turn their new borns. Voluntary consent will be obtained both from mothers and their husbands.

2. Mothers will be vaccinated either with **Streptococcal Pneumonia** or **Meningicoccal meningitidis** polysaccharide vaccines. Both vaccines have proven to be safe causing little or no side effects.

3. Mothers suffering from headaches, common cold etc. will not be enrolled Diabetics, mothers with known history of difficult previous child birth will not be enrolled.

4. The study will be a randomized double blind trial. All information will be coded and kept under lock and key.

5. The project field staff coming in contact with mothers and infants will be females. Female workers will help in collection of breast milk, cord blood etc.

   a. A written informed consent will be administered to both the mother and her husband.

   b. Other than the type of vaccination being administered no information will be withheld from the subjects.

   c. There is minimum potential risk to the subjects. Treatment will be given for respiratory infectious and diarrhoeal diseases.

6. The initial interview will take 15-20 min and will be done at the anti-natal clinics where the mothers will be enrolled. All subsequent interviews will be done at home and will take 5-10 minutes.

7. There is minimum risk involved and biological samples will be collected with appropriately trained personnel. This project will provide information on placental transmission of pneumococcal antibody which will provide biological rationale for further studies on the efficacy of maternal immunization.

8. Activities involve the use of obstetrical records, mother and infant sera, cord blood, colostrum and breast milk samples and throat swab specimen.

APPENDIX 1

STUDY PROCEDURES FOR MOTHERS

Gestation	32W	36W	40W	Birth	1M	2M	3M	4M	5M
Vaccine	V	-	-	-	-	-	-	-	-
Serum	S	S	-	S	-	-	-	-	-
B/Milk*	-	-	-	B	B	-	B	-	B

* Colostrum; day 3, 7, 15 also breast milk will be collected.

Vaccine - either Spn or (Nm)

APPENDIX 1A

STUDY PROCEDURES FOR INFANTS

Weeks after birth

	Birth	2	4	6	8	10	12	14	16	18	20	22
Serum	Cord		S				S				S	
T/Swab	x	x	x	x	x	x	x	x	x	x	x	x

* Colostrum, day 3, 7, 15 also

APPENDIX II - Calculation of Sample Size

Sample size required to detect the specified differences, with a power of 80% and a statistical significance of 0.05 (alpha=0.05, beta=0.20):

A true difference of 45% (5% vs. 50%) or greater in the proportion of women who respond to the vaccine with putative protective levels or $\geq$4 fold increase in titer.

N = 19 in each group; using the formula:

$$N = \frac{[Za\sqrt{2(P_1+P_2/2)(1-(P_1+P_2/2)} + Zb\sqrt{P_1+(1-P_1) + P_2(1-P_2)}]^2}{(P_1-P_{2)2}}$$

Where:

P_1 = % of A vaccinees seroconverting

P_2 = % of B vaccinees seroconverting

Za= Z value for alpha

Zb= Z value for beta

(formula from Meinert C, Clinical Trials, Oxford 1986:82,84)

APPENDIX III

I Serum sample

Subject	Time	No.	Where drawn
a. Mothers (5 ml-IV)	At vaccination	60	Antenatal clinic
	1M Post Vacc	60	"
	At delivery 60		Hospital
	Total samples	180	
b. Infants (2ml-IV)	Cord	60	Hospital
	Im	60	ICDDR,B Clinic
	3m	60	" "
	5m	60	" "
		240	

Total - 420 serum samples

II Breast Milk Samples (5ml)

Colostrum	1-2d	60	Hospital
Breast Milk	3d	60	Hosp/Home
"	7d	60	Home
"	15d	60	"
"	28d	60	"
"	3m	60	"
"	5m	60	"
	Total:	420	

Total - 420 milk samples

III Nasopharyngeal (NP) cultures of infants
Intervals - at birth and every fortnight through age 5 months
Total NP cultures - 60 X 12 samples = 720

APPENDIX IV

Assays to be done

420 Sera Elisa ICDDR,B & Dr. Siber'S lab
IgG to Spn serotypes 3 & 6 plus 4 common Bangladesh serotypes
IgG to (Nm) polysaccharide -

420 Colostrum & Breast milk ICDDR,B & Dr. Siber's lab
Elisa
IgA to Spn - same 6 pneumocci serotypes (see above)
IgA to (Nm)

720 infants nasopharyngeal swabs - ICDDR,B
Indentification only of Spn

APPENDIX V

Mouse Virulence Test - Since the pneumoccus is extremely virulent for the mouse it can be isolated from mixed cultures by infecting a mouse intraperitoneally, and then culturing the peritoneal fluid and heart blood of the mouse (Type 14 pneumococcus is not virulent for the mouse), within 24-48 hours.

