INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
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CENTRE
FOR HEALTH AND
POPULATION RESEARCH

Memorandum

PHSD
2000

27 March 2000

To : Dr. Abdullah-Hel Baqui
Public Health Sciences Division

From: Professor Mahmudur Rahman
Chairman, Ethical Review Committee

Sub : Approval of Protocol # 2000-003

This has reference to your memo of 27th March 2000 attaching a modified copy of your protocol # 2000-003 entitled "An effectiveness of *Haemophilus influenzae* type b (Hib) vaccine". I am pleased to inform you that the protocol is hereby approved upon your appropriate addressing of the issues raised by the Committee in its meeting held on 23rd February 2000.

Thanking you and wishing you success in running the said study.

cc: Division Director
Public Health Sciences Division

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CENTRE FOR HEALTH AND POPULATION RESEARCH
Mail : ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh
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CENTRE
FOR HEALTH AND
POPULATION RESEARCH

To : Professor Mahmudur Rahman
Chairman, Ethical Review Committee

From : Abdullah H Baqui
PI, An effectiveness study of
Haemophilus influenzae type b (Hib) vaccine

Date: March 27, 2000

Subject: Response to the comments of the Ethical Review Committee

Thank you for your 29 February 2000 memo communicating the valuable observations of the Ethical Review Committee (ERC) on the above mentioned protocol. We have carefully reviewed the observations made by your committee. To comply with the observations we have modified the protocol as appropriate and we are providing the documentation you requested. Please find below our responses to your observations:

a) We will use DTP-PRP-T or TetractHib vaccine manufactured by Aventis Pasteur International (API), [formarly known as Pasteur-Mérieux Connaught (PMC)]. DPT-PRP-T has been shown to be safe, immunogenic, and efficacious in several studies, including in developing countries. The relevant references are attached for your kind review (Amir et al, 1997; Bell et al, 1996; Mulholland et al, 1997).

b) The vaccine is lecensed for free sell in France, the country of origin. The certificate from the company is attached.

c) We agree with this comment. It was proposed that, since no new invasive procedure (other than routine immunization procedures) will take place during vaccination, the vaccinator should take verbal consent before vaccination and then document it by an initial of the parent or guardian on the EPI register. Written consent will be taken from the parents of the children from whom blood or CSF (if necessary) will be taken. Also, written consent will be taken from the parents/legal guardian of the children enrolled in the immunogenicity sub-study.

d) PRP-T does not adversely influence on immunogenecity of DPT or vice-versa. Studies showed that the immunogenicity of the combined vaccine is similar to the immunogenicity of the vaccines administered separately (Begg et al., 1995; Kaplanet al, 1994).

e) We regret the inconsistency between the text of the protocol (page 19) and consent forms regarding the amount of blood to be drawn. About 2.5 ml (not 5 ml) of blood will be collected from the patients for culture and other laboratory tests.

f) We agree that 2 ml of blood will be adequate to determine anti-PRP antibody and accordingly we will collect 2 ml blood for this assay.

g) It is true that Hib is isolated from only 2-4% of the pneumonia cases by blood culture. However, as mentioned in the protocol, the reported rates of Hib pneumonia based on positive blood cultures are gross underestimates of the true incidence of Hib pneumonia because pneumonia and other localized infections are characterized by intermittent bacteriemia and low order of magnitude of bacteriemia. Thirty six to 50% of pneumonia cases were found to be bacterial in some studies in which antigen detection was used to make the diagnosis of Hib and pneumococcal pneumonia (Barker et al., 1989; Berman et al., 1983; Wall et al., 1986; Weinberg et al., 1989; Jacobs & Harris, 1979; Namba et al., 1988; Azmi et al., 1987). Another alternative approach recently recommended by WHO is to use radiologically defined pneumonia as an end point in the evaluation of respiratory vaccines. This approach was used in the evaluation of a Hib conjugate vaccine (PRP-T) in the Gambia and the efficacy of the vaccine for the prevention of radiologically defined pneumonia at any time after enrollment was 21.1% (Mulholland et al., 1997). This finding suggests that about one-fifth of the radiologically defined pneumonia in developing countries could be due to Hib infections. Therefore, this study proposes to use radilogically defined pneumonia as an outcome in addition to culture proven Hib pneumonia.

May we request you to kindly approve the modified protocol.

Best regards.

References:

Amir J., Melamed R., Bader J., et. Al. (1997). Immunogenicity and safety of a liquid combination of DT-PRP-T vs lypolized PRP-T reconstituted with DPT. Vaccine. 15:149-54.

Bell F, Martin A, Blondeaus C., Thornton C, Chaplais J, Adam Finn. Combined diphtheria, tetanus, pertussis and *Haemophilus influenzae* type b vaccines fpr primary immunisation. Arch Dis Child. 1996;75:298-303.

Begg NT, Miller E., Fairley CK., et al. Antibody responses and symptoms after DTP and either tetanus or diphtheria *Haemophilus influenzae* type b conjugate vaccines given for primary immunisation by separate or mixed injections. Vaccine, 1995;13:1547-50.

Kaplan SL, Laurer BA, Ward MA, et al. Immunogenicity of *Haempophilus influenzae* type b-tetanus protein conjugate vaccine alone or mixed with diphtheria-tetanus-pertussis vaccine in infants. J Pediatr, 1994; 124:323-7.

Mulholland K., Hilton S., Adegbola R., et al. Randomised trial of *Haemophilus influenzae* type-b tetanus protein conjugate for prevention of pneumonia and meningitis in Gambian infants. Lancet. 1997;349:1191-7.

RHÔNE-POULENC

Rhône-Poulenc Rorer Bangladesh Ltd.

FACSIMILE TRANSMISSION

To : Dr. Abdullah H Baqui	Fax no : 8823116
From : Dr. Rezaul Farid Khan	
Date : 23 March, 2000	Total Page : 1 of 2

Subject: Free Sales Certificate of TETRAct-HIB

We have received Free Sales Certificate of the country of origin of TETRAct- HIB. Please find enclose here with.

Thanking you

Dr. Rezaul Farid Khan

CERTIFICAT DE LIBRE VENTE

FREE SALE CERTIFICATE
CERTIFICADO DE LIBRE VENTA

Il est certifié que les laboratoires
It is hereby certified that Laboratories
Se certifica que los laboratorios

PASTEUR MERIEUX
Sérums et Vaccins
58, Avenue Leclerc
69007 - LYON
FRANCE

sont autorisés à fabriquer et / ou à vendre des spécialités pharmaceutiques.
are authorized to manufacture and to sell pharmaceutical specialities.
estan autorizados para la fabricación y la venta de especialidades farmacéuticas.

Ils fabriquent et / ou exploitent notamment les ..... médicaments ci-après désignés, dont
They manufacture and exploit, in particular, the ..... medicaments mentioned hereafter,
Fabrican y explotan particularmente los ..... medicamentos mencionados abajo, cuyas

les formules sont jointes au présent document (pièces numérotées de 1 à ....).
the formulae of which are attached to this document (schedules numbered from 1 to ...).
formulas se adjutan al presente documento (documentos numerados de 1 a ....).

Ces spécialités ont fait l'objet d'une autorisation de mise sur le marché en France (visa).
These pharmaceutical specialities have received an authorization for their marketing in France (visa).
Estas especialidades han recibido una autorización para su venta en el mercado francés (visado).

Indépendamment de l'application éventuelle des règlements relatifs aux substances vénéneuses, ladite autorisation n'est assortie d'aucune autre restriction ou précaution d'emploi que celles figurant, le cas échéant, sur les pièces ci-annexées.
Independently of the possible application of the regulations relating to poisonous substances, the said authorization is subject to no restriction or precaution with respect to its use other than those shown, as the case may be, on the documents attached as schedules hereto.
Independientemente de la aplicación eventual de los reglamentos que conciernen las sustancias venenosas, dicha autorización no tiene otra restricción o precaución de empleo que las que figuran en los documentos adjuntos, si se presenta el caso.

TETRAct-HIB, poudre et suspension pour suspension injectable (IM), en flacon et seringue pré-remplie
Vaccin contre la diphtérie, le tétanos, la coqueluche, la méningite à haemophilus influenzae type b.

Fait à Saint-Denis, le 25 AOUT 1999

Pour le Directeur Général et par délégation
Le Chef de l'Unité Autorisation d'Importation et d'Exportation

Le Directeur Général
de l'Agence du Médicament
France ROUSSELLE

AGENCE FRANÇAISE DE SÉCURITÉ SANITAIRE DES PRODUITS DE SANTÉ
143-147, bd Anatole France
93285 SAINT-DENIS CEDEX

APPROVED COPY

International Centre for Diarrhoeal Disease Research, Bangladesh

RESEARCH PROTOCOL

1. **Project Title: An Effectiveness Study of *Haemophilus Influenzae* type b Vaccine**

2. Investigators :

2a. Name of the Principal Investigator including position and qualifications:

Abdullah H Baqui, MBBS, MPH, DrPH
Senior Epidemiologist and Head, Child Health Program (CHP)
Public Health Sciences Division (PHSD), ICDDR,B

2b. Name of Co-Investigators:

ICDDR,B: SE Arifeen, K Zaman, Manirul Islam, N Shahid, S Hossain, M Rahman, and Lars Ake Persson

2c. Collaborating Investigators:

Shishu Hospital: Samir K Saha, Ruhul Amin, and Manzoor Hussain

Radda MCH-FP Centre: Bushra Amena

Association pour l'Aide a la Medecine Preventive (AMP), France: Bradford Gessner

Johns Hopkins University, USA: Mathuram Santosham, Robert E Black

London School of Hygiene and Tropical Medicine: J. Patrick Vaughan

3. Name of the Division/ Branch / Programme of ICDDR,B under which the study will be carried out: Child Health Programme, PHSD

4. Dates of Proposed Period of Support (Day, Month, Year):

April 1, 2,000 - March 31, 2003

5. Cost Required for the Budget Period:

5a. Direct Cost : US$ 536,337 Total Cost : US$ 625,502*

6. Sponsor(s): Asian Development Bank financed Urban Primary Health Care Project of the Government of Bangladesh and Aventis Pasteur International (API) [formerly known as Pasteur Merieux Connaught (PMC)], Lyon, France

* In addition, Aventis Pasteur International (API), Lyon, France, the company that produces Hib conjugate, absorbed diphtheria, tetanus and pertussis combined vaccine (TetractHib) confirmed that the company will make a donation to ICDDR,B up to 60,000 doses of TetractHib to be used in this study. The market price of this vaccine is about US$ 420,000.

7. Approval of the Project by the Division Director of the Applicant

The above-mentioned project has been discussed and reviewed at the Division level as well by the external reviewers. The protocol has been revised according to the reviewer's comments and is approved.

Professor Lars Ake Persson

Name of the Division Director	Signature	Date of Approval

8. Certification by the Principal Investigator

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

9. Signature of PI: _____ Date: _____

Table of contents

PROJECT SUMMARY: Describe in concise terms, the hypothesis, objectives, and the relevant background of the project. Describe concisely the experimental design and research methods for achieving the objectives. This description will serve as a succinct and precise and accurate description of the proposed research is required. This summary must be understandable and interpretable when removed from the main application. **(Type Text Within the Space Provided and should not exceed 350 words).**

Principal Investigator: Dr. Abdullah H Baqui

Although the routine use of *Haemophilus influenzae* type b (Hib) conjugate vaccine has led to the near disappearance of invasive Hib diseases in developed countries, these diseases (e.g. pneumonia, meningitis) remain important causes of morbidity and mortality in infants and young children in developing countries including Bangladesh. There has been considerable interest in incorporating Hib conjugate vaccine into the routine childhood immunization programme in Bangladesh. However, a policy decision has not yet been taken since it is a relatively expensive vaccine and the data documenting its effectiveness in Bangladesh are lacking. The Asian Development Bank funded Urban Primary Health Care Project has provided an opportunity to assess the effectiveness of this vaccine in Bangladesh.

The proposed study is designed to introduce a Hib conjugate, adsorbed diphtheria, tetanus and pertussis combined vaccine (TetractHib) manufactured by Aventis Pasteur International (API), Lyon, France in Zones 7 and 8 of Dhaka city. These two Zones having an estimated annual birth cohort of 27,000 will constitute the study area. The vaccine will be introduced through the existing immunization centres of a non-governmental organization, the Radda MCH-FP Centre. Clinical and laboratory-based surveillance for pneumonia and meningitis will be set up in the entire study area. All the curative care providers in this area will be encouraged to refer children with clinically severe pneumonia and meningitis to Shishu Hospital where cases will be ascertained by blood (and CSF in case of meningitis) culture and chest radiographs. Two to four age- and sex-matched controls will be randomly selected per case from the study area. The effectiveness of the vaccine in <2 year old children will be assessed using an incident case-control approach. The primary outcomes for vaccine effectiveness will be the adjusted exposure odds ratios, comparing cases and matched controls for the groups: Hib pneumonia, radiologically defined pneumonia, and Hib meningitis.

This study will provide data on the effectiveness and cost of introduction of Hib conjugate vaccine in Bangladesh. If it can be shown that the introduction of Hib conjugate vaccine substantially reduces the burden of pneumonia and meningitis in <2 year old children in Bangladesh, its inclusion in routine EPI will considerably improve child health and survival.

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be tested:

Concisely list in order, in the space provided, the hypothesis to be tested in the proposed study. Provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

After introduction of a Hib conjugate, adsorbed diphtheria, tetanus and pertussis combined vaccine (TetractHib) manufactured by Aventis Pasteur International (API), Lyon, France in a large area of Dhaka city with an estimated annual birth cohort of about 27,000, compared to <2 year old unimmunized children, immunized children will have:

a) 75% lower incidence of Hib pneumonia;
b) 85% lower incidence of Hib meningitis; and
c) 20% lower incidence of radiologically defined pneumonia.

Specific Aims:

Describe the specific aims of the proposed study. State the specific parameters, biological functions/ rates/ processes that will be assessed by specific methods **(Type within limits).**

Primary Aim:

The primary aim of this study is to measure the effectiveness of 2-3 doses Hib conjugate, adsorbed diphtheria, tetanus and pertussis combined vaccine (TetractHib) given at 6, 10, and 14 weeks of age on: a) Hib pneumonia, b) radiologically defined pneumonia, and c) Hib meningitis.

Secondary aims:

a) To determine what proportion of infants develops protective level of anti-PRP antibody and what proportion maintains the protective level of anti-PRP antibody up to 15 months of age after immunization with three doses of Hib conjugate, adsorbed diphtheria, tetanus and pertussis combined vaccine given at 6, 10 and 14 weeks of age;

b) To study the epidemiology of Hib infections e.g., incidence, age and gender distribution, clinical presentations, severity, and seasonality.

Background of the Project including Preliminary Observations

Describe the relevant background of the proposed study. Discuss the previous related works on the subject by citing specific references. Describe logically how the present hypothesis is supported by the relevant background observations including any preliminary results that may be available. Critically analyze available knowledge in the field of the proposed study and discuss the questions and gaps in the knowledge that need to be fulfilled to achieve the proposed goals. Provide scientific validity of the hypothesis on the basis of background information. If there is no sufficient information on the subject, indicate the need to develop new knowledge. Also include the **significance and rationale** of the proposed work by specifically discussing how these accomplishments will bring benefit to human health in relation to biomedical, social, and environmental perspectives. **(Do not exceed 5 pages, use continuation sheets).**

Introduction

Invasive diseases due to *Haemophilus influenzae type b* (Hib) infections, particularly bacterial meningitis, was one of the leading cause of morbidity and mortality in developed countries prior to the availability of the Hib conjugate vaccines (Shapiro et al., 1991). The development of a number of safe and highly efficacious Hib conjugate vaccines and subsequent licensure and routine use of these vaccines in infancy have virtually eliminated the disease from developed countries. Before the Hib conjugate vaccines became available, Hib caused approximately 20,000 cases of meningitis per year in US alone. In contrast, currently, the reported number of cases are fewer than 200 per year.

Although data on the incidence of Hib diseases in developing countries are limited, available data suggest that Hib is an important cause of morbidity and mortality in developing countries as well. Several studies documented a high prevalence of Hib meningitis among children admitted to hospitals in various parts of tropical Africa (Autret et al., 1979; Ntihinyurwa et al., 1979). High incidence rates of invasive Hib diseases were also documented in Papua New Guinea (Shann et al., 1984; Lehman, 1990) and the Gambia (Forgie et al., 1991a; Forgie et al., 1991b). Reported rates of Hib meningitis in the Gambia were as high as 297/100,000 for children one year of age and about 60/100,000 for children under five years of age (Bijlmer et al., 1990). The rates reported from some Asian countries are lower than in developed countries (Ishikawa et al., 1996; Wang & Lin, 1996; Tee & Lin, 1996; Munson et al., 1989). These rates of disease in Asian countries are probably gross under estimates of the true incidence of the Hib disease because of the widespread use of antibiotics to treat patients with childhood illnesses prior to performing cultures. Worldwide, it has been estimated that more than 500,000 cases of Hib meningitis occur each year and this accounts for significant morbidity, mortality and health care expenditure (Schwartz, unpublished data).

The epidemiology of invasive Hib disease seen in developed and developing countries differ greatly in several important respects. The age distribution of Hib disease in developing countries is different from developed countries. In most developed countries less than 20% of the cases occur prior to six months of age, whereas in developing countries approximately 50% of the cases occur prior to six months of age and 80% occur prior to one year of age (Funkhouser et al., 1991). This difference in disease burden by age has important implications on age at which the vaccine should be given. Since the peak incidence of Hib diseases is around 5 to 6 months in developing countries, the vaccine should be administered as soon after birth as possible. The clinical presentation of the disease also differs. In developing countries, pneumonia is the most common manifestation of Hib disease (Greenwood,

1992). Therefore, any evaluation of Hib conjugate vaccine in a developing country must carefully evaluate its effect on pneumonia in addition to its effect on meningitis.

Acute lower respiratory tract infection (ALRI) is one of the leading cause of morbidity and mortality in developing countries resulting in 3 to 4 million deaths each year among children under five years of age. Majority of these deaths are due to pneumonia (WHO, 1996). Unfortunately, given the great difficulty of establishing an aetiologic diagnosis of bacterial pneumonia in children, the reported Hib disease burden undoubtedly underestimates the incidence of Hib pneumonia. When cultures of lung aspirates were performed in children with pneumonia, *Streptococcus pneumoniae* and *Haemophilus influenzae* were found to be the leading causes of pneumonia in developing countries (Forgie et al., 1991a; Forgie et al, 1991b; Shann et al., 1984). In these countries, 50%-70% of lung aspirates from patients who did not previously receive antibiotics were positive for bacteria (Klein, 1969; Rapkin, 1975; Shann et al., 1984; Mimica et al., 1971; Abdel-Khalik et al., 1938; Diakparomre & Obi, 1981; Escobar et al., 1976; de Olarte et al., 1971; Hughes et al., 1969; Hughes et al., 1966; Kalra et al., 1981; Riely et al., 1983; Gohar & Abboud, 1933; Silverman et al., 1977; Kumar & Ayyagari, 1984). The isolation rates varied significantly ranging from 92% in Egypt to 13% in Nigeria. The proportion of bacterial isolates that were *Haemophilus influenzae* also varied significantly ranging from 0% to 100%.

Data on invasive Hib diseases from Bangladesh are few. However, in Bangladesh, about 25% of deaths in under five year old children and 40% of deaths in infancy are associated with ALRI (Baqui et al., 1998). A community-based cohort study in rural Matlab, Bangladesh observed an annual ALRI incidence of 30/100 child-years in children less than two years of age (Zaman et al., 1997). A hospital based study conducted in the ICDDR,B's Dhaka hospital between 1986-88 investigated 401 ALRI cases in children less than five years of age for respiratory pathogens. The most common manifestation was pneumonia (93%). A respiratory pathogen was detected in 30% of the patients; a bacterial pathogens was detected from only 10% of the cases; Hib was detected in 3% of the cases. The most commonly detected pathogen was respiratory syncytial virus (RSV) which was detected in 14% of the cases. The case fatality rate was 14% in bacterial pneumonia and 3% in viral pneumonia (Rahman et al., 1990).

As mentioned earlier, the reported rates of Hib pneumonia based on positive blood cultures are probably gross underestimates of the true incidence of Hib pneumonia because pneumonia and other localized infections are characterized by intermittent bacteriamia and low order of magnitude of bacteriemia. To overcome the intermittency and the low order of magnitude, it has been recommended that blood be obtained for two to three separate blood cultures (Washington and Ilstrup, 1986). Repeated cultures are, however, not feasible in large scale field studies. An alternative approach is to use antigen detection test which has a much higher sensitivity. Thirty six to 50 % of pneumonia cases were found to be bacterial in some studies in which antigen detection was used to make the diagnosis of Hib and pneumococcal pneumonia (Barker et al., 1989; Berman et al., 1983; Wall et al., 1986; Weinberg et al., 1989; Jacobs & Harris, 1979; Namba et al., 1988; Azmi et al., 1987). Another alternative approach recently recommended by WHO is to use radiologically defined pneumonia as an end point in the evaluation of respiratory vaccines. This approach was used in the evaluation of a Hib conjugate vaccine (PRP-T) in the Gambia and the efficacy of the vaccine for the prevention of

radiologically defined pneumonia at any time after enrollment was 21.1% (Mulholland et al., 1997). This finding implies that about one-fifth of the radiologically defined pneumonia in developing countries could be due to Hib infections. However, the interpretation of chest x-rays is notoriously non-standardized with relatively high intra- and inter-observer variations ((Davies et al., 1996). To develop and evaluate a practical system for use in the standardization of reading of the chest radiographs for the detection of 'obvious radiological pneumonia', WHO Pneumococcal Vaccine Trial Group organized a radiology workshop in May 1999 in Baltimore, USA. The principal investigator (AHB) and four co-investigators (SEA, KZ, MS, BG) of the proposed study participated in this workshop. WHO in collaboration with the Johns Hopkins University, USA is now setting up an Internet x-ray interpretation and standardization (IXIS) system in Johns Hopkins University.

Acute bacterial meningitis is also a common cause of morbidity and mortality in Bangladesh among children less than five years of age. A laboratory-based study of diagnosed bacterial meningitis in the Shishu Hospital (a national pediatric hospital in Dhaka) identified 852 meningitis cases during the period 1987-94 (Saha et al., 1997). This 345 bed teaching hospital with a catchment population of about 16 million admitted about 1300 patients per month during the study period. Bacterial meningitis was diagnosed if a bacterial pathogen was isolated from cerebro-spinal fluid (CSF) or if the CSF contained more than 100 white cells/cubic mm. Of the 587 culture-positive cases, *Haemophilus influenzae* was isolated from 47% and *Streptococcus pneumoniae* was isolated from another 32% of the cases. Sixty eight percent of the *Haemophilus influenzae* cases occurred below one year of age and another 24% in the second year of life. *Haemophilus influenzae* showed a remarkable increase of 700% during the study period. Ninety eight percent of the *Haemophilus influenzae* strains were type b (Hib). Antigen detection in 72 culture negative cases in the last two years of the study also revealed the predominance of *Haemophilus influenzae* (49%).

Current status of Hib vaccines:

The first Hib vaccine available for prevention of Hib disease contained the pure polysaccharide - polyribosylribitol phosphate (PRP) capsule of the organism. This vaccine was found to be efficacious in children above 18 months of age but not below that age (Peltola et al., 1977). In addition, it was demonstrated that the immune responses of 24 month old Apache infants to the pure polysaccharide (PRP) vaccine was ten fold lower compared to infants in a general US population (Siber et al., 1991). In the last decade a number Hib conjugate vaccines have been manufactured by linking the pure PRP of Hib to different protein carriers. Unlike the pure polysaccharide (PRP) vaccine, the conjugate vaccines induce a T cell dependant response. Thus, repeated injections with the conjugate vaccines induces a memory cell response. The Hib conjugate vaccines have been shown to be immunogenic from one to three months of age and protective after two to three doses (Santosham et al., 1991; Eskola et al., 1990). The American Academy of Pediatrics recommends that Hib conjugate vaccination should be initiated at 6 weeks to two months of age (1991). The available Hib conjugate vaccines, the protein carrier used for manufacturing each of the vaccines and their respective manufacturers is shown in the following table:

Distinct Properties of the Four licenced *Haemophilus influenzae* type b (Hib) Conjugate Vaccines				
Product name	PRP-D	HBOC	PRP-OMP	PRP-T
Trade name	ProHibit	HibTITER*	PedvaxHib	ActHib**
Manufacturer	Connaught Lab.	Lederle-Praxis	Merck Sharp and Dohme	Aventis Pasteur International (API)
Indicated ages for vaccination (months) in developed countries	>15	2, 4, 6, 12-15	2, 4, 12-15	2, 4, 6, 12-15
Indicated ages for vaccination (weeks) in developing countries	-	-	-	6-8, 10-12, 14-16
Carrier protein	Diphtheria toxoid	CRM (a non-toxic mutant of Diphtheria toxoid)	OMPC (outer membrane protein complex of the *Neisseria meningitidis* serotype B)	Tetanus toxoid

* Also available in combination with diphtheria-tetanus-pertussis as TETRAMUNE

** Also available in combination with DPT (TetractHib) which will be used in the proposed trial

All of the Hib conjugate vaccines have been shown to be efficacious in preventing Hib disease in developed countries. However, the level of efficacy appears to vary between populations. For example, one of the Hib conjugate vaccines, PRP-D, manufactured by Connaught laboratories by linking the PRP to the diphtheria toxoid was shown to be over 90% efficacious in preventing Hib disease among infants in Finland (Eskola et al., 1990) but the efficacy was less than 40 % among Alaskan Indian infants (Ward et al., 1990). The vaccine manufactured by Merck Research Laboratories (PRP-OMP) has been shown to be over 90% efficacious among the Navajo Indian population in Arizona when 2 doses of the vaccine were given two months apart in infants aged 2 months (Santosham et al., 1991). Comparative immunogenicity studies of the different Hib vaccines have demonstrated that the PRP-OMP vaccine induces a "protective" immune response (anti-capsular antibody levels above 1 mcg/ml) in over 50% of infants after one dose. However, the peak antibody levels after 3 doses is higher in infants who are given the HBOC vaccine or the PRP-T vaccine compared to infants who are given the PRP-OMP (Bulkow et al., 1993; Decker et al., 1992).

The vaccine manufactured by Aventis Pasteur International (API) by linking the PRP to the tetanus toxoid (PRP-T) is the only vaccine that has been shown to be efficacious in a population from a less developed country. A large randomized clinical trial (RCT) of PRP-T was conducted in 42,848 infants in the Gambia between 1993 and 1995. In the Gambian trial, the efficacy of the PRP-T vaccine for the prevention of all invasive Hib disease was shown to be 95% when three doses of the vaccine was given (PRP-T vaccinees 1, control 19), for the prevention of Hib pneumonia after two or three doses was 100% (vacinees 0, control 10), and for the prevention of radiologically defined pneumonia at any

time after enrollment was 21.1% (vacinees 198, controls 251) (Mulholland et al.1997). This finding underscores that for assessment of the impact of Hib vaccine on pneumonia one should not rely on blood cultures alone since the bacteremia in Hib pneumonia is infrequent and intermittent. As alluded earlier, WHO has recommended that radiologically defined pneumonia should be an outcome measure in all Hib and pneumococcal vaccine trials. The findings of the Gambian trial that the introduction of Hib vaccine can reduce radiologically defined or severe pneumonia by about 20% has important public health significance where pneumonia is the leading cause of childhood morbidity and mortality such as in Bangladesh.

Although the use of Hib conjugate vaccines in industrialized countries has led to the near disappearance of invasive Hib disease in these countries, the vaccine could not be introduced in most developing countries, primarily due to its high cost. Only three developing countries (Uruguay, Chile, and Qatar) had incorporated Hib conjugate vaccine into their Expanded Programme on Immunization by January, 1997 (Levine and Levine, 1997). Since Hib diseases cause significant morbidity, long-term sequelae, and mortality among infants and young children in developing countries and since the Hib conjugate vaccines are highly efficacious, despite high cost, the introduction of Hib conjugate vaccine is likely to be a highly cost-effective intervention. The ease with which Hib conjugate vaccine can be combined with other vaccines, such as DPT, should also be considered (Avendano et al., 1993). Finally, the fundamental market pressure that may lead to a fall in the vaccine cost should also be considered. If several developing countries introduce Hib conjugate vaccine combined with DPT, the global market would be substantially expanded which should reduce the cost of the vaccine.

The proposed study:

Considering the high disease burden due to Hib infections, there has been considerable interest in incorporating Hib conjugate vaccine into the routine childhood immunization programme in developing countries including in Bangladesh. The PRP-T vaccine is currently being given in Bangladesh by private sector providers but the coverage is negligible. A policy decision to incorporate the Hib conjugate vaccine in the routine childhood immunization has not yet been taken considering its cost and for lack of data showing the effectiveness of this vaccine in Bangladesh children. The ADB funded Urban Primary Health Care Project of the Government of Bangladesh (GoB) has provided the opportunity to introduce this vaccine in selected urban areas and assess the cost and effectiveness of this vaccine.

We would like to introduce a Hib conjugate vaccine (PRP-T) combined with diphtheria, tetanus and pertussis vaccine (TetractHib) manufactured by PMC, Lyon, France (PRP-T) in selected areas of Dhaka city by working with an NGO, Radda MCH-FP Centre. We chose PRP-T because this is the only Hib conjugate vaccine that has been evaluated for efficacy in developing countries.

The randomized clinical trial (RCT) has been the gold standard for evaluation of health care interventions because randomization minimizes confounding that can easily occur with other designs. While RCTs are the best approach for evaluation of both therapeutic and preventive interventions, it is not always feasible and/or ethical. Since Hib conjugate vaccine is routinely used in developed

countries and has been shown to be highly efficacious even in a developing country, the Gambia, we believe that a randomized clinical trial (RCT) of Hib conjugate vaccine may not be ethical. Therefore, we propose to introduce the vaccine in a defined area of Dhaka city and conduct a case-control study to evaluate the effectiveness of the vaccine using various outcomes. Case-control studies have had an increasing role in the post-licensure evaluation of vaccine efficacy and effectiveness. Mills and Rhoads (1996) reviewed the contribution of case-control studies to the evaluation of vaccines for *Streptococcus pneumoniae* and *Haemophilus influenzae* type b and compared the findings of these studies with the findings of clinical trials of these vaccines. They concluded that post-marketing case-control studies provide the best evidence that these vaccines have actually worked. For both Hib and pneumococcal vaccines, the estimates of efficacy from meta-analysis of case-control studies were more conservative than the results of original trials. Comstock (1994) argued that despite the disadvantage of some selection bias, the evaluation from a case-control study may be more realistic since controlled trials are often conducted under conditions that are difficult to achieve. We, however, propose a case-control study to evaluate the effectiveness of Hib conjugate vaccine instead of a randomized clinical trial primarily based on ethical consideration.

RATIONALE: Describe the relevance of the proposed study to national health priorities and relationship of the objectives to existing scientific knowledge on the subject.

As mentioned in an earlier section, available data suggest that both Hib pneumonia and meningitis are important public health problem in Bangladesh and there is considerable interest in incorporating Hib conjugate vaccine into the routine childhood immunization programme. However, a policy decision could not be taken since this is a relatively expensive vaccine and the data documenting the effectiveness of this vaccine in Bangladesh is not available. The Asian Development Bank (ADB) funded Urban Primary Health Care Project (UPHCP) of the Government of Bangladesh (GoB) has provided an opportunity to introduce this vaccine in selected urban areas and evaluate the effectiveness and cost of introduction of this vaccine.

Ideally, population-based epidemiologic data on disease burden due to Hib infections should form the basis of an effectiveness trial of Hib vaccine. Such data are not readily available from Bangladesh. Yet, an effectiveness trial in Bangladesh seems justifiable for the following reasons:

a) Population-based data on childhood morbidity and mortality from Bangladesh reveal that acute lower respiratory infections (ALRI), particularly pneumonia, is the most important cause of morbidity and mortality in children under-five years of age. This data when combined with the hospital-based aetiologic data of pneumonia and meningitis provide adequate evidence that Hib disease is common in Bangladeshi children.

b) Highly efficacious vaccines against Hib disease are available. It seems more appropriate to introduce the vaccine in a defined population to evaluate the effectiveness of the vaccine and at the same time collect the necessary epidemiologic data as part of the study. The data from the non-vaccinated population should provide an estimate of disease burden in the general population. This approach will save time and reduce cost than conducting studies in phases.

METHODOLOGY: Describe the design of the study, methods and procedures in sufficient detail to enable assessment of how they will contribute towards achievement of the stated objectives and to permit proper appraisal of the budget. Plan for data analysis should be included if relevant and important. This section should contain details of the research methods. Enough detail should be given to evaluate whether the methods are already tested and feasible. Discuss the limitations and difficulties of the proposed procedures and sufficient justify the use of them. Point out safety procedures to be observed for protection of individuals during any situations or materials that may be injurious to human health. The methodology section should be sufficiently descriptive to allow the reviewers to make valid and unambiguous assessment of the project (do not exceeds 10 pages).

Study design:

We propose to conduct an incident case-control study to evaluate the effectiveness of a Hib conjugate vaccine in less than two year old children in Dhaka city, Bangladesh.

The plan is to introduce a Hib conjugate, adsorbed diphtheria, tetanus and pertussis combined vaccine (TetractHib) manufactured by Aventis Pasteur International (API) in Zones 7 and 8 of Dhaka city through the existing immunization centres of a non-governmental organization (NGO), set-up clinical and laboratory-based surveillance to identify cases of invasive Hib diseases (pneumonia and meningitis) and radiologically defined pneumonia occurring in the study area, select two to four age-matched controls for each case, also from the study area, and evaluate the effectiveness of the vaccine.

Study setting and subjects:

The proposed study will be conducted in Dhaka city, the capital of Bangladesh by ICDDR,B in partnership with the Dhaka Shishu Hospital and a NGO- the Radda MCH-FP Centre. The current estimated population of the Dhaka City Corporation (DCC) area is about 5.6 million. According to the 1991 census, the enumerated population of DCC area was about 3.2 million and the post-enumeration adjusted population was about 3.8 million. The figure of 5.6 million is based on the assumption that the population has been growing approximately at a rate of 5-6% per year since the 1991 census. The city corporation has divided the city into 10 administrative zones and 100 wards. The average estimated population of DCC zones is about 560,000, although the actual population sizes of different zones vary quite a bit. About 25% or an estimated 1.25 million people live in more than 3,000 slums and squatter settlements of varying sizes.

The crude birth rate (CBR) of Dhaka city is 27/1,000 population; CBR is higher in slums (35/1,000) than in non-slum (24/1000) areas. The overall infant mortality rate (IMR) is 92/1,000 live births; 99/1,000 in slum areas and 85/1,000 in non-slum areas. The overall, slum, and non-slum death rates in children in the second year of life are 11.2, 22.1 and 1.4 per 1,000 populations respectively (Baqui et al., 1997).

Vaccinations are given from about 107 EPI centres; most of these centres are managed either directly by the city health department or by non-governmental organizations (NGOs) with assistance from DCC. Vaccinations are also given from private facilities. According to a survey conducted in one zone of Dhaka city in 1995, about 91% of the non-slum and 74% of the slum infants received at least one dose of DPT/OPV (Perry et al, 1997). The three dose completion rate of DPT/OPV was about 70%; this rate was only 54% in the slum areas (Perry et al, 1997). Curative care for childhood illnesses is

provided by about 94 MOH/DCC dispensaries, 144 MCH-FP Centres managed NGOs and GoB-FP directorate, GoB operated tertiary care hospitals, Shishu Hospital and from about 207 private hospitals (Mazumder et al., 1997). In addition, care is provided by many single practitioners, pharmacists, and different types of traditional healers.