Throat swab specimens will be diluted in 1ml of phosphate buffered saline, vortexed and centrifuged. The swab will be removed and the fluid injected intraperitoneally into the mouse. After 24 hours the abdomen will be tapped and peritoneal fluid will be cultured on blood agar plates for identification of pneumococci using standard techniques(33). In mice that have died within 48 hrs, heart blood will be cultured.

SPN VACCINE STUDY

Form I (ENROLMENT)

1. Subject #.....:_____
3. Date of Interview (dd/mm/yy).....:_____
4. Age of the subject (in yrs).....:_____

5. Place of Enrolment.....:1=TAC 2=KB 3=AM 7=Other

6. Address:_____
_____

7. Husband's age.....:_____ years

8. Head of the family.....:1=Husband 2=Self 3=Son

9. Obstetric History:
   a. LMP Date (dd/mm/yy).....:_____
   b. Gravida.....:_____
   c. Para.....:_____
   d. Previous history
      1. Live birth.....:1=Yes 2=No
      2. precipitated labor.....:1=Yes 2=No
      3. abortion.....:1=Yes 2=No

10. Age of the last child (0=No child).....:_____ months
11. Blood Group of the subject:
   a. Group.....:1=A 2=B 3=AB 4=O
   b. Rh type.....:1=+ve 2=-ve

12. Blood Group of the husband
   a. Group.....:1=A 2=B 3=AB 4=O
   b. Rh type.....:1=+ve 2=-ve

5. Has TT been given during this pregnancy:1=Yes 2=No
5. Date TT given.....:_____
3. Date of vaccine given.....:_____
4. Vaccine code.....:_____

5. Reaction to the vaccine immediately after given vaccine (24-36 hrs):

   a. Rash.....:1=Yes 2=No
   b. Itching.....:1=Yes 2=No
   c. Erythema.....:1=Yes 2=No
   d. Pain.....:1=Yes 2=No
   e. Fever.....:1=Yes 2=No
   f. Others.....:1=Yes 2=No

   g. Body temperature.....:_____ .C

5. Blood collected.....:Yes=1 No=2

7. Has previous child got breastfeeding...:No child=0 Yes=1 No=2

3. Interviewer's Id #.....:_____

SPN VACCINE STUDY

Form II (SOCIOECONOMIC STATUS)

1. Study #.....:_____

2. Date of Interview (dd/mm/yy).....:_____

3. Any side effect of vaccine.....:1=Yes 2=No

4. Date side effect (dd/mm/yy).....:_____

5. Subject's years of schooling.....:_____
6. Husband's years of schooling.....:_____

7. Is subject an earning member.....:1=Yes 2=No

8. No. of the members eating together...:_____
9. No. of the rooms in the house.....:_____

10. Does family own:

a. AC.....:1=Yes 2=No
b. Fan.....:1=Yes 2=No
c. TV.....:1=Yes 2=No
d. VCP/VCR.....:1=Yes 2=No
e. Refrigerator.....:1=Yes 2=No
f. Luxery Cot.....:1=Yes 2=No

11. Type of fuel used for cooking.....:1=Gas 2=Electricity
3=Firewood 4=Cowdung/leaves
5=Misc.scraps

12. Light source at night.....:1=Electricity 2=Kerosine lamp

13. Keeps chicken/duck/dog.....:1=Yes 2=No

14. Chicken/duck/dog enters into kitchen.:1=Yes 2=No

15. Disposal site of garbage.....:1=Courtyard
2=Outside the house

16. Husband smokes.....:_____ no.sticks/day
17. Subject smokes.....:_____no.sticks/day
18. Caretaker smokes.....:_____no.sticks/day
19. Other member smokes.....:_____no.sticks/day

20. Is husband a Zarda/Tobacco taker.....:1=Yes 2=No
21. Is subject a Zarda/Tobacco taker.....:1=Yes 2=No
22. Is caretaker a Zarda/Tobacco taker...:1=Yes 2=No
23. Is other member a Zarda/Tobacco taker:1=Yes 2=No