For the proposed trial, ICDDR,B in partnership with a large NGO - Radda MCH-FP Centre will introduce Hib vaccine in the catchment area of Radda MCH-FP Centre through their existing immunization clinics in Zones 7 and 8 of DCC. Radda MCH-FP Centre operates seven clinics [until recently they had three fixed clinics and three mobile teams (MT)]. They have a long track record of providing both preventive and curative care services in large areas of Zones 7 and 8 of DCC. The estimated catchment population of Radda Centre (Zones 7 and 8) is about one million. The estimated annual birth cohort will be about 27,000. The following is the volume of services provided by Radda Centre during the period July 1998-June 1999:

Type of service	Clinic Section-10	Clinic Section -1	Clinic Section - 12	MT-1	MT-2	MT-3	Total
DPT 1	5,860	4,086	2,836	1,181	787	418	15,168
DPT 2	5,621	3,842	2,765	1,055	776	413	14,472
DPT 3	5,298	3,698	2,492	1,037	759	371	13,655
Number of <5 children seen	27,878	21,436	11,537	6,107	3,788	1,652	72,398
Number of <5 children seen with clinical ALRI	4,417	2,341	2,084	1,142	459	242	10,685
Number of <5 children seen with severe pneumonia	440	245	247	50	25	7	1,014

Although Radda is the main primary health care provider in this area, the area is also served by other NGOs including Pragati Samaj Kallan Prathistan (PKSP), Bangladesh Women's Health Coalition (BWHC) and Marie Stopes Clinic Society and other private providers/facilities. The above service statistics imply that Radda MCH-FP Centre alone immunizes about 50% of the babies born in their catchment area. Since the three dose DPT completion rate is about 70%, some infants receive vaccine from other sources and some remain non-vaccinated.

The Zones 7 and 8 of DCC will form the study area for the proposed study. This area has already been mapped (please see the attached map) but will be more carefully delineated and mapped before beginning the study. As mentioned above, clinical and laboratory-based surveillance will be set up in all of Zones 7 and 8 to identify as many pneumonia and meningitis cases as possible from the study area. Both cases and controls will be selected from this pre-defined trial area to minimize biases. The surveillance method and methods for selection of cases and controls have been described in later sections.

Systems will be developed to refer all potential Hib cases to the Dhaka Shishu Hospital for ascertainment of the cases. Shishu Hospital has agreed to collaborate with ICDDR,B in the proposed study. Shishu Hospital is the largest pediatric hospital of the country. This hospital provides primary, secondary as well as tertiary care. It has a large outpatient department and an inpatient facility with 345 beds. The hospital provides treatment to patients from Dhaka city as well as from other parts of the country with both acute and chronic conditions. Majority of the inpatient beds are free so that the poor patients can access the services. The inpatient case-mix includes a wide range of clinical conditions. Infectious diseases (e.g., pneumonia, meningitis, sepsis) are very important causes of admission in infancy. The hospital has x-ray, clinical pathology and microbiology laboratories. Dr. Samir K Saha, Head of Shishu Hospital Microbiology laboratory and a co-investigator of this project has extensive experience in isolation and characterization of *Haemophilus influenzae*. As part of the outpatient services, the Shishu Hospital provides limited community health care and follow-up services to neonates and patients with tuberculosis and meningitis living in the adjoining slums. About 10% of the patients seen in the OPD are admitted in different units of the hospital.

Outcome variables and case definitions:

The study will have the following three outcome variables or end points:

a) Hib pneumonia;
b) Radiologically defined pneumonia; and
c) Hib meningitis.

The following case definitions will be used:

Hib pneumonia: Clinical pneumonia (cough or difficult breathing with one or more of the following: elevated respiratory rate, chest in-drawing, auscultatory findings or chest x-ray compatible with pneumonia), and either Hib cultured from blood or Hib antigen detected from blood by latex agglutination test.

Radiologically defined pneumonia: Clinical pneumonia and 'obvious radiographic pneumonia' as defined by WHO. [WHO in collaboration with the Johns Hopkins University, USA has set up an Internet x-ray interpretation and standardization (IXIS) system in Johns Hopkins University. The collaborating investigators from Johns Hopkins University will assist with standardization of reading of chest radiographs].
Hib meningitis: Signs and symptoms consistent with meningitis (fever and convulsion with or without neck rigidity and Kernig's sign) and CSF finding consistent with meningitis [>100 polymorphonuclear leukocytes (PMN) per cubic mm of CSF] and either Hib cultured from blood or cerebro-spinal fluid (CSF) or detection of Hib antigen from CSF or blood by latex agglutination test.

Sample size:

Sample sizes have been calculated using standard formula for matched-pair case control studies and assuming that the vaccine efficacy will be similar to what was observed in the Gambia. In the Gambian trial, Mulholland et al (1997) found that the efficacy of the vaccine was 95% for the prevention of all invasive Hib disease and 100% for the prevention of Hib pneumonia after three doses of PRP-T vaccine, and 21.1% for the prevention of radiologically defined pneumonia at any time after enrollment. We anticipate decrease of Hib pneumonia by 75%, Hib meningitis by 85%, and radiologically defined pneumonia by 20% in the proposed study.

In the first step, the number of Hib pneumonia cases and controls that will be required have been calculated assuming a 50% exposure of the controls to three doses of the Hib vaccine. For a type I error of 0.05, type II error of 0.2, assumed correlation coefficient of 0.1 for exposure between matched cases and controls, odds ratio of 4.0, and for one control per case patient, the sample size requirement will be 42 cases and 42 controls. If two controls are selected per case, 31 cases and 62 controls will be required.

Similar calculations can be done for other outcomes. The following table provides the number of cases and controls required for the three outcomes to be measured:

Outcome	P_0	Type I error	Type II error	PHI	M	OR	Cases	Controls
Hib pneumonia	0.5	0.05	0.2	0.1	2	4.0	31	62
Radiologically defined pneumonia	0.5	0.05	0.2	0.1	4	1.25	830	3320
Hib meningitis	0.5	0.05	0.2	0.1	4	6.7	15	60
Note: P0=Probability of exposure in controls, PHI=Correlation coefficient for exposure between matched cases and controls, M=Number of matched controls per case patient, OR=Odds Ratio.								

In the next step, the size of the study population in which the vaccine will have to be introduced has been estimated using community-based pneumonia morbidity and hospital-based pneumonia and meningitis etiology data from Bangladesh. We realize that despite strengthening of surveillance, many pneumonia and meningitis cases may not report to the study centres and thus, we will not ascertain all the pneumonia and meningitis cases. Some cases will be treated by private providers, in peripheral facilities, and by traditional and spiritual healers. This should not pose any problem as long as there is no bias in reporting of pneumonia and meningitis cases between the vaccinated and non-vaccinated groups and the number of cases identified is sufficient to detect the assumed differences between the vaccinated and non-vaccinated groups.

The reported incidence rate of ALRI in <2 children in Bangladesh is 30 episodes per 100 child years observed. We wanted to be conservative and assumed a pneumonia incidence rate of 25/100 child

years. If 3% of these episodes are due to Hib, then the expected rate of Hib pneumonia in the non-vaccinated population will be 0.0075/child/year and in the vaccinated population will be 0.001875.

75% protection implies a relative risk (in this case odds ratio) of 4.0 in the non-vaccinated group. That means of the 31 cases assembled, about 24 to 25 are expected to be from the non-vaccinated group and about 6 to 7 from the vaccinees. If we apply the expected rates of Hib pneumonia in vaccinees and assume that we will be able to ascertain only 40% of the cases in the study population, to assemble 7 cases in vaccinees, about 9334 infants will have to vaccinated. Since only about 75% of the infants who receive the first dose complete the three doses, about 12,445 infants should be attempted vaccination. Similarly, to assemble 25 cases from non-vacinees there has to be about 8,334 non-recipients of Hib vaccine in the study population.

To detect 85% protection from Hib meningitis we need to identify about 15 cases of which 12-13 are assumed to be in the non-vaccinated group and 2-3 in the vaccinated group. The Gambian study followed 42,828 infants for 5-36 months of which half were vaccinated. The study identified 28 Hib meningitis cases. Assuming that the incidence and care seeking pattern for meningitis are similar in Bangladesh and Gambia, 15 cases are expected in 23,000 infants.

Similar calculation can be done for radiologically defined pneumonia assuming that about 15-20% of the severe pneumonia cases will have radiological evidence of pneumonia (estimated incidence = 4-5/100 child year). At this rate, a cohort of 23,000 infants should provide adequate number of cases.

As the infant mortality rate (IMR) is high, to have a study cohort of 23,000 infants in a year, a birth cohort of about 25,000 is needed. The estimated annual birth cohort of the study area is about 27,000 which should be adequate for assessment of all the outcomes.

Vaccination and record keeping:

All Hib vaccinations will take place through the existing clinics of Radda MCH-FP Centre. During the study period, the DPT vaccine in these clinics will be replaced by Hib conjugate, adsorbed diphtheria, tetanus and pertussis vaccine (TetractHib).

All children presenting for vaccination at one of the Radda immunization clinics in Zones 7 & 8 will be eligible for enrollment. Children who have not completed a primary DTP series (i.e., three doses of DTP) will be enrolled and will receive the study vaccine. If a child has received one or two doses of DTP vaccine before enrollment in the study, that child will receive only enough study vaccine to complete the primary DTP series. This policy will result in a minimum of confusion with vaccine distribution and necessitate the use of only one type of vaccine in a particular clinic. While the majority of children enrolled in the study will receive three doses of study vaccine, some children will receive only one or two doses of study vaccine. The effect of less than complete vaccination on the study outcomes will be examined during analysis.

Guardians of all infants coming to these clinics for immunization will be approached by a study assistant and take verbal consent to give TetractHib to the child instead of DPT. Those who will give consent will receive TetractHib, and those who will not give consent will receive regular DPT. Vaccination records will include: EPI card number, child's name, date of birth, gender, father's name, address and date of immunization (please see Form 1, Annex 2). These information will be recorded in the EPI registrar currently in place. The addresses of vaccinated children will be carefully recorded so that the children can be located if needed.. A research assistant will extract these data from the EPI registrars and will enter in a computer to create a master database of all Hib vaccine recipients. This will help determine the vaccination status of Hib cases as they occur.

Vaccine type and storage

We will use liquid Tetract-Hib (DTP-PRP-T) vaccine manufactured by PMC. This vaccine has been shown to be safe and immunogenic in both animal and human studies.

Vaccines willll be stored at a temperature ranging from +2°C to +8°C (in a refrigerator) (to be adapted according to product). Temperature should be monitored and documented on the appropriate form during the entire trial.

In case of deep freezing or accidental disruption of the cold chain, vaccines will not be administered and the investigator or the responsible person should contact the API to receive further instructions.

Surveillance for pneumonia and meningitis:

The vaccine will be given over a year period. Surveillance will be instituted which will continue for a second year to allow evaluation of the effectiveness of the vaccine during the first two years of life. There will be some promotional activities in the study communities to improve care-seeking for severe pneumonia and suspected meningitis. The promotional activity will target all children <2 years of age regardless of their vaccination status.

Many of the pneumonia cases will be presented in peripheral facilities where x-rays and blood culture will not be feasible. To maximize case ascertainment, all curative care providers in the trial area will be requested to participate in the surveillance. The providers will be given standardized case definitions of severe pneumonia and clinical meningitis, will be requested to use the standardized definitions and keep simple records of all such cases. They will be requested to refer all severe pneumonia and suspected meningitis cases to the Shishu Hospital. The study physicians will periodically visit these providers/facilities to assess the quality of diagnosis made and to reinforce use of case definitions and referral of all suspected meningitis and severe pneumonia cases to Shishu Hospital. If parents/guardian of a potential case can not go to Shishu Hospital for lack of conveyance, his/her conveyance will be provided from the project fund.

Evaluation of completeness of surveillance:

To assess the completeness of pneumonia and meningitis surveillance, a study physician will visit each curative care provider/facility once in a week. The physician will review the record of the providers/facility to collect information on the number of severe pneumonia and meningitis cases seen and the number of cases referred by them to Shishu Hospital. These data will be analyzed fortnightly to identify areas with low case load and/or low level of referral. The data will be matched with the Shishu Hospital attendance registrar to estimate what proportion of the referred cases actually complied with the referral. This will help identify areas with low level of compliance with referral. Efforts will be made where needed to improve case identification, referral and compliance with referral.

Case Ascertainment and Selection of Cases:

Case detection will be carried out in the Shishu Hospital. A study Medical Officer will check the residence and age of all 'potential' Hib infection cases between 3 to 23 months of age (the actual eligible age range for enrollment will depend on the week of the study -- please see form 3) presenting to the Shishu Hospital. The lower age limit of 3 months is chosen because given the immunization schedule in Bangladesh (6, 10, 14 weeks) infants below this age are unlikely to receive at least two doses which is needed for protection. All potential cases fulfilling the residency and age criteria will be enrolled in the clinical and microbiological surveillance after obtaining consent from their parents/guardians.

Potential cases from the study area with signs and symptoms suggestive of meningitis will be admitted to the Shishu Hospital and subjected to a lumbar puncture. Blood samples will be collected from all severe pneumonia and meningitis cases. All blood and CSF specimens will be sent to the Shishu Hospital laboratory for microscopy and culture. In addition, each patient will have a chest x-ray done. Blood and CSF samples will be saved for antigen detection using latex agglutination test. Clinical and laboratory data will be collected from all enrolled cases using standardized forms. Data from each potential case will be carefully reviewed by a study physician who will make the final categorization. This physician will not have access to vaccination status information of the cases since this information can influence the diagnosis.

Parents/guardians of all cases with Hib pneumonia, Hib meningitis, and radiologically defined pneumonia from the study area will be approached in person by a research assistant to participate in the case-control study. A verbal consent statement will be obtained from parents of cases. Data collected from each case child will include demographic, socioeconomic, and other risk factor variables (e.g., mother's education and occupation, father's education and occupation, type of housing, family size, monthly household expenditure, ages of siblings alive, ages of siblings dead, parental smoking), type of heath care provider (public, NGO, private) and vaccination status. Vaccination status will be verified for all case children by checking their immunization cards or by checking the provider's record. Since the EPI card retention rate in the study area has been reported to be more than 90% (Dr. Bushra Amena, Program Manager, Radda MCH-FP Centre, personal communication), it should not be difficult to determine the immunization status of children and the

source of immunization. Only children who received two doses of the vaccine □14 days before the date of hospital admission or date of positive culture results will be considered as vaccinated. In addition, the attending physicians will be asked about any underlying conditions that might predispose the child to serious infection, regardless of vaccination status.

All patients presenting to the Dhaka Shishu Hospital with sign and symptoms of pneumonia will receive all necessary care including medications free of cost.

Blood samples handling

1. Obtaining Serum Samples

About 2.5 ml of blood will be collected in vacutainer tubes. This will be used for blood culture and other clinical pathological tests for the diagnosis of disease and its treatment.

Immediately prior to blood sampling, the person in charge of the procedure will verify subject's identity and will check that the name and study ID on the laboratory request form are those of the subject. Then, the subject's name and ID# will be recorded on all labels of the corresponding band. One label will be affixed onto the vacutainer tube immediately prior to blood sample drawing.

The blood collected will immediately be transferred to the Dhaka Shishu Hospital Laboratory for processing.

There will be two aliquots, one for the use in DSH laboratory and the second one for quality check in the reference laboratory of Johns Hopkins University.

The analysis will be performed initially in Dhaka Shishu Hospital laboratory for microbiologic, antigen detection and serologic examination. A sub-sample of the serum samples will be sent to the Johns Hopkins University for quality check.

Handling Serum Samples

A. Aliquoting: Aliquoting will be performed subject by subject to avoid mixing blood tubes. The following procedure must be followed:

1. After centrifugation, the person responsible for aliquoting should carry out the operation by taking the tubes one by one from the centrifuge.
2. The operator will only place in a rack two Nunc tubes (Nalgene cryovials) for aliquoting and will affix the completed labels onto the tubes.
3. Aliquoting will be performed according to the required volume, the number of tubes, and the priority of titrations.
4. Pre-labelled Nunc tubes that would not have been filled for lack of serum will be removed.
5. The subject's identification number and initials, the date of sampling, the number of aliquots

obtained, and the date and time of aliquoting will be specified on the sample identification list.

6. Comments may be made on the quality of samples (haemolysed, contaminated, etc.).
7. The next sample will be taken out of the centrifuge only when the steps 1 through 6 are completed.

B. Storage Conditions: Each aliquot will be frozen at -20°C to -80°C. The temperature will be monitored and documented on the appropriate form during the entire trial.

Selection of control children:

Age and gender matched control children will be randomly selected from the study area for each case of Hib pneumonia, Hib meningitis, and radiologically defined pneumonia. The number of controls will be two for each Hib pneumonia case, four for each radiologically defined pneumonia case, and four for each Hib meningitis case.

To ensure that the controls are representative of the study population which generates the cases, they will be selected randomly. To facilitate random selection, using GIS software and maps of zones 7 and 8, the study area will be divided into 400-600 squares by overlay grids before the beginning of the study. The north-south columns of the grid will be numbered alphabetically while the east-west rows numerically. Each cluster (square) will thus have an unique identifier. Since one control will be selected from a cluster at a time, if four controls are needed, four clusters will be randomly selected. At the first stage in the selection of controls the required number of clusters will be randomly selected [this is not probability proportionate to size (PPS) and it assumes uniform population density - but will ensure that there is no areal bias]. Once a cluster is selected , that square will be further divided into smaller clusters of approximately 10,000 sq meters each using the GIS software. One of the smaller cluster will then be randomly selected. A team of one male and one female research assistants will first attempt to identify the selected cluster on the ground using a geo-positioning system (GPS) unit. If this cluster is physically unapproachable or not-identifiable then a second small cluster will be selected using the same method. Once a small cluster is physically located, the team will list all the households in that cluster and randomly select one as the starting household. The team will move from one household to the next nearest (front door to front door) household until they find an eligible control child whose parents/guardian agree to have the child enrolled in the study. We expect that this sampling method will be feasible and provide an unbiased sample of controls. However, we may need to make adjustments based on initial experience and after discussions with relevant experts.

The control child should be of the same sex as the case and as tightly matched for age as possible but must be within ±1 month of age of the case child. Verbal consent will be obtained from the parents of control children. After obtaining consent, the same information collected from the case children will also be collected for each control child. The information on the vaccination status of the control children will be collected using the same method as in case children only after the control children are finally selected.

Sub-study to Assess Immunogenecity and the Persistence of Anti-PRP Antibody:

We plan to assess the immunogenecity and persistence of anti-PRP antibody after receiving three doses of TetractHib at 6, 10, and 14 weeks of age in a sample of children. The primary outcome will be the proportion of infants with serum anti-polyribosylribitol phosphate (PRP) concentrations of 0.15 μg/ml or more at 9 and 15 months of age since this level of antibody is considered protective. On the basis of data generated in other settings we assume that 95% of the infants receiving the full three-dose primary immunization schedule will maintain sero-protection at the age of 9 months and at least 80% will maintain sero-protection at the age of 15 months. The vaccine will be considered adequate if at least 70% of the infants maintain sero-protection at the age of 15 months. To determine this with 95% significance level, 61 infants are needed. We will enroll 70 infants to allow for 10-15% drop out.

The immunogenecity study will be done only in the main clinic of Radda MCH-FP Centre beginning in the second month of the study. Written consent will be obtained from parents/ guardians of infants eligible for DPT vaccination. About 2 ml of blood will be collected before the first dose, one month after the third dose, at 9 months and at 15 months of age. Serum will be separated and saved in -20° C until the antibody assays are done.

Laboratory method:

Blood culture will be done by using trypticase soy broth. After inoculation of blood, the broth will be incubated at 37 degree Celcius for 7 days with subcultures on McConkey and chocolate agar plates on the day 2, 4 and 7 (Washington and Ilstrup, 1986).

CSF will be analysed for cytology (total and differentional count) and biochemistry (protein and sugar) following the standard methods. Portion of the fluid will be inoculated on chocolate, blood and McConkey agar plates and incubated at 37 degree Celcius for 24-48 hours.

Identification of *H. influenzae*: The suspected colonies of *H. influenzae* will be Gram stained to see the Gram negative cocco bacilli. The organism will finally be identified on the basis X and V factor requirements by putting discs inpregnated with X and XV factor (Difco) on Tryricase soy agar swabbed with the suspension (0.5 Mcfarland density) of *H. influenzae* (Manual of Clinical Microiology). *H. inlfuenzae* will be serotyped and identified as type b by slide agglutination with the specific antisera against it.

Antigen detection test: The CSF specimens with >100 leucocytes/cmm with polymorph predominance, raised protein and reduced sugar do not show any bacteria by Gram stains, the fluid will be subjected to antigen detection test. Antigen of Hib will be detected by slide agglutination test of heat treated CSF specimen with the latex particles, sensitised with the Hib antisera.

The anti-PRP antibody level in serum samples from infants in the sub-study on immunogenecity will be measured using a quantitative ELISA method (Phipps et al., 1990).

Adverse events reporting

Definitions

* Adverse event (AE):
  Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

* Serious adverse event (SAE):
  A serious adverse event (experience) is any untoward medical occurrence that at any dose:
  - results in death
  - is life-threatening. The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event, which hypothetically might have cause death, if it were more severe.
  - requires inpatient hospitalization or prolongation of existing hospitalization
  - results in persistent or significant disability/incapacity.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.

* Adverse drug reaction (ADR):
All noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions. The phrase "responses to a medicinal product" means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

* Unexpected adverse drug reaction:
An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved investigational medicinal product).

Collection and follow-up of adverse events

Adverse events will be collected as indicated in the Case Report Forms (CRF).

Adverse events likely to be related to the product, whether serious or not, which persist at the end of

the trial will be followed up by the investigator until their complete disappearance. The investigator will inform the Medical Director or the Monitor of the date of final disappearance of the adverse event and will document it on a correction sheet.
Moreover, any serious adverse event likely to be related to the product and occurring after trial termination will be reported by the investigator to AVENTIS PASTEUR INTERNATIONAL according to the procedure described below.

Reporting of Serious Adverse Events

Every serious adverse event occurring throughout the trial will be reported to the Chairman, ERC, ICDDR,B, and the Medical Director, API by the investigator as soon as he is alerted of it, i.e., within 24 hours, even if the investigator considers that the adverse event is not related to treatment:
Notification should be made:

-by phone; then the investigator should immediately send the completed alert form to ICDDR,B and API
or by faxing the completed alert form.

The alert form will be sent to:

Chairman, ERC, ICDDR,B, and
The Medical Director: Dr François Bompart, AVENTIS PASTEUR INTERNATIONAL France Tel: 33 4 37 37 71 34 / Fax: 33 4 37 37 78 30. In his absence, to the person in charge of the pharmacovigilance of the product: Dr. Françoise PIGNATO, AVENTIS PASTEUR INTERNATIONAL, France. Tel: 33 4 37 37 77 94 / Fax: 33 4 37 37 71 32

The investigator will then fill in the SAE reporting form as soon as possible, i.e., within five working days or seven calendar days. This form will be signed by the investigator and sent to the Chairman, ERC, ICDDR,B, and by fax or express mail to the Medical Director of API or the person in charge of the pharmacovigilance of the product.

Any relevant information concerning the adverse event that becomes available after the SAE reporting form has been sent (outcome, precise description of medical history, results of the investigation, copy of hospitalization report, etc.) will be forwarded as soon as possible to the Medical Director. The anonymity of the subjects shall be respected when forwarding this information.

The causal relationship between the SAE and the product will first be evaluated by the investigator with the following scale:

0 . NO RELATIONSHIP :

Inconsistent timely relationship (too long interval between injection and onset of symptom or symptom appeared before injection) or evidence that a symptom is definitely related to an etiology other than the study drug.

1 . POSSIBLE :

Has a timely relationship to the study drug; however, a potential alternative etiology which may

be responsible for the symptom has not been investigated.

2. PROBABLE :
   Has a relevant timely relationship to the study drug, a suggestive symptomatology and a potential alternative etiology is not apparent.

3. DEFINITE :
   Has a relevant timely relationship to the study drug and no alternative etiology is apparent (after investigation other etiologies have been ruled out) or positive rechallenge with a suggestive symptomatology or local reaction at the site of injection.

Then, according to the available information and the current medical knowledge, the Medical Director and the person in charge of the pharmacovigilance of the product will also assess the causal relationship to the product. The decision to modify or discontinue the trial, or to break individual or all study codes may be made after mutual agreement between the sponsor and the investigator.

Blood sampling

A blood sample to be taken as soon as possible might be requested in case of serious adverse event if it can help in analyzing the SAE. Five milliliters of blood will be taken in a dry tube.

Hospital care

All children under 2 years of age from the study area will with suspected meningitis or pneumonia will get medical care (including diagnostics and medicine) at Dhaka Shishu Hospital free of cost. The medical care will be provided by the attending physician as necessary. The physician will take decision to start treatment solely on the welfare of the patient. But attempts should be made to collect specimens necessary for the study before starting any antibiotic.

Data entry and management:

All questionnaires and data forms will be reviewed by the investigators for accuracy, consistency and completeness. Whenever necessary, the study assistants will make additional visits to clarify inconsistencies or collect missing information. After editing, the data will be entered in databases using on-line custom-designed data entry programs. Necessary range and consistency checks will be in-built. Data will be periodically checked by running and reviewing frequency distributions and cross-tabulations.

Data Analysis:

For descriptive purposes, the relative frequencies of the demographic, socioeconomic, matching, clinical, and other study variables will be obtained for the total group and case-control sub-groups. Statistical significance of differences and association between cases and controls for these variables will be assessed with t-tests for continuous variables and chi-squared test analysis for categorical variables. The ninety-five percent confidence interval around the Mantel-Hanenszel estimate of the odds ratio will be derived using the test-based method of Miettinen (Schlesselman, 1982).

To adjust for multiple confounding variables as well as to evaluate effect modification, conditional logistic regression method designed for matched studies will be used (Breslow and Day, 1980). This analysis will provide adjusted odds ratios and its confidence intervals. Since perfect age-matching may be difficult, age may remain a potential confounder because it is related to both disease and likelihood of vaccination. To handle this potential problem, vaccine efficacy estimates will be age adjusted by including a linear term for age in month in all models. Estimates of vaccine effectiveness will be calculated using the formula: VE=(1-matched OR)X100, where OR indicates the odds ratio for Hib vaccination of cases and controls (Smith et al., 1984).

The primary outcome of the immunogenecity component of the study will be the proportion of children that maintained protective Hib antibody response at 15 months of age. The proportion with concentration above 0.15 mcg/ml will be calculated, along with standard 95% confidence intervals. Geometric mean concentrations will also be estimated with the corresponding appropriate confidence intervals. Similar calculations will be made for the responses at each serum sample time in order to characterize the immunogenicity dynamics over time.

Data from the surveillance component of the study will be analyzed to quantify the magnitude of the incidence of Hib infections and to determine the clinical and epidemiological characteristics of Hib pneumonia and meningitis.

UTILIZATION OF RESULTS: Describe in brief how you perceive that the results from this study may contribute to health development and health policy of the Country.

This trial will provide data on effectiveness of Hib vaccine in Bangladeshi children which is needed for a policy decision regarding its inclusion in routine EPI. If it can be shown that Hib vaccine substantially reduces pneumonia which is the most important cause of death in <2 children in Bangladesh, its inclusion in routine EPI will considerably improve child health and survival.

FACILITIES: Resources equipment chemicals subjects (human, animal) etc. required for the study.

Facilities available:

Zones 7 and 8 of Dhaka city corporation together provide an estimated annual birth cohort of 27,000. This cohort size will be adequate for the study. About half of the babies in the study area should receive the Hib conjugate vaccine (TetractHib). Introduction of the vaccine through the seven existing clinics of Radda MCH-FP Centre should ensure this. ICDDR,B will work with all the major clinical care providers of zones 7 and 8 of DCC to set up pneumonia and meningitis surveillance. Case ascertainment will be done in Shishu Hospital. The out-patient, in-patient and microbiology laboratory facilities of Shishu Hospital will be used. The immunological assay will be done in the Immunology laboratory of ICDDR,B.

Aventis Pasteur International (API), Lyon, France, one of the companies that produces Hib conjugate vaccine confirmed that the company will make a donation to ICDDR,B up to 60,000 doses of Hib conjugate, absorbed diphtheria, tetanus and pertussis combined vaccine to be used in this study. The market price of this vaccine is about US$ 400,000.

Additional facilities required:

Resources will be required to pay for the time devoted by the investigators and other staff of ICDDR,B, Shishu Hospital, and Radda MCH-FP Centre to this project. Additional staff will have to be recruited. Patient cost, drug cost, travel cost, cost of equipment, reagents, other supplies, and other services will have to be covered (please see the budget).

Ethical Assurance for Protection of Human Rights

Describe in the space provided the justifications for conducting this research in human subjects. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how subject's rights are protected and if there is any benefit or risk to each subject of the study.

The vaccine that will be used in the proposed study has been shown to be safe and efficacious in preventing Hib disease in both developed and developing countries. This is a licensed vaccine and it is routinely used in many developed countries and is also approved for use in Bangladesh. The vaccine is not associated with any serious side effect. The research methods outlined in the protocol do not create any major potential risks. There is a very small risk of infection through blood collection, though all possible aseptic precautions will be taken. No sensitive information will be collected in this study.

ETHICAL ISSUES

Developed countries, such as the United States, have justified the introduction of Hib vaccine based on its impact against Hib meningitis. Hib meningitis caused a considerable amount of morbidity and

mortality relative to other causes of childhood illness, and the sequelae of meningitis (mental retardation, seizures, and deafness) resulted in enormous costs related to special education, long-term medical care, and disability. In many developing countries, however, these conditions may not hold. In areas with high IMRs, meningitis may be a relatively unimportant cause of morbidity and mortality. Additionally, in financially-poor countries, few resources may be devoted to providing long-term care and education to survivors of meningitis.

In contrast to meningitis, in many developing countries, pneumonia is the most common cause of childhood morbidity and mortality, resulting in significant medical costs related to provision of acute care. Consequently, if Hib vaccine prevents a significant proportion of pneumonia, a more powerful justification is found for vaccine introduction in the EPI programme. To date, though, only one study, in Gambia, has documented an impact of Hib vaccine against pneumonia. It is entirely unclear whether Hib vaccine will have an impact against pneumonia in other parts of the world, including Bangladesh.

Currently, Hib vaccine is not routinely provided as part of the EPI schedule in Bangladesh because of a lack of data on the impact of disease due to Hib. Additionally, based on existing data, the Bangladesh Government has no definite plan to provide Hib vaccine as part of routine EPI.

With the above as background, we believe our study meets the following ethical criteria:

1. *Patient risk.* The study is designed so that no study participant will receive any extra injection. Thus, there is no additional risk for study participants other than that associated with Hib vaccine itself. The vaccine which we will use has been demonstrated to be safe, with minimal side effects in actual field testing in developing countries (Mulholland, et al., 1997).
2. *Autonomy.* Informed consent will be obtained from a parent or guardian of all study participants. Parents will be informed that they are free to elect to have their child not participate in the study, and that medical care for their child will not suffer if they elect not to participate (Appendix I) and their child will get regular DPT vaccine if they are not willing to participate.
3. *Beneficence.* The study addresses beneficence in several ways:
    - Intervention children will benefit from receipt of Hib vaccine. If Hib vaccine is demonstrated to be efficacious in preventing the study outcomes, policy recommendation will be made to include this vaccine in routine EPI programme.
    - All study participants, as well as the children in the neighbourhood will benefit from improved diagnosis, referral (and reimbursement of conveyance to the needy people), and treatment of pneumonia and meningitis in a specialized hospital —benefits which would not be available to those who can not attend the Shishu Hospital due to financial constraints.
    - The study does not collect redundant information. In particular, the study does not attempt to measure whether Hib vaccine is efficacious against Hib disease, as this information is readily available in the medical literature. Instead, vaccine will be used to determine the burden of

disease due to Hib infection, and effectiveness of Hib vaccine when introduced through existing service delivery program, information on which do not exist for Southeast Asia.

LITERATURE CITED: Vancouver style to be followed. Please consult J. Annals of Internal Medicine 1988 108: 258-265. All citations should be .referenced in the reference section/bibliography.

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Annex – 2 (1)

Invitation to accept *haemophilus influenzae* type b (Hib) vaccine
CONSENT FORM

Title: An effectiveness study of *Haemophilus Influenzae* type b vaccine.

Principal investigator: Dr. Abdullah H. Baqui, Head, Child Health Programme, Public Health Sciences Division, ICDDR,B., Mohakhali, Dhaka – 1212.

Study location: Mirpur, Dhaka, Bangladesh.

Pneumonia is a major killer disease in the children under five years of age. On average, every year about a third of the under 2 year old children develop pneumonia. Pneumonia accounts for about 25% of <5 deaths and about 40% of infant deaths. *Haemophilus Influenzae* type b (Hib) is the second most important bacterial cause of childhood pneumonia in Bangladesh. *Haemophilus Influenzae* type b also causes meningitis which is a deadly disease in young children.

As a parent / legal guardian of a child, you are requested to allow your child to participate in a study to assess the effectiveness of a vaccine against *Haemophilus Influenzae* type b. This is a Hib conjugate, adsorbed diphtheria, tetanus, and pertussis combined vaccine (TetractHib). This vaccine is licensed and is being widely used in many countries. It is now also being provided in Bangladesh by private providers. Many studies have been shown this vaccine to be safe and effective. There is no or very little chance of side effects of this vaccine, those are not different from the side effects of other vaccines and occur very rarely. There is no chance of any other physical risk from participating in this study.

Before participation, you should understand that: (1) participation of your child in this study is completely voluntary. You may not allow your child to participate at all, or may withdraw from the study at any time. This will not prevent you, your child or any of your family members from getting the usual care you receive from ICDDR,B, Radda-Barnen, or Dhaka Shishu Hospital facilities; (2) your child may be benefited from this study by getting immunity against *Haemophilus Influenzae* type b; (3) The findings from this study will allow us to make policy decision regarding inclusion of this vaccine in routine EPI.

If your child participates in this study, he/she will get the vaccines given under EPI programme, the DPT vaccine will be replaced by TetractHib (combined Hib conjugate with diphtheria, tetanus and pertussis vaccines). If you decide not to participate, your child will get the normal DPT vaccine. if your child develops any sign of pneumonia or mningitis, you should take your child to the Dhaka Shishu Hospital. Your child will get all proper treatment and investigations needed free of cost.

Your identity will remain strictly confidential, but the records may be reviewed by representatives of the authorities supporting this study. Thank you very much for your co-operation.

CONSENT OF PARENT /LEGAL GUARDIAN

Would you like to allow your child to participate in this study? YES / NO.

(For vaccinator: If YES, Please take initials of the guardian on the EPI register, and proceed with TetractHib. Thank you)

শিশুকে টিকা দেবার সম্মতিপত্র

প্রজেক্টের নাম: **An effectiveness study of *Haemophilus Influenzae* type b vaccine.**

Principal investigator: Dr. Abdullah H. Baqui, Head, Child Health Programme, Public Health Sciences Division, ICDDR,B., Mohakhali, Dhaka – 1212.

Study location: Mirpur, Dhaka, Bangladesh.