24. Interviewer's Id #.....:_____

CHD:SPN\F2
DOI:7/1992

SPN VACCINE STUDY

Form III (DELIVERY RELATED INFORMATION)

1. Subject #.....:_____

2. Date of Interview (dd/mm/yy).....:_____

3. Where was child delivered.....:1=Clinic 2=Hospital
3=Home 7=Other

4. Date of delivery (dd/mm/yy).....:_____

5. Time of delivery (hh/mm).....:_____

6. Who delivered.....:1=Consultant 2=Physician
3=Trained TBA 4=Untrained TBA
7=Other

7. Mode of delivery.....:1=Normal 2=C/S
3=Forceps 4=Episiotomy

8. Was the mother give GA.....:1=Yes 2=No

9. Was the mother give LA.....:1=Yes 2=No

10. Birth weight.....:_____ kg.

11. When was the baby put to the breast..:_____ hrs after delivery

12. Is cord blood collected.....:1=Yes 2=No

13. Is colostrom collected.....:1=Yes 2=No

14. Is throat swab collected.....:1=Yes 2=No

15. Interviewer's Id #.....:_____

CHD:SPN\F3
DO:7/1992

SPN VACCINE STUDY

Form IV (ASSESMENT OF FETAL MATURATION):PAGE-1

Subject #.....:_____

Date of Interview (dd/mm/yy).....:_____

Neuromuscular Maturity:

a. Posture.....:0=Arms and legs extended
1=Beginning of flexion of hips and knees,arms extended
2=Stronger flexion of legs, arms extended
3=Arms slightly flexed & legs are flexed and abducted
4=Full flexion of arms &legs

b. Square Window (in degrees).....:0=900 1=60 2=45
3=30 4=0

c. Arm Recoil (in degrees).....:0=180 2=100-180
3=90-100 4=<90

d. Popliteal Angle (in degrees).....:0=180 1=160 2=130
3=110 4=90 5=<90

e. Scarf Sign.....:0=Elbow reaches opposite side
1=Elbow between midline & opposite axillary line
2=Elbow reaches midline
3=Elbow will not reach midline

f. Heel to ear.....:0=According to the sampled diagram
1=According to the sampled diagram
2=According to the sampled diagram
3=According to the sampled diagram
4=According to the sampled diagram

Physical Maturity:

a. Skin.....:0=Gelatious red,transparent
1=Smooth pink,visible veins
2=Superficial peeling,&/or rash, few veins
3=Cracking pale area,rare veins
4=Parchment deep cracking, no vessels
5=Leathery cracked wrinkled

b. Lanugo.....:0=None
1=Abundant
2=Thinning
3=Bald areas
4=Mostly bald

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Form IV (ASSESMENT OF FETAL MATURATION): PAGE-2

. Subject #.....:_____

. Physical Maturity(contd.):

c. Plantar Crease.....:0=No crease
1=Faint red marks
2=Anterior tranverse crease only
3=Creases ant.2/3
4=Creases cover entire sole

d. Breast.....:0=Barely percept
1=Flat areola no bud
2=Stippled areola 1-2mm bud
3=Raised areola 3-4mm bud
4=Full areola 5-10mm bud

e. Ear.....:0=Pinna flat, stays folded
1=Slightly curved pinna;soft with slow recoil
2=Weel-curve pinna;soft but ready coil
3=Formed & firm with instant recoil
4=Thick cartige ear stiff

f. Genitals (in males).....:0=Scrotom empty no rugae
2=Testes descending, few rugae
3=Testes down, good rugae
4=Testes pendulous, deep rugae

g. Genitals (in females).....:0=Prominant clitoris & labia minora
2=Majora & minora equally prominant
3=Majora large,minora small
4=Clitoris & monora completely covered

5. Interviewer's Id #.....:_____

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Form V (FORTNIGHTLY VISIT FORM)

1. Subject #.....:_____
2. Date of Interview (dd/mm/yy).....:_____
3. T/S taken.....:1=Yes 2=No
4. Morbidity of this child in past 7 days:
   a. Fever.....:1=Yes 2=No
   b. Cough/sneezing/running nose.....:1=Yes 2=No
   c. Rapid respiration/breathery difficulty:1=Yes 2=No
   d. Any other complaints.....:1=Yes 2=No
   e. Diarrhea.....:1=Yes 2=No
5. Interviewer's Id #.....:_____

CHD:SPN\F5
DO:7/1992