৫ বছরের কম বয়সী শিশুদের মৃত্যুর প্রধান কারণ হলো শিশুদের নিউমোনিয়া। আমাদের দেশে প্রতি বছর গড়ে প্রতি তিনজন ২ বছরের কম বয়সী শিশুর মধ্যে একজন নিউমোনিয়ায় আক্রান্ত হয়। আমাদের দেশে যতো শিশু মারা যায় তার মধ্যে ৫ বছরের কম বয়সী শিশুদের শতকরা ২৫ জন এবং ১ বছরের কম বয়সী শিশুদের শতকরা ৪০ জন মারা যায় নিউমোনিয়ার জন্য। ***Haemophilus Influenzae* type b** (Hib) নামে এক ধরণের জীবাণু আমাদের দেশে শিশুদের নিউমোনিয়ার একটি প্রধান কারণ। এই জীবাণুটি মেনিনজাইটিস নামে একটি মস্তিস্কের অসুখও সৃষ্টি করে যা শিশুদের মারা যাওয়ার আরেকটি প্রধান কারণ।

আমরা Hib নামের এই জীবাণুটির প্রতিরোধে একটি টিকা কতোখানি কার্য্যকর তা জানার জন্য একটি গবেষণা করছি। এই টিকাটি ডিপথেরিয়া, টিটেনাস ও হুপিং কাশির (DPT) টিকার সাথে দেয়া হয় এবং পৃথিবীর বিভিন্ন দেশে ব্যবহার হচ্ছে। বাংলাদেশেও এটি অনেক ডাক্তার শিশুদের দিয়ে থাকেন। এই টিকাটির পার্শ্ব-প্রতিক্রিয়া প্রায় নেই বললেই চলে এবং অন্য টিকাগুলির চেয়ে নতুন বা আলাদা কিছু নয়। এবং এই টিকা দেবার ফলে অন্য কোনোরকম শারীরিক সমস্যা হবার সম্ভাবনা নেই।

আপনি একজন শিশুর পিতা/মাতা/অভিভাবক হিসেবে আমরা আপনাকে অনুরোধ করছি যে, আপনি আপনার শিশুকে এই গবেষণা কাজে অংশগ্রহণ করতে অনুমতি দিবেন। এটা আপনার বোঝা অত্যন্ত প্রয়োজন যে, (ক) এই গবেষণায় আপনার শিশুর অংশগ্রহণ সম্পূর্ণ স্বেচ্ছামূলক, (খ) আপনি যে কোনো সময়ে আপনার শিশুকে এই গবেষণা হতে সরিয়ে নিতে পারেন। যে কোনো ক্ষেত্রেই, আই-সি-ডি-ডি-আর, বি (কলেরা হাসপাতাল), রাড্ডা-বার্নেন, বা ঢাকা শিশু হাসপাতালে আপনার শিশুর বা আপনার পরিবারের কারো কোনো সময়ে প্রয়োজনীয় চিকিৎসার কোনো ত্রুটি হবে না, (গ) আপনার শিশু সরসরি এই টিকার দ্বারা উপকৃত হবে, এবং (ঘ) এই গবেষণায় অর্জিত তথ্য আমাদেরকে দেশের শিশুদের "ই-পি-আই"-এর মাধ্যমে এই টিকাটি দেয়ার বিষয়ে সিদ্ধান্ত নিতে সাহায্য করবে।

যদি আপনি আপনার শিশুকে এই গবেষণায় অংশ নিতে অনুমতি দেন তবে সে "ই-পি-আই" কর্মসূচীর সবগুলি টিকা-ই পাবে, শুধুমাত্র "ডি-পি-টি" টিকার বদলে অন্য একটি টিকা দেয়া হবে যাতে "ডি-পি-টি" এবং Hib-এর টিকা একত্রিত করা আছে। আপনি যদি আপনার শিশুকে এতে অংশ নিতে না দেন তবে আপনার শিশু সাধারণ "ডি-পি-টি" টিকা পাবে। আপনার শিশু যে টিকা-ই পাক না কেন, সে যদি কোনো সময় নিউমোনিয়ায় আক্রান্ত হয় - আপনি/ আপনারা তাকে ঢাকা শিশু হাসপাতালে নিয়ে যাবেন। আমরা বিনামূল্যে তার সব রকম চিকিৎসা করবো।

এই গবেষণায় অংশগ্রহণ করলে আপনার বা আপনার শিশুর পরিচয় প্রকাশ হয়ে পড়ার কোনো সম্ভাবনা নেই। আপনার বা আপনার শিশুর নাম বা পরিচিতিমূলক কোনো তথ্য বাইরে কোথাও প্রকাশ করা হবে না। শুধুমাত্র কাজের প্রয়োজনে আমাদের নিজস্ব প্রতিনিধি এই সব কাগজপত্র পরীক্ষা করতে পারেন।

অভিভাবকের সম্মতি:

আপনি কি আপনার শিশুকে এই গবেষণায় অংশ নিতে অনুমতি দিলেন?হ্যাঁ / না

Vaccinator: যদি অভিভাবক সম্মতি দিয়ে থাকেন তবে সব তথ্য লিপিবদ্ধ করুন, রেজিষ্টার বইয়ে তাঁর সই নিন এবং শিশুকে TetractHib টিকা দিন।

আপনার সহযোগিতার জন্য আপনাকে অসংখ্য ধন্যবাদ।

Annex – 2 (2)

CONSENT FORM FOR ENROLLMENT OF POTENTIAL CASES IN PNEUMONIA AND MENINGITIS SURVEILLANCE

Title: **An effectiveness study of *Haemophylus Influenzae* type b vaccine**

Principal investigator: Dr. Abdullah H. Baqui, Head, Child Health Programme, Public Health Sciences Division, ICDDR,B., Mohakhali, Dhaka – 1212.

Study location: Mirpur, Dhaka, Bangladesh.

Pneumonia is a major killer disease in the children under five years of age. On average, every year about a third of the under 2 year old children develop pneumonia. Pneumonia accounts for about 25% of <5 deaths and about 40% of infant deaths. *Haemophilus Influenzae* type b (Hib) is the second most important bacterial cause of childhood pneumonia in Bangladesh. *Haemophilus Influenzae* type b also causes meningitis which is a deadly disease in young children.

Your child is suffering from pneumonia/meningitis. As part of a Hib vaccine effectiveness study, we are conducting surveillance to identify Hib pneumonia, x-ray defined pneumonia, and Hib meningitis cases. We are requesting you to allow your child to participate in this study. If you agree to participate, we will ask you some questions regarding the health and illness, and immunisation status of your child and some questions regarding your family's health and illness; take chest X-rays, about 2.5 ml of blood, and if necessary, cerebrospinal fluid from your child. It will take about 15-20 minutes for you to answer the questions. As you are aware, blood and other tests are normally necessary for proper treatment of your child's disease. There is very little risk of injury to your child, and proper precautions will be taken to minimise any risk. You may decide not to participate in this study at all. Your decision not to participate will not affect your child's right of getting proper treatment here, but your participation will help us in making policy decision regarding inclusion of the vaccine in the routine EPI programme.

If you agree to allow your child to participate, your child's identity will remain strictly confidential, but the records may be reviewed by representatives of the authorities supporting this study. Thank you very much for your co-operation.

CONSENT OF PARENT/ LEGAL GUARDIAN.

The study has been completely described to me. All of my questions were answered satisfactorily. I understand that participation of my child is completely voluntary. By signing this form, I allow my child (name) _____, (age) _____ months as a participant of this study. I also allow the investigators to perform all necessary investigations, X-rays, blood and CSF tests.

Signature : _____ (Father / Mother / Legal guardian). Date: _____

Name in Full:_____

Name of the child (ALL CAPS): _____

Age: _____ mo. Date of birth: ____/____/_____

Witness: Name: _____ Signature: _____

Person taking this consent: _____ Study ID #: _____

(**For study Medical Officer**: If YES, enrol the child and ensure that all necessary data and specimens have been collected. If NO, thank the guardian of the child and extract the needed information from patient record at a later time)

শিশুকে গবেষণায় অংশ নিতে অনুমতি দেবার সম্মতিপত্র

(For Cases of Hib disease)

প্রজেক্টের নাম: **An effectiveness study of *Haemophilus Influenzae* type b vaccine.**

Principal investigator: Dr. Abdullah H. Baqui, Head, Child Health Programme, Public Health Sciences Division, ICDDR,B., Mohakhali, Dhaka – 1212.

Study location: Mirpur, Dhaka, Bangladesh.

৫ বছরের কম বয়সী শিশুদের মৃত্যুর প্রধান কারণ হলো শিশুদের নিউমোনিয়া। আমাদের দেশে প্রতি বছর গড়ে প্রতি তিনজন ২ বছরের কম বয়সী শিশুর মধ্যে একজন নিউমোনিয়ায় আক্রান্ত হয়। আমাদের দেশে যতো শিশু মারা যায় তার মধ্যে ৫ বছরের কম বয়সী শিশুদের শতকরা ২৫ জন এবং ১ বছরের কম বয়সী শিশুদের শতকরা ৪০ জন মারা যায় নিউমোনিয়ার জন্য। ***Haemophilus Influenzae* type b** (Hib) নামে এক ধরণের জীবাণু আমাদের দেশে শিশুদের নিউমোনিয়ার একটি প্রধান কারণ। এই জীবাণুটি মেনিনজাইটিস নামে একটি মস্তিস্কের অসুখও সৃষ্টি করে যা শিশুদের মারা যাওয়ার আরেকটি প্রধান কারণ।

আমরা Hib নামের এই জীবাণুটির প্রতিরোধে একটি টিকা কতোখানি কার্য্যকর তা জানার জন্য একটি গবেষণা করছি। এই টিকাটি ডিপথেরিয়া, টিটেনাস ও হুপিং কাশির (DPT) টিকার সাথে দেয়া হয় এবং পৃথিবীর বিভিন্ন দেশে ব্যবহার হচ্ছে। এটির কার্যকারীতা জানার জন্য আমাদের কিছু নিউমোনিয়া ও মেনিনজাইটিস-এ আক্রান্ত শিশুর তথ্য নেয়া প্রয়োজন। আপনার শিশুটি নিউমোনিয়া / মেনিনজাইটিস-এ আক্রান্ত। এখন, আমরা আপনার শিশুকে এই গবেষণায় অংশ নিবার জন্য অনুমতি দিতে অনুরোধ করছি। আপনি যদি রাজী থাকেন তবে আমরা আপনাকে আপনার শিশুর এবং আপনার পরিবারের অসুখ ও স্বাস্থ্য বিষয়ে ১৫-২০ মিনিট কিছু প্রশ্ন করবো, বুকের একটি এক্স-রে করবো, পরীক্ষার জন্য আধা চা-চামচ (২.৫ মিঃ লিঃ) পরিমাণ রক্ত নেবো এবং আপনার শিশুর চিকিৎসার জন্য দরকার হলে অন্যান্য পরীক্ষা করবো। আপনি জানেন, এই ধরণের পরীক্ষাগুলি আপনার শিশুর চিকিৎসার জন্য প্রয়োজন। এতে আপনার শিশুর ক্ষতি হবার কোনো সম্ভাবনা নেই, বরং আপনার শিশু আরো ভালো চিকিৎসা পাবে।

এটা আপনার বোঝা অত্যন্ত প্রয়োজন যে, (ক) এই গবেষণায় আপনার শিশুর অংশগ্রহণ সম্পূর্ণ স্বেচ্ছামূলক, (খ) আপনি যে কোনো সময়ে আপনার শিশুকে এই গবেষণা হতে সরিয়ে নিতে পারেন। যে কোনো ক্ষেত্রেই, আই-সি-ডি-ডি-আর, বি (কলেরা হাসপাতাল), রাড্ডা-বার্নেন, বা ঢাকা শিশু হাসপাতালে আপনার শিশুর বা আপনার পরিবারের কারো কোনো সময়ে প্রয়োজনীয় চিকিৎসার কোনো ত্রুটি হবে না। এই গবেষণায় পাওয়া জ্ঞান থেকে আমরা আগামীতে শিশুদের নিউমোনিয়ার টিকা দেবার বিষয়ে সিদ্ধান্ত নিতে পারবো - যা ভবিষ্যতে অন্য শিশুদের নিউমোনিয়ার কবল থেকে রক্ষা করবে।

এই গবেষণায় অংশগ্রহণ করলে আপনার বা আপনার শিশুর পরিচয় প্রকাশ হয়ে পড়ার কোনো সম্ভাবনা নেই। আপনার বা আপনার শিশুর নাম বা পরিচিতিমূলক কোনো তথ্য বাইরে কোথাও প্রকাশ করা হবে না। শুধুমাত্র কাজের প্রয়োজনে আমাদের নিজস্ব প্রতিনিধি এই সব কাগজপত্র পরীক্ষা করতে পারেন।

অভিভাবকের সম্মতি:

আমাকে এই গবেষণার বিষয়বস্তু ঠিকমতো বোঝানো হয়েছে। আমার সকল প্রশ্নের সন্তোষজনক উত্তর পেয়েছি। আমি বুঝেছি যে, আমার বাচচার এই গবেষণায় অংশগ্রহণ স্বেচ্ছামূলক। আমি আমার শিশু (নাম): _____ বয়স: _____- কে এই গবেষণায় অংশ নিতে অনুমতি দিলাম।

স্বাক্ষর: _____ তারিখ: _____

পুরো নাম: _____ সম্পর্ক: _____

শিশুর পুরো নাম: _____

সাক্ষীর স্বাক্ষর: _____ তারিখ: _____

সাক্ষীর পুরো নাম: _____

Study ID#: _____

Study Physician: যদি অভিভাবক সম্মতি দিয়ে থাকেন তবে সব তথ্য লিপিবদ্ধ করুন এবং নিশ্চিত করুন যে, * সবগুলি ফরম ঠিকমতো পূরণ করা হয়েছে এবং * প্রয়োজনীয় পরীক্ষাগুলি করা হয়েছে।

আপনার সহযোগিতার জন্য আপনাকে অসংখ্য ধন্যবাদ।

শিশুর কিছু তথ্য দেবার মৌখিক সম্মতিপত্র (For Control Child)

প্রজেক্টের নাম: **An effectiveness study of *Haemophilus Influenzae* type b vaccine.**

Principal investigator: Dr. Abdullah H. Baqui, Head, Child Health Programme, Public Health Sciences Division, ICDDR,B., Mohakhali, Dhaka – 1212.

Study location: Mirpur, Dhaka, Bangladesh.

৫ বছরের কম বয়সী শিশুদের মৃত্যুর প্রধান কারণ হলো শিশুদের নিউমোনিয়া। আমাদের দেশে প্রতি বছর গড়ে প্রতি তিনজন ২ বছরের কম বয়সী শিশুর মধ্যে একজন নিউমোনিয়ায় আক্রান্ত হয়। আমাদের দেশে যতো শিশু মারা যায় তার মধ্যে ৫ বছরের কম বয়সী শিশুদের শতকরা ২৫ জন এবং ১ বছরের কম বয়সী শিশুদের শতকরা ৪০ জন মারা যায় নিউমোনিয়ার জন্য। ***Haemophilus Influenzae* type b** (Hib) নামে এক ধরণের জীবাণু আমাদের দেশে শিশুদের নিউমোনিয়ার একটি প্রধান কারণ। এই জীবাণুটি মেনিনজাইটিস নামে একটি মস্তিস্কের অসুখও সৃষ্টি করে যা শিশুদের মারা যাওয়ার আরেকটি প্রধান কারণ।

আমরা Hib নামের এই জীবাণুটির প্রতিরোধে একটি টিকা কতোখানি কার্য্যকর তা জানার জন্য একটি গবেষণা করছি। এই টিকাটি ডিপথেরিয়া, টিটেনাস ও হুপিং কাশির (DPT) টিকার সাথে দেয়া হয় এবং পৃথিবীর বিভিন্ন দেশে ব্যবহার হচ্ছে। এটির কার্যকারীতা জানার জন্য আমাদের কিছু নিউমোনিয়ায় আক্রান্ত শিশু এবং একই বয়সী কিছু স্বাস্থ্যবান শিশুর তথ্য নেয়া প্রয়োজন। আপনার এলাকার একটি শিশু নিউমোনিয়ায় আক্রান্ত। এখন, আমরা আপনার শিশুকে এই গবেষণায় অংশ নিবার জন্য অনুমতি দিতে অনুরোধ করছি। আপনি যদি রাজী থাকেন তবে আমরা আপনাকে আপনার শিশুর এবং আপনার পরিবারের অসুখ ও স্বাস্থ্য বিষয়ে ১৫-২০ মিনিট কিছু প্রশ্ন করবো। এতে আপনার শিশুর বা আপনার কোনো উপকার হবে না, কিন্তু এই গবেষণায় পাওয়া জ্ঞান থেকে আমরা আগামীতে শিশুদের নিউমোনিয়ার টিকা দেবার বিষয়ে সিদ্ধান্ত নিতে পারবো – যা ভবিষ্যতে অন্য শিশুদের নিউমোনিয়ার কবল থেকে রক্ষা করবে।

এই গবেষণায় আপনার শিশুর অংশগ্রহণ সম্পূর্ণ স্বেচ্ছামূলক। আপনি ইচ্ছা করলে অংশ না-ও নিতে পারেন। অথবা আপনার পছন্দ না হলে কোনো প্রশ্নের উত্তর না-ও দিতে পারেন। এতে আপনার বা আপনার শিশুর কোনো ক্ষতি হবে না।

এই গবেষণায় অংশগ্রহণ করলে আপনার বা আপনার শিশুর পরিচয় প্রকাশ হয়ে পড়ার কোনো সম্ভাবনা নেই। আপনার বা আপনার শিশুর নাম বা পরিচিতিমূলক কোনো তথ্য বাইরে কোথাও প্রকাশ করা হবে না। শুধুমাত্র কাজের প্রয়োজনে আমাদের নিজস্ব প্রতিনিধি এই সব কাগজপত্র পরীক্ষা করতে পারেন।

মৌখিক সম্মতি:

আপনি কি আপনার শিশুকে এই গবেষণায় অংশ নিতে অনুমতি দিলেন?হ্যাঁ / না

Research Assistant: যদি অভিভাবক সম্মতি দিয়ে থাকেন তবে সব তথ্য লিপিবদ্ধ করুন এবং প্রশ্নপত্র পূরণ করুন।

আপনার সহযোগিতার জন্য আপনাকে অসংখ্য ধন্যবাদ।

Annex – 2 (3)

REQUEST TO PARTICIPATE AS A CONTROL CHILD
CONSENT FORM

Title: **An effectiveness study of *Haemophylus Influenzae* type b vaccine**

Principal investigator: Dr. Abdullah H. Baqui, Head, Child Health Programme, Public Health Sciences Division, ICDDR,B., Mohakhali, Dhaka – 1212.

Study location: Mirpur, Dhaka, Bangladesh.

Pneumonia is a major killer disease in the children under five years of age. On average, every year about a third of the under 2 year old children develop pneumonia. Pneumonia accounts for about 25% of <5 deaths and about 40% of infant deaths. *Haemophilus Influenzae* type b (Hib) is the second most important bacterial cause of childhood pneumonia in Bangladesh. *Haemophilus Influenzae* type b also causes meningitis which is a deadly disease in young children.

We are conducting a study to assess the effectiveness of Hib conjugate vaccine in Bangladeshi children. To assess the effectiveness of this vaccine, we need to identify cases with Hib disease (Hib pneumonia/ radiologically defined pneumonia/Hib meningitis) and need to select age-matched healthy children to serve as control. A child in your area of age similar to your child with Hib disease has been identified. We are requesting you to allow your child to participate in the study as a control child. If you agree, we'll ask you some questions regarding the health and illness, and immunisation status of your child and some questions regarding your family's health and illness. It will take about 15-20 minutes to answer the questions. There is no risk to your child. Your participation is completely voluntary. There is no benefit to you or your child from this study and you may decide not to participate in this study at all, but your participation will help us in making policy decision regarding inclusion of the vaccine in the routine EPI programme.

If you agree to participate, please answer to the questions. You may or may not answer any question if you feel, but we'll appreciate your help in getting all information. Your identity will remain strictly confidential, but the records may be reviewed by representatives of the authorities supporting this study. But there is no way that your identity will be given to any one not related to this study.

Thank you very much for your co-operation.

VERBAL CONSENT OF PARENT/ LEGAL GUARDIAN

Do you agree to participate in this study? YES / NO.

(**For study Research Assistant:** If YES, enrol the child and ensure that all necessary data have been collected. Thank you)

শিশুকে গবেষণায় অংশ নিতে অনুমতি দেবার সম্মতিপত্র

(For Immunogenicity Study)

প্রজেক্টের নাম: **An effectiveness study of *Haemophilus Influenzae* type b vaccine.**

Principal investigator: Dr. Abdullah H. Baqui, Head, Child Health Programme, Public Health Sciences Division, ICDDR,B., Mohakhali, Dhaka – 1212.

Study location: Mirpur, Dhaka, Bangladesh.

৫ বছরের কম বয়সী শিশুদের মৃত্যুর প্রধান কারণ হলো শিশুদের নিউমোনিয়া। আমাদের দেশে প্রতি বছর গড়ে প্রতি তিনজন ২ বছরের কম বয়সী শিশুর মধ্যে একজন নিউমোনিয়ায় আক্রান্ত হয়। আমাদের দেশে যতো শিশু মারা যায় তার মধ্যে ৫ বছরের কম বয়সী শিশুদের শতকরা ২৫ জন এবং ১ বছরের কম বয়সী শিশুদের শতকরা ৪০ জন মারা যায় নিউমোনিয়ার জন্য। ***Haemophilus Influenzae* type b** (Hib) নামে এক ধরণের জীবাণু আমাদের দেশে শিশুদের নিউমোনিয়ার একটি প্রধান কারণ। এই জীবাণুটি মেনিনজাইটিস নামে একটি মস্তিস্কের অসুখও সৃষ্টি করে যা শিশুদের মারা যাওয়ার আরেকটি প্রধান কারণ।

আমরা Hib নামের এই জীবাণুটির প্রতিরোধে একটি টিকা কতোখানি কার্য্যকর তা জানার জন্য একটি গবেষণা করছি। আপনি আপনার শিশুকে এই টিকা দিতে রাজী হয়েছেন। আমরা দেখতে চাই, আপনার শিশুর শরীরে এই টিকাটি কতোখানি রোগ-প্রতিরোধ ক্ষমতা সৃষ্টি করে। এখন, আমরা আপনার শিশুকে এই গবেষণার এই পর্যায়ে অংশ নিবার জন্য অনুমতি দিতে অনুরোধ করছি। আপনি যদি রাজী থাকেন তবে আমরা আপনাকে আপনার শিশুর এবং আপনার পরিবারের অসুখ ও স্বাস্থ্য বিষয়ে ১৫-২০ মিনিট কিছু প্রশ্ন করবো, এবং প্রতিবার আধা চা-চামচ-এরও কম (২ মিঃ লিঃ) পরিমাণ রক্ত নেবো - একবার এখন, একবার সবগুলি টিকা দেয়া হয়ে যাবার ১ মাস পর, আরেকবার শিশুর ৯ মাস ও শেষবার শিশুর ১৫ মাস বয়সে। এতে আপনার শিশুর ক্ষতি হবার কোনো সম্ভাবনা নেই, এবং আমরা চেষ্টা করবো যেন আপনার শিশুর অন্য কোনো অসুবিধা না হয়।

এটা আপনার বোঝা অত্যন্ত প্রয়োজন যে, (ক) এই গবেষণায় আপনার শিশুর অংশগ্রহণ সম্পূর্ণ স্বেচ্ছামূলক, (খ) আপনি যে কোনো সময়ে আপনার শিশুকে এই গবেষণা হতে সরিয়ে নিতে পারেন। যে কোনো ক্ষেত্রেই, আই-সি-ডি-ডি-আর, বি (কলেরা হাসপাতাল), রাড্ডা-বার্নেন, বা ঢাকা শিশু হাসপাতালে আপনার শিশুর বা আপনার পরিবারের কারো কোনো সময়ে প্রয়োজনীয় চিকিৎসার কোনো ত্রুটি হবে না। এই গবেষণায় পাওয়া জ্ঞান থেকে আমরা আগামীতে শিশুদের নিউমোনিয়ার টিকা দেবার বিষয়ে সিদ্ধান্ত নিতে পারবো - যা ভবিষ্যতে অন্য শিশুদের নিউমোনিয়ার কবল থেকে রক্ষা করতে সাহায্য করবে।

এই গবেষণায় অংশগ্রহণ করলে আপনার বা আপনার শিশুর পরিচয় প্রকাশ হয়ে পড়ার কোনো সম্ভাবনা নেই। আপনার বা আপনার শিশুর নাম বা পরিচিতিমূলক কোনো তথ্য বাইরে কোথাও প্রকাশ করা হবে না। শুধুমাত্র কাজের প্রয়োজনে আমাদের নিজস্ব প্রতিনিধি এই সব কাগজপত্র পরীক্ষা করতে পারেন।

অভিভাবকের সম্মতি:

আমাকে এই গবেষণার বিষয়বস্তু ঠিকমতো বোঝানো হয়েছে। আমার সকল প্রশ্নের সন্তোষজনক উত্তর পেয়েছি। আমি বুঝেছি যে, আমার বাচচার এই গবেষণায় অংশগ্রহণ স্বেচ্ছামূলক। আমি আমার শিশু (নাম): _____ বয়স: _____- কে এই গবেষণায় অংশ নিতে অনুমতি দিলাম।

স্বাক্ষর: _____ তারিখ: _____

পুরো নাম: _____ সম্পর্ক: _____

শিশুর পুরো নাম: _____

সাক্ষীর স্বাক্ষর: _____ তারিখ: _____

সাক্ষীর পুরো নাম: _____

Study ID#: _____

আপনার সহযোগিতার জন্য আপনাকে অসংখ্য ধন্যবাদ।

Annex – 2 (4)

REQUEST TO PARTICIPATE IN THE IMMUNOGENECITY SUB-STUDY CONSENT FORM

Title: **An effectiveness study of *Haemophilus Influenzae* type b vaccine.**

Principal investigator: Dr. Abdullah H. Baqui, Head, Child Health Programme, Public Health Sciences Division, ICDDR,B., Mohakhali, Dhaka – 1212.

Study location: Mirpur, Dhaka, Bangladesh.

Pneumonia is a major killer disease in the children under five years of age. On average, every year about a third of the under 2 year old children develop pneumonia. Pneumonia accounts for about 25% of <5 deaths and about 40% of infant deaths. *Haemophilus Influenzae* type b (Hib) is the second most important bacterial cause of childhood pneumonia in Bangladesh. *Haemophilus Influenzae* type b also causes meningitis which is a deadly disease in young children.

We are conducting a study to assess the effectiveness of a Hib conjugate vaccine. We invited you to allow your child to participate in this study and you agreed. We also need to assess the immunogenicity of this vaccine in a sample of children and requesting you to allow your child to participate in this sub-study. If you agree, we'll ask you a few questions regarding the health and illness of your child and regarding your family. Also, we'll take about 2 ml blood from your child for 4 times: now, one month after the third dose of the vaccine, and at 9 and 15 months of age. It will take about 10 minutes for you to answer the questions. There is very little risk of injury to your child, and proper precautions will be taken to minimise any risk. You may decide not to participate in this study at all. Your decision not to participate will not affect your child's right of getting proper treatment anywhere, but your participation will help us in making policy decision regarding inclusion of the vaccine in the routine EPI programme.

If you agree to allow your child to participate, please sign this form. Your identity will remain strictly confidential, but the records may be reviewed by representatives of the authorities supporting this study. By signing this form, you agree to such inspection and disclosure. Also, by signing this form you allow us to take blood samples from your child.

Thank you very much for your co-operation.

CONSENT OF PARENT/ LEGAL GUARDIAN

The study has been completely described to me. All of my questions were answered satisfactorily. I understand that participation of my child is completely voluntary. By signing this form, I allow my child (name) _____, (age) _____ months as a participant of this study. I also allow the investigators to take blood for immunogenicity tests.

Signature : _____

Relationship: Father / Mother / Legal guardian. Date: _____

Name in Full:_____

Name of the child (ALL CAPS): _____

Witness Name: _____ signature: _____

Study ID number: _____ Date: _____

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Immunogenicity and safety of a liquid combination of DT-PRP–T vs lyophilized PRP–T reconstituted with DTP

Jacob Amir*, Rimma Melamed†, Juma Bader*, Chantal Ethevenaux‡, Bernard Fritzell‡, Jean R. Cartier‡, François Arminjon‡ and Ron Dagan†§

The immunogenicity and safety of a combined diphtheria, tetanus, pertussis and Haemophilus influenzae *type b–tetanus conjugate vaccine (DTP–PRP–T) was compared to the same combination obtained by the reconstitution of* H. influenzae *type b–tetanus conjugate vaccine lyophilized (PRP–T) with liquid diphteria–tetanus–pertussis vaccine (DTP). Two hundred and sixty-two healthy infants were randomized to receive a intramuscular injection of 0.5 ml of one of the above combination vaccines at 2, 4 and 6 months of age, and a subgroup of 134 infants received a booster dose at 12 months. Serum antibody levels to each vaccine component were measured at ages 2, 6, 7, 12 and 13 months. Systemic and local reactions were assessed during the first 3 days after each injection by diary cards distributed to the parents. After the third dose and booster administered at 12 months of age, significant equivalence between the groups was observed, and the geometric mean titers of anti* H. influenzae *type b capsular polysaccharide (Hib-CP) antibodies were 5.9 and 32.6 μg ml^{-1} for the liquid combination group and 5.8 and 19.4 for the lyophilized group, respectively. After the third dose, anti-Hib-PC antibody levels of ≥1.0 μg ml^{-1} and 0.15 μg ml^{-1} were seen in 94% and 100%, respectively, of the liquid combination group and 90 and 99%, respectively of the lyophilized group. After the booster dose, levels of ≥1.0 μg ml^{-1} were observed in 100% and 93.5% of the liquid combination group and the lyophilized combination group, respectively. Systemic and local reactions to the vaccination were generally mild and did not differ significantly between the groups. We conclude that the liquid combination of DTP–PRP–T is safe and at least as immunogenic as the lyophilized preparation. This liquid preparation, like other combined vaccines may be helpful for planning vaccination programs with a reduced number of injections. Copyright © 1997 Elsevier Science Ltd.*

Keywords: *Haemophilus influenzae* b; immunization; combination vaccines

The introduction of *Haemophilus influenzae* type b (Hib) conjugate vaccines beginning at 2 months of age has dramatically affected the incidence of invasive Hib disease in young children[1]. The simultaneous administration of Hib vaccines with the other routine childhood vaccines has been shown to be safe and efficacious[2]. However, in order to decrease the number of injections given at the same time, investigators have examined the use of various combinations of vaccines in the same syringe.

Initial studies revealed that the combination of diphtheria–tetanus pertussis (DPT) vaccine with lyophilized conjugate Hib vaccine was satisfactory[3–13], although there were contradictory reports of a possible decrease in the response of pertussis agglutinin antibodies[8,12,14,15]. Only two liquid preparations of the combination of DTP and conjugate Hib vaccine were tested and licensed (DTP–HbOC; Tetrammune; Lederle and DTP–PRPD; Connaught)[16,17].

The present study was designed to evaluate the safety and immunogenicity of the new liquid combination of DTP with a conjugate Hib–tetanus vaccine (PRP–T) in young infants, by comparison to a previously licensed combination obtained by reconstituting lyophilized PRP–T with DTP[4].

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SUBJECTS AND METHODS

This study was designed as a multicenter comparative randomized trial and approved by the local and national ethics committees. Informed consent was obtained from the parents before subject enrollment.

Subjects

Healthy infants of mean age 2 months (range, 8–11 weeks), born at term were recruited in eight Mother and Child health centers located in two cities in Israel: Beer Sheva in the South and Petah Tiqva in the Center.

Vaccines

DTP. Each 0.5 ml dose of the vaccine (Pasteur-Mérieux Sérums et Vaccins, Lyon, France, batch No. S-2851) contained purified diphtheria toxoid (≥ 30 IU), purified tetanus toxoid (≥ 60 IU) and inactivated *Bordetella pertussis* suspension (≥ 4 IU). The vaccine also contained aluminum hydroxide, thiomersal and buffer solution.

PRP–T. PRP–T (Pasteur-Mérieux Sérums et Vaccins, Lyon, France, batch No. S-0427) was composed of Hib capsular polysaccharide (PRP) conjugated to tetanus toxoid. The content of the tetanus protein was 24 μg per dose conjugated to 10 μg PRP and presented in a lyophilized form. Each dose also contained 0.6 mg of Tris(hydroxymethyl aminomethane) and 42.5 mg of sucrose.

DTP–PRP–T liquid combination. The antigens included in this vaccine were identical to those in the DTP and PRP–T product, except that the PRP–T was not freeze-dried but directly derived from the prelyophilization stage (batch No. S-2862).

Vaccination protocols

Infants were given identification numbers and allocated to one of the vaccination groups according to a randomization list that followed the chronological order of enlistment into the trial. In the liquid combination vaccine group, the solution was injected intramuscularly (i.m.) into the antero-lateral surface of the right thigh with the prefilled syringe (0.5 ml). In the lyophilized group, the lyophilized PRP–T was reconstituted with the DTP at the time of injection and the reconstituted solution (0.5ml) was injected i.m. at the same site, in the right thigh.

Injections were administered in both groups at 2, 4 and 6 months. Half of the children were to receive a fourth injection at 12 months. The same batch numbers were used for all four injections.

In addition all infants received the mandatory, routine childhood vaccines according to the immunization program in Israel, as follows: An enhanced potency inactivated polio vaccine (IPV) at 2, 4, and 12 months; live oral polio trivalent vaccine (OPV) at 4, 6 and 12 months; and a third dose of hepatitis B vaccine at 6 months (the first two doses were given at birth and at 1 month).

Venous blood was obtained from all infants before immunization at age 2 months, before the third immunization at age 6 months and 1 month after the third immunization, at age 7 months. Further samples were taken from a subgroup of infants at age 12 and 13 months before and after the booster dose.

Adverse reactions

Each child was observed for 30 min after the injection for immediate reactions. The parents of participating infants were given a special form to record any local reaction (pain, redness, duration) and systemic reaction (fever, irritability, unusual crying, loss of appetite, insomnia, lethargy, diarrhea, vomiting) that occurred during the first 3 days after injection, including time of the appearance, duration, intensity, and treatment administered. Parents were also asked to record rectal temperature twice daily during these 3 days and to report the highest daily temperature. They were asked to notify the study team of any visits to a doctor or nurse during the month after the injection.

Serologic testing

Global anti-Hib-CP antibody response was assessed with a Farr-type radioimmunoassay[18] using ^{125}I-labeled polysaccharide according to the specifications of C.E. Frasch (Food and Drug Administration, Center for Biologic Evaluation and Research). The results were expressed as μg ml^{-1}. Each series tested included three sera with a known anti-PRP antibody content.

Anti-diphtheria and anti-tetanus toxin antibodies were measured by a radioimmunoassay and results were expressed as IU ml^{-1} with reference to an international standard[19,20].

Agglutinin antibodies against *B. pertussis* were assayed by a conventional technique[21]. Results were expressed as a reciprocal dilution.

All assays were performed at the Pasteur-Mérieux Sérums et Vaccins Laboratories, Marcy l'Etoile, France. We used a special number for each serum; the code was unknown to the technician performing the laboratory test.

Statistical analysis

The statistical analysis was performed using SAS Software (SAS Institute, Carry, NC, USA).

Analysis of primary vaccination. The main judgement criterion was the percentage of infants with an anti-Hib-CP antibody titer ≥ 1 μg ml^{-1} after three doses. For this criterion (and ≥ 0.15 μg ml^{-1}), a test of equivalence was performed, based on χ^2 or normal distribution. Equivalence was defined by a difference of $\leq 11.5\%$ in the proportion of subjects with anti-Hib-CP titer ≥ 1 μg ml^{-1} after three doses.

Analysis of booster injection. The judgment criterion corresponding to the assessment of the immunogenicity was the geometric mean titer (GMT) of anti-Hib-CP antibodies 1 month after the booster dose. A test of equivalence was performed, based on the normal distribution calculated under the null hypothesis. Equivalence was defined by a GMT in liquid combination group at least equal to 50% of the lyophilized combination group one.

Table 1 Anti-Hib-CP antibody concentrations in 262 infants immunized with either liquid or lyophilized preparations of DTP–PRP–T combination vaccine administered at 2, 4, 6, and 12 months

	Before first dose (age=2 months)		Post second dose (age=6 months)		Post third dose (age=7 months)		Pre-booster (age=12 months)		Post-booster (age=13 months)	
	LIQU	LYOPH	LIQU	LYOPH	LIQU	LYOPH	LIQU	LYOPH	LIQU	LYOPH
Number of samples	130	132	125	124	119	119	51	45	50	46
% with ≥0.15 $\mu g\ ml^{-1}$	44	56	98	93	100	99	94	87	100	100
% with ≥1.0 $\mu g\ ml^{-1}$	9	11	69*	55*	94	90	55	53	100	94
GMT $\mu g\ ml^{-1}$	0.15	0.22	1.90	1.38	5.92	5.79	1.14	1.10	32.59	19.43

LIQU, liquid formulation; LYOPH, lyophilized formulation; GMT, geometric mean titer. *P=0.02 (test of independence)

Table 2 Anti-pertussis agglutinin reciprocal titer in 262 infants immunized with either liquid or lyophilized preparations of DTP–PRP–T combination vaccine administered at 2, 4, 6, and 12 months

	Before first dose (age=2 months)		Post second dose (age=6 months)		Post third dose (age=7 months)		Pre-booster (age=12 months)		Post-booster (age=13 months)	
	LIQU	LYOPH	LIQU	LYOPH	LIQU	LYOPH	LIQUI	LYOPH	LIQU	LYOPH
Number tested	130	132	125	124	119	119	51	46	50	46
GMT (dilutions)	21	15	134	80	601	597	1087	1006	2560	2447
% with ≥40	42	34	83	71	98	98	100	100	100	100
% with ≥320	10	4	42	32	87	88	98	98	100	100

LIQU, Liquid formulation; LYOPH, lyophilized formulation; GMT, geometric mean titer

Proportional data were compared using the χ^2 or Fisher's exact test when required by sparse data. Log transformed serologic antibody titers were compared with the Student t-test. Negative values were given the value of one-half of the difference between the cut-off value of the test and zero.

The chosen sample allowed to test equivalence between the main judgment criterion (anti-Hib-CP antibody ≥1 $\mu g\ ml^{-1}$ after three doses) of each group a P<0.05 (one tailed), a 90% power and a 11.5% delta value (maximum value to conclude to the equivalence).

RESULTS

A total of 262 infants were enrolled in the study between November 1993 and March 1994; of these 130 were randomly assigned to the liquid combination group and 132 to the lyophilized group. These included 133 males and 129 females in a homogeneous sex distribution. The mean (±S.D.) age at the time of the first dose was 65±6 days in the liquid group and 65±5 days in the lyophilized group. The second and third injections were given at 120±9 and 120±8 days and 175±12 and 177±13 days, respectively.

Two hundred and forty-two (92%) infants completed the first part of the study (122 in the liquid combination group and 120 in the lyophilized group) and had their third blood sample taken at the age of 7 months. One hundred and thirty-four infants (66 in the liquid group and 68 in the lyophilized group) were included in the booster dose subgroup (fourth and fifth blood sample at 12 and 13 months).

Twenty infants did not complete the study: eight in the liquid combination group for refusal to continue (five cases), technical failures (two cases) and non compliance (one case); 12 in the lyophilized group for refusal to continue (nine cases), technical failures (two cases) and unexpected adverse event after the first injection (one case).

Serologic response to PRP–T

The anti-PRP antibody concentration in the two study groups is presented in *Table 1*. The GMTs of anti-Hib-CP before immunization were 0.15 and 0.22 $\mu g\ ml^{-1}$ in the liquid and lyophilized group, respectively. After the second immunization, a significantly higher percentage of infants in the liquid group had an anti-Hib-CP titer ≥1.0 $\mu g\ ml^{-1}$ (69% vs 55%, P=0.02). After third immunization 100% of the liquid preparation recipients and 99% of the lyophilized preparation recipients had anti-Hib-CP antibodies ≥0.15 $\mu g\ ml^{-1}$ (P<0.0001; test of equivalence). Likewise, after the booster dose, the GMT of the anti-Hib-CP antibody was similar in the liquid combination and the lyophilized groups: 32.59 and 19.43 $\mu g\ ml^{-1}$, respectively (P<0.0001; test of equivalence).

Serologic response to pertussis

No significant differences between the two groups were noted after the third immunization (*Table 2*). The GMTs of pertussis agglutinin titers after the third dose were 601 and 597 in the liquid and lyophilized groups, respectively. A threshold titer of 320 was achieved in 87% and 88%, respectively, and 98% of the infants in both groups had levels ≥40. After the booster dose, all infants in both groups had agglutinin titers of equal to or 320.

Serologic response to diphtheria and tetanus

All infants in both groups achieved protective concentrations (0.01 IU ml^{-1}) of diphtheria and tetanus antitoxin after the second immunization (*Table 3*). No difference was shown for the GMTs of the diphtheria and tetanus antitoxin were similar between the group after the second, third, and booster injections. Ninety-nine percent in both groups had anti-diphtheria

Table 3 Anti-tetanus and anti-diphtheria antibody concentrations in 262 infants immunized with either liquid or lyophilized preparation DTP–PRP–T combination vaccine administered at 2, 4, 6, and 12 months

	Before first dose (age=2 months)		Post second dose (age=6 months)		Post third dose (age=7 months)		Pre-booster (age=12 months)		Post-booster (age=13 months)	
	LIQU	LYOPH	LIQU	LYOPH	LIQU	LYOPH	LIQU	LYOPH	LIQU	LYOPH
Diphtheria antitoxin										
Number tested	79	82	124	120	119	116	42	44	48	46
GMT (IU ml^{-1})	0.02	0.03	0.55	0.51	1.56	1.35	0.19	0.13	4.45	3.21
% with 0.01 IU ml^{-1}	72	71	100	100	100	100	100	93	100	100
% with 0.1 IU ml^{-1}	11	21	95	89	99	99	71	61	100	100
Tetanus antitoxin										
Number tested	128	128	125	124	119	117	48	45	50	46
GMT (IU ml^{-1})	0.09	0.10	1.20	1.25	4.91	5.13	0.52	0.59	11.45	12.04
% with 0.01 IU ml^{-1}	94	92	100	100	100	100	100	100	100	100
% with 0.1 IU ml^{-1}	47	54	99	100	100	100	100	93	100	100

LIQU, Liquid formulation; LYOPH, lyophilized formulation; GMT, geometric mean titer

Table 4 Local and systemic adverse reactions in the first 72 h following immunizations in 262 infants immunized with either liquid or lyophilized preparations of DTP–PRP–T combination vaccine administered at 2, 4, 6, and 12 months

	First dose (age=2 months)		Second dose (age=4 months)		Third dose (age=6 months)		Booster dose (age=12 months)	
Number of children	LIQU 130	LYOPH 130	LIQU 129	LYOPH 127	LIQU 125	LYOPH 122	LIQU 68	LYOPH 66
Local reactions								
% with any local reactions	60	71	44	44	45	38	28	25
% with pain	51	55	35	36	33	23	16	19
% with redness	29	39	30	27	27	28	18	13
% with induration	34	44	30	21	29	30	16	7
Systemic events								
% with any systemic events	70	69	50	48	45	43	40	28
% with unusual crying	45	45	30	26	26	21	18	7
% with fever	19*	35*	18	24	18	22	15	13
% with irritability	48	42	36	28	29	22	24	18
% with loss of appetite	29	29	16	15	15	12	16	9
% with insomnia	25	25	21	15	20	12	10	16

LIQU, Liquid formulation; LYOPH, lyophilized formulation; *P=0.003

antibody titer 0.1 IU ml^{-1} after the third immunization and 100% in both groups had anti-tetanus antibody titer 0.1 IU ml^{-1} after the third immunization.

Adverse reactions

No infants had any immediate reaction within 30 min of injection. Local reactions were common in both groups (*Table 4*); however, all were mild and resolved without medical intervention. The percentage of infants with a local reaction varied from 60% to 28% in the liquid group and from 71% to 25% in the lyophilized group, with a tendency to show less reaction with increasing dose. The most common reported local reaction was pain in the injection site, ranging from 51% to 33% in the liquid group and 55% to 19% in the lyophilized group. Rates for the other types of local reactions (redness and induration) were also similar in the two groups. No significant differences in local reaction after the first, second, third, and booster doses were found between the two study groups.

Systemic events were also common in both groups, especially after the first dose (70% of the liquid group, 69% of the lyophilized group) (*Table 4*). Unusual crying and irritability were the most frequent systemic reactions after each infection in the two groups.

The only significant difference between the groups was in the incidence of a fever (38°C), which was higher in the lyophilized group after the first immunization (P=0.003). High fever (defined as ≥39°C) was also more frequent after the first injection in the lyophilized group [13/130 (10%)] than in the liquid group [4/130 (3%)] (P=0.02). Otherwise systemic reactions did not differ in frequency, severity or duration between the two groups.

One serious adverse effect was reported in the course of this trial. A 2-month old infant in the liquid group suffered a hyporesponsive-hypotonic episode after the first immunization which resolved spontaneously. This infant was excluded from further follow-up.

DISCUSSION

The reconstitution of lyophilized PRP–T with DTP or DTP IPV has already been validated and registered in many countries. The advantage of a liquid combination is to avoid a manipulation of the products. In the present study we have shown that the liquid preparation of DTP PRP–T was at least as safe and immunogenic as the lyophilized preparation. Anti-Hib-CP concentrations ≥0.15μg ml^{-1} (the level accepted as protective) were obtained in all infants who received three doses of

liquid combination vaccine. No difference was wn between GMTs of both groups[23]. The liquid mbination vaccine was shown to confer good priming mst Hib as very high GMT was detected after booster dose administered at age 12 months ($\mu g\ ml^{-1}$), and all the infants receiving this paration had $\geq 1.0\ \mu g\ ml^{-1}$ anti-Hib-CP or more. ese results are in agreement with the study of Dagan coworkers[5], performed previously with lyophilized paration among the same population.

For combinations in which the individual components already licensed and their efficacy is well established, ch as in the present study, there is usually no need to form an efficacy study to confirm their usefulness. herefore the design for comparison with an already censed vaccine or combination is planned in such a way at equal immunogenicity and safety may be demonrated. We designed our study to be able to show that no more than 11.5% difference exists between the two mpared combinations. Because of the specific design nd choice of the study group size, although the liquid mbination showed better results in immunogenicity hen anti-Hib-CP-antibody levels were tested, we had to conclude that the two tested combinations had similar results.

To date, only two liquid combinations of DTP and conjugate Hib vaccines exist commercially, namely a liquid combination of DTP and Hib-CP conjugated to diphtheria toxoid (DTP–PRP–D) and a liquid preparation of DTP with Hib oligosaccharid conjugated to a mutant diphtheria toxin (DTP–HbOC). The anti-Hib-CP antibody levels achieved in our study are comparable to those achieved with the DTP–HbOC[16,17]. No post-booster data were shown in the report describing DTP–HbOC but the result of GMT of 32.59 mg ml^{-1} achieved in our study with 100% of the children having values ≥ 1.0 mg ml^{-1} is in the range for which any higher immunogenicity is of questionable additional benefit.

With regards to anti-diphtheria and anti-tetanus antibody, the results of the liquid DTP–PRP–T preparation show results in the same range of magnitude as those of DTP–HbOC, with higher antibody levels of pertussis agglutinins.

The local and systemic reactions were all mild except in one child, and were in the expected range for the whole cell pertussis component. Furthermore, we found that fever was significantly less frequent among recipients of the liquid formulation than among those having received the lyophilized vaccines.

In regard to liquid preparations of combined vaccines, the issue of stability is of utmost importance. Thus, the clinical results were supplemented by laboratory data through a stability study conducted at +4°C during 24 months: physico-chemical parameters as well as the PRP–T potency in animals were proven to be satisfactory. Furthermore a clinical study has been conducted with the same batch of vaccine, 18–24 months old, with immunogenicity results for all the valences (unpublished data).

In summary, the present study shows that the liquid combination vaccine of DTP–PRP–T in a single formulation is immunogenic and safe in young infants. It is at least as immunogenic as the lyophilized PRP–T preparation reconstituted with DPT. Being a combined vaccine, its use would decrease the number of injections given to infants during routine immunizations. This preparation should be considered in planning vaccination programs.

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Combined diphtheria, tetanus, pertussis, and *Haemophilus influenzae* type b vaccines for primary immunisation

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Abstract

A total of 146 infants were immunised at ages 2, 3, and 4 months with a combined diphtheria, tetanus, pertussis (DTP) - *Haemophilus influenzae* type b (Hib) tetanus toxoid conjugate (PRP-T) vaccine (Pasteur Merieux) to assess the antibody response and adverse events associated with immunisation. Adverse events, including fever, were recorded by parents in a diary for three days following each injection. Blood was taken before the first immunisation and four weeks after the third immunisation to assess antibody response. Data were compared with those from historical controls who had received DTP and PRP-T vaccines by separate injection. The combined vaccine was well tolerated. Rates of local and general reactions were similar to those reported for infants immunised by separate injection. All infants achieved protective antibody titres (> 0.01 IU/ml) for diphtheria and tetanus; 98% acquired Hib (PRP) antibody > 0.15 μg/ml and 82.5% > 1.0 μg/ml. Pertussis antibody titres (pertussis toxin, filamentous haemagglutinin, total agglutinins, and agglutinins 2 and 3) showed appreciable rise following immunisation. DTP and PRP-T vaccines provide similar antibody responses and adverse effects whether mixed in the same syringe or administered by separate injection. The vaccines could be combined for use in the United Kingdom primary immunisation schedule.

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Keywords: diphtheria tetanus pertussis vaccine, *Haemophilus influenzae* type b vaccine, immunisation.

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The introduction of *Haemophilus influenzae* type b (Hib) immunisation to the universal schedule in the United Kingdom in 1992 has led to a dramatic reduction in reported rates of invasive haemophilus disease in children.[1] However, extending the protection offered by immunisation doubled the number of injections given to infants in the primary schedule as Hib and diphtheria, tetanus, and pertussis (DTP) vaccines have been given separately at each immunisation visit. Combining the injected antigens of the primary schedule into a single injection would reduce discomfort for infants, so might improve parental acceptability and vaccine uptake. In the United Kingdom a combined DTP-Hib vaccine would reduce the number of immunising injections by two million each year. Healthcare staff would welcome the time saved by carrying out the simpler procedure. Demonstrating the safety and immunogenicity of vaccine combinations will become increasingly important as new antigens are introduced into the universal primary immunisation schedule.

Several conjugate vaccines derived from Hib capsular polysaccharide have been developed and considered for primary immunisation of infants. Previous studies, using polyribosylribitol phosphate conjugated with tetanus toxoid (PRP-T) given in the same syringe as DTP, have shown the combination to be well tolerated.[2-5] However, some reports have suggested a reduction in vaccine immunogenicity,[6,7] particularly in the response to pertussis agglutinins.[8-10] It is unclear how much variation is attributable to the mixing of antigens and still more difficult to assess the clinical significance of minor changes in mean antibody titre. A previous study mixing these vaccines identified lower levels of PRP antibody in the group receiving the combined vaccine.[6] Mean titres in the mixed group were still several times higher than the level thought to confer long term protection, and all infants achieved levels above this threshold. Protective thresholds have not been established for pertussis, which makes interpreting differences in immune response more difficult.

Concerns about impaired antibody response may be particularly relevant in the United Kingdom in view of the earlier completion of the accelerated schedule at 4 months, which may itself interfere with optimal immune response,[11,12] and in the absence of booster doses of pertussis or Hib vaccines beyond this age.

We sought to establish whether combined administration of DTP with PRP-T (a Hib conjugate vaccine in current use in the United Kingdom) would provide satisfactory protection for infants with an acceptable profile of adverse events. Accepted protective antibody thresholds exist for Hib,[13-16] diphtheria, and tetanus,[17-19] allowing comparisons to be made with previous studies of infants immunised in the United Kingdom accelerated schedule.[11,12,20-22]

We immunised infants with a vaccine combining DTP and Hib (PRP-T) mixed in a

'bypass' syringe given at age 8, 12, and 16 weeks, assessed the frequency and severity of adverse events and examined the antibody response to immunisation.

Methods

Parents of healthy infants were approached on the postnatal wards of the two obstetric units serving the city of Sheffield, England and given information about the study. Families who expressed interest by returning a reply slip were seen later at home, where written informed consent for the study was obtained. Babies born at less than 37 weeks' gestation or weighing less than 2500 g at birth were excluded. Infants of families taking part were scheduled to receive immunisations at home at age 8, 12, and 16 weeks. All received a single injection at each visit combining Hib-tetanus toxoid conjugate (PRP-T) and adsorbed diphtheria, tetanus, and whole cell pertussis vaccines (Pasteur Merieux). This was given by intramuscular injection in the right anterolateral thigh using a 25G needle. Antigens were mixed immediately before administration using a 'bypass' syringe which allows liquid DTP vaccine in the upper barrel to reconstitute dried PRP-T in the lower portion of the syringe, resulting in an injected volume of 0.5 ml. Single batches of PRP-T and DTP vaccines were supplied by the manufacturer for the study. Oral polio vaccine (Wellcome) was given at each immunisation visit.

Parents were given a surface activated liquid crystal thermometer (Body-temp, Tommee Tippee, Cramlington, UK) and a diary in which to record temperature twice daily, with details of other adverse events for the three days following immunisation. They were asked to note redness or swelling at the injection site (greater than 2.5 cm diameter), to assess infant crying, activity, and feeding, and to record any use of paracetamol. Parents were advised to give paracetamol if their baby seemed to be in discomfort or had a temperature of 38°C or above. We contacted parents by telephone at one and three days following immunisation to ask about unwanted effects and to confirm diary information.

Venous blood was taken before the first injection and again four weeks after the third immunisation to assess antibody responses. A local anaesthetic cream (EMLA, Astra Pharmaceuticals, Kings Langley, UK) was applied before blood sampling at 5 months. Sera were analysed for antibody to diphtheria and tetanus toxoids, PRP, and pertussis antigens (pertussis toxin (PT), filamentous haemagglutinin (FHA), total agglutinins and agglutinins 2 and 3 (fimbrial antigens)). Antibody to PRP was measured by radioimmunoassay. Diphtheria and tetanus antitoxins, antibodies to PT, FHA, and agglutinins 2 and 3 were measured by enzyme linked immunosorbent assay (ELISA). Total pertussis agglutinins were determined by serial dilution of serum until precipitation with pertussis suspension was no longer observed.

All assays except for pertussis agglutinins 2 and 3 were performed at Val de Reuil, France (laboratory 1). There are no universally accepted standards or methods for pertussis antigen assays, so postimmunisation sera were additionally assayed (ELISA)[23] for pertussis toxin and FHA at the Centre for Applied Microbiology and Research, Porton Down (laboratory 2) to allow comparison with previous reports from the United Kingdom. This laboratory was also responsible for preimmunisation and postimmunisation assays for agglutinins 2 and 3.[21] Where results lay below the limit of detection for the assay, a value of one half the lower limit was used to calculate geometric mean titres.

STATISTICS

Antibody levels following immunisation were compared in groups of infants with low and high preimmunisation titres by the Mann-Whitney U test for non-parametric data. Analyses were performed using SPSS for Windows Release 6.0 (SPSS, Chicago, Illinois, USA). Categorical data were compared using the χ^2 test or Fisher's exact test where fewer than five infants were expected in any group. Approval for the study was obtained from the North and South Sheffield research ethics committees.

Results

One hundred and forty six infants (71 girls) were recruited to the study. Twenty four per cent of the parents approached agreed to take part. All immunisations and blood tests took place on or within two weeks of the dates when these were due. Median ages at immunisation and at final blood sampling were 8.1, 12.4, 16.6, and 20.9 weeks. Two infants did not complete the study: temporary loss of contact with one family led to a delay between second and third immunisations, and a second family moved away before the final blood sample was taken.

One infant developed swelling involving the major part of the thigh following second immunisation, precluding administration of a third dose of the combined vaccine. She remained otherwise well; swelling and erythema had resolved by four days. Immunisation was completed with DT, Hib, and polio vaccines and this infant excluded from serological analysis. No other adverse effects met Department of Health criteria for severe reactions to immunisation.[24] A minority of infants developed appreciable erythema or swelling at the immunisation site (table 1). Modest rates were reported for prolonged crying and for disturbed feeding (table 1). Up to one half of all infants were febrile at some time in the three days following immunisation. Temperatures $\geq$ 38°C were recorded less frequently with succeeding doses (table 1).

Serum was available from 143 infants following immunisation with three doses of combined vaccine. Insufficient blood was obtained from one infant for preimmunisation analysis. All achieved protective antibody titres (over 0.01 IU/ml) for diphtheria and tetanus (table 2). Three infants failed to achieve protective titres for PRP (table 2). Geometric mean antibody titres for these antigens before

Table 1 Number (percentage) of infants experiencing adverse events in the 3 days following immunisation (n=140-146)

	Dose		
	1	2	3
Erythema ≥ 2.5 cm	6 (4)	12 (8)	9 (6)
Swelling ≥ 2.5 cm	16 (11)	15 (10)	13 (9)
Crying			
> 1 hour	16 (11)	9 (6)	14 (10)
Inconsolable > 4 hours	0 (0)	2 (1)	1 (1)
Disturbed feeding			
Fed less than usual	59 (41)	30 (21)	33 (23)
Missed feeds	17 (12)	6 (4)	10 (7)
Fever ≥ 38°C			
On first evening	52 (36)	26 (18)	17 (12)
Any (over 3 days)	77 (53)	44 (31)	35 (24)

and after immunisation are shown in table 3. There were marked increases in titres of antibody to the four pertussis antigens measured (table 4).

The influence of maternally acquired antibody was assessed by comparing antibody titres before and after immunisation. Infants with titres above protective threshold for tetanus and diphtheria before immunisation achieved lower titres at 5 months than those with initial levels of antibody below these titres (table 5). This was more clearly seen for tetanus, 38 infants (27%) experiencing a fall in titre between 2 and 5 months of age despite immunisation. Similarly, infants with low antibody levels before immunisation achieved higher titres for pertussis toxin, FHA, and agglutinins 2 and 3. No effect of preimmunisation titre on antibody levels at 5 months was shown for PRP or for total pertussis agglutinins.

Discussion

Combining the antigens administered by separate injections at one visit would be welcomed by infants, parents, health workers, and administrators. As new vaccines are proposed for inclusion in the primary immunisation schedule, it will continue to be necessary to demonstrate the safety and effectiveness of vaccine combinations in order to maximise protection against infectious diseases without an unacceptable increase in immunisation visits or injections.

Several studies have addressed the immunogenicity and tolerance of a number of DTP vaccines mixed with PRP-T in other settings, given by more extended schedules.[2-10] Those that have considered PRP-T in the United Kingdom schedule, either combined or as a separate injection, have used the Wellcome (Evans) DTP vaccine.[20 22] Certain DTP/Hib conjugate vaccine combinations have been licensed, are in use in the USA,[25] and have recently been recommended in the United Kingdom.[26]

There was no obvious bias in maternal age, race, social class, or geographical distribution within the city in the sample recruited to this study. The combined PRP-T/DTP vaccine given at 2, 3, and 4 months was well tolerated by these infants. Only one child failed to complete immunisation for all antigens because of a local reaction following the second dose of the combined vaccine. Rates of redness (4-8%) and swelling (9-11%) at the injection site were similar to those reported for PRP-T combined with Wellcome DTP[22] and lower than those reported for DTP when given either in a separate limb from PRP-T (redness 12-27%, swelling 12-24%),[22] or given alone without Hib vaccine (redness 10-26%, swelling 10-32%).[21 27]

No serious systemic reactions were encountered, although over 50% of infants had fever ≥ 38°C occurring at any time during the three days after the first dose of vaccine compared with the low rates (3-10%) reported previously.[22] However, direct comparisons are difficult as the methods for analysis of temperature data in that study are unclear. In another study, 6% of infants receiving vaccines as two separate injections had fever after the first dose when parents measured temperatures once daily.[20] Rates of fever for subsequent doses in our study (31% following the second and 24% after the third) compare with reports of 16% for both doses for combined vaccine recipients[22] or 5-16% and 6-24% respectively, for infants receiving vaccine by separate injection.[20 22]

Our results may have been influenced by the use of a forehead thermometer reading to the nearest whole degree (allowing interpolation to nearest 0.5°C) which may have overestimated the number of infants with temperature above 38°C as temperatures were rounded up. One previous study has reported a higher rate of fever in infants receiving one of two batches of PRP-T combined with DTP,[10] but no other has shown an increase in febrile responses among combined vaccine recipients, and an earlier United Kingdom study found similar rates in historical controls given vaccine by separate injection.[22] Differences in response might also be explained by the use of DTP from different manufacturers,[28] or by greater use of paracetamol in other studies. Paracetamol was given by parents after 55% of immunisations in this study, but these data have not been recorded in previous reports. None of the febrile infants in this study were seriously unwell, and no child required admission to hospital or intervention other than with simple antipyretics.

Several studies have addressed the immunogenicity of mixed PRP-T/DTP in more extended schedules. Some have identified modest reductions in antibody titre to PRP,[6] tetanus,[7 9] and pertussis,[8-10] while others have found no evidence for reduced immunogenicity.[2-5] One study showed an enhanced response to diphtheria for combined vaccine recipients,[3] and in another, infants immunised on a 2, 3, 4 month schedule showed higher antibody titres to tetanus.[22]

Infants in our study acquired satisfactory antibody titres for each vaccine constituent. All achieved diphtheria and tetanus titres above protective threshold. Geometric mean titres (GMT) for diphtheria and tetanus were similar to those reported for United Kingdom infants immunised with DTP in the accelerated schedule (table 3).[11 12 21 22]

The mean titre for PRP in this group of infants is similar to the figure reported by Begg

Table 2 Number (percentage) of infants with antibody levels above protective threshold compared with those reported previously for infants receiving vaccines by separate injections[11 20]

	Preimmunisation PRP-T/DTP combined	*Postimmunisation PRP-T/DTP combined*	*Postimmunisation PRP-T and DTP* separate sites*[11 20]
Diphtheria			
> 0.01 IU/ml	46/134 (34)	143/143 (100)	† (100)
> 0.1 IU/ml	7/134 (5)	142/143 (99)	
Tetanus			
> 0.01 IU/ml	133/142 (94)	143/143 (100)	† (100)
> 0.1 IU/ml	109/142 (77)	143/143 (100)	89/91 (98)
PRP			
> 0.15 μg/ml	43/142 (30)	140/143 (98)	105/107 (98)
> 0.1 μg/ml	1/142 (1)	118/143 (82)	97/107 (91)

* Wellcome (Evans) DTP.
† Numbers not given in report.
DTP = absorbed diphtheria, tetanus, pertussis vaccine; PRP-T = polyribosylribitol phosphate tetanus toxoid conjugate vaccine; PRP = polyribosylribitol phosphate.

Table 3 Geometric mean antibody titres (95% confidence limits for mean) for diphtheria, tetanus, and polyribosylribitol phosphate (PRP), compared with those reported previously for infants receiving vaccines by separate injections[11 20]

	Preimmunisation PRP-T/DTP combined (n=142)	*Postimmunisation PRP-T/DTP combined (n=143)*	*Postimmunisation PRP-T and DTP** separate sites*[11 20] *(n=103)*
Diphtheria (IU/ml)	0.007* (0.006 to 0.009)	1.07 (0.95 to 1.21)	3.87
Tetanus (IU/ml)	0.25 (0.19 to 0.33)	1.01 (0.87 to 1.16)	0.70
PRP (μg/ml)	0.12 (0.11 to 0.14)	3.65 (2.92 to 4.56)	5.01

* 134 samples available for preimmunisation diphtheria serology.
** Wellcome (Evans) DTP.
DTP = absorbed diphtheria, tetanus, pertussis vaccine; PRP-T = polyribosylribitol phosphate tetanus toxoid conjugate vaccine; PRP = polyribosylribitol phosphate.

Table 4 Geometric mean antibody titres (95% confidence limits for mean) for pertussis antigens compared with those reported previously for infants receiving vaccines by separate injections[11]

	Preimmunisation PRP-T/DTP combined laboratory 1 (n=142)	*Postimmunisation PRP-T/DTP combined laboratory 1† (n=143)*	*Postimmunisation PRP-T/DTP combined laboratory 2† (n=143)*	*Postimmunisation PRP-T and DTP** separate sites laboratory 2*[11] *(n=102)*
Pertussis toxin (EIAU/ml)	1.2 (1.0 to 1.5)	41 (31 to 55)	6700 (5250 to 8540)	850
FHA (EIAU/ml)	5.7 (4.7 to 7.0)	40 (35 to 47)	6110 (5270 to 7080)	6200
Total agglutinins (reciprocal of dilution)	20 (16 to 24)	420 (360 to 480)	—	—
Agglutinins 2 and 3 (reciprocal of dilution)	195* (140 to 270)	—	33 490* (26 610 to 42 150)	38 900

† Preimmunisation and postimmunisation serology for agglutinins 2 and 3 performed in laboratory 2.
** Wellcome (Evans) DTP.
† Different assays used for antibody to pertussis toxin and FHA in laboratories 1 and 2. Results cannot be compared between laboratories.
DTP = absorbed diphtheria, tetanus, pertussis vaccine; PRP-T = polyribosylribitol phosphate tetanus toxoid conjugate vaccine; FHA = filamentous haemagglutinin; EIAU = enzyme immunoassay (that is, arbitrary) units.

et al from Gloucester, mixing PRP-T with DTP.[22] Both are a little lower than titres reported for PRP-T administered in a separate limb: infants in our study achieved a mean titre of 3.65 μg/ml compared with 3.4 μg/ml for the combined vaccine in Gloucester.[22] Infants given vaccine by separate injection achieved GMT of 4.5 μg/ml in that study and 5.0 μg/ml in the first United Kingdom study of the PRP-T vaccine in the Oxford region.[20] Three infants in our study failed to achieve minimum protective antibody titres four weeks after the primary course was complete. The same proportion (2%) remained unprotected in Oxford,[20] an expected response to Hib conjugate vaccines in infancy. Follow up from a large trial of the earlier Hib polysaccharide (unconjugated) vaccine found that PRP titres above 1 μg/ml three weeks after immunisation were associated with continued protection against invasive Hib disease in an immunised population.[14-16] Eighty two per cent of our infants achieved titres above 1.0 μg/ml, while 91% given vaccine by separate injections reached this threshold in Oxford.[20] The proportion achieving this titre in our study—although lower than that reported from Oxford—is consistent with experience in industrialised countries of administering PRP-T by separate injection.[29] Two studies have shown equivalent GMT for PRP following immunisation with DTP and PRP-T vaccines when combined or given at separate sites[5 10]; several others have found lower mean values in the combined vaccine group,[3 9 22] although this only achieved statistical significance in one study from Chile.[9]

Infants completing the current study with PRP titres below 0.15 μg/ml were offered an additional dose of vaccine at 8-10 months of age. All three subsequently achieved antibody levels above 1 μg/ml, suggesting effective priming by earlier immunisation.

There were substantial rises in antibody titre to each of the pertussis antigens measured. Mean titres following immunisation were higher (pertussis toxin) or close (FHA, agglutinins 2 and 3) to figures from previous reports from the accelerated schedule (table 4).[11 21 22] Those studies used a different DTP vaccine; the response to pertussis antigens, particularly pertussis toxin, may vary between vaccines from different manufacturers.[30 31]

The 'accelerated' United Kingdom primary schedule is unusual in beginning immunisation at age 2 months with completion by 4 months in the absence of subsequent reinforcing doses of pertussis or Hib vaccines. The timing of the schedule has raised concerns about interference from maternal antibody still present at the time of first immunisation.[11] We found no evidence for substantial interference in this population, although infants with high levels of maternal antibody to tetanus and diphtheria achieved lower titres at 5 months than those with lower initial levels of antibody (table 5). The clinical significance of this inhibition is uncertain. In the short term at least, all infants achieved levels of tetanus and diphtheria antitoxin held to confer protection against disease.[17 19] The effect on response to diphtheria was less marked, with preimmunisation titres much lower than for tetanus, reflecting maternal immunisation experience. Until recently the last dose of diphtheria toxoid has been given in the United Kingdom at age 4 years (school entry). Since 1994, combined tetanus/diphtheria immunisation has been recommended for school leavers. As diphtheria antibody levels rise in the young adult population, interference with the response to primary immunisation may become more important.

Inhibition from passive antibody has previously been demonstrated in the accelerated schedule for tetanus, pertussis toxin, and for

Table 5 Effect of preimmunisation titres on subsequent antibody levels. Infants were divided into two groups (above and below threshold indicated) on the basis of initial antibody titre. Postimmunisation titres for these groups were compared by Mann-Whitney test

	Threshold preimmunisation	$n_{low\ pre-}$	$n_{high\ pre-}$	Mean titre $_{low\ pre-}$	Mean titre $_{high\ pre-}$	p Value
Diphtheria	0.01 IU/ml	85	49	1.25	0.83	< 0.01
Tetanus	0.1 IU/ml	33	109	1.62	0.88	< 0.001
PRP	0.15 μg/ml	99	43	3.52	3.91	0.68
Pertussis toxin*	Median	71	71	91.5	18.0	< 0.001
FHA*	Median	71	71	50.3	32.5	< 0.01
Total agglutinins	≤ 10/ ≥ 20	62	80	438	408	0.91
Agglutinins 2 and 3	Median	71	71	48 591	22 504	< 0.01

* Assays performed in laboratory 1.
$n_{high\ pre-}$ = number of infants with initial antibody below threshold indicated; $n_{low\ pre-}$ = number of infants with initial antibody above threshold indicated; mean titre $_{low\ pre-}$ = final geometric mean antibody titre in group with 'low' initial titres; mean titre $_{high\ pre-}$ = final geometric mean antibody titre in group with 'high' initial titres; PRP = polyribosylribitol phosphate; FHA = filamentous haemagglutinin.

pertussis agglutinins 2 and 3.[11] High initial titres of antibody to tetanus, the PRP-T carrier protein, have also been associated with lower antibody response to PRP in infants immunised with two doses of PRP-T.[12] In contrast, we found higher PRP responses in infants with preimmunisation tetanus antibody above 0.1 IU/ml ($p < 0.001$, Mann-Whitney test). These infants were more likely to achieve protective thresholds for PRP; three infants with low initial tetanus titres failed to reach 0.15 μg/ml for PRP, while no infant with higher initial titres remained unprotected ($p = 0.01$, Fisher's exact test). Similarly, 61% of those with low preimmunisation tetanus titres achieved PRP antibody of 1.0 μg/ml or more, compared with 89% for those with high initial tetanus titres $p < 0.001$, χ^2 test).

Factors influencing the antibody response to the primary series—such as age at immunisation, the mixing of antigens, or the nature of the conjugate vaccine carrier protein—may all have implications for continued vaccine protection. Concerns about impaired antibody response to vaccines may increase as new antigens, for example acellular pertussis vaccines, are included and mixed in the primary schedule. This may require reappraisal of the need for booster doses of vaccine in the second year. We intend to investigate the persistence of protective antibody in a study measuring antibody levels at age 13 months and at school entry.

Despite these observations, immunisation in the manner described conferred high levels of protection with few adverse effects. Combining the injected antigens of the primary schedule in the same syringe provides similar antibody responses to those achieved by administering DTP and Hib (PRP-T) vaccines by separate injection. The combined vaccine improves acceptability and provides a basis for including new immunising antigens in the future.

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Randomised trial of *Haemophilus influenzae* type-b tetanus protein conjugate for prevention of pneumonia and meningitis in Gambian infants

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Summary

Background In developing countries, pneumonia and meningitis due to *Haemophilus influenzae* type b (Hib) are common in children under age 12 months and the mortality from meningitis is high. Protein-polysaccharide conjugate vaccines have brought Hib disease under control in industrialised countries. We did a double-blind randomised trial in The Gambia to assess the efficacy of a Hib conjugate vaccine for the prevention of meningitis, pneumonia, and other invasive diseases due to Hib.

Methods Between March, 1993, and October, 1995, 42 848 infants were randomly allocated the conjugate vaccine Hib polysaccharide tetanus protein (PRP-T) mixed with diphtheria-tetanus-pertussis vaccine (DTP), or DTP alone at age 2 months, 3 months, and 4 months. Children who presented with signs of invasive Hib were investigated by blood culture and, where appropriate, by lumbar puncture, chest radiograph, or percutaneous lung aspirate. Children were followed up for between 5 and 36 months.

Findings The median ages at which children received the study vaccine were 11 weeks, 18 weeks, and 24 weeks. 83% of children enrolled received all three doses of vaccine. 17 cases of culture-positive Hib pneumonia, 28 of Hib meningitis, and five of other forms of invasive Hib disease were detected amongst the study children. The efficacy of the vaccine for the prevention of all invasive disease after three doses was 95% (PRP-T vaccinees 1, controls 19 [95% CI 67–100]), for the prevention of Hib pneumonia after two or three doses, 100% (vaccinees 0, controls 10 [55–100]), and for the prevention of radiologically defined pneumonia at any time after enrolment, 21·1% (PRP-T vaccinees 198, controls 251 [4·6–34·9]).

Interpretation PRP-T conjugate Hib vaccine prevented most cases of meningitis and pneumonia due to Hib in Gambian infants. The reduction in the overall incidence of radiologically defined pneumonia in PRP-T vaccinees suggests that about 20% of episodes of pneumonia in young Gambian children are due to Hib. The introduction of Hib vaccines into developing countries should substantially reduce childhood mortality due to pneumonia and meningitis.

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Introduction

Invasive disease due to *Haemophilus influenzae* type b (Hib) remains a major problem in developing countries, where the epidemiology of the disease differs from that seen in industrialised countries in several important respects. In developing countries, the incidence of Hib disease is greater than in industrialised countries, and the disease occurs in younger children, with most cases occurring before 12 months of age and almost half before the age of 6 months.[1,2] The clinical pattern of disease also differs: in developing countries, pneumonia is commoner than meningitis, whereas epiglottitis is not seen.[3] The outcome of Hib meningitis in developing countries is poor. In sub-Saharan Africa, 20–40% of patients who reach health-care facilities die from the disease and neurological sequelae occur in a significant proportion of survivors.[3] Nasopharyngeal carriage of Hib is commoner and occurs in younger children in developing than in industrialised countries.[1,4]

Protein-polysaccharide conjugate vaccines against Hib have been highly effective in reducing Hib disease and carriage in industrialised countries.[5,6] Although, before the present study, no randomised double-blind efficacy study of the Hib polysaccharide-tetanus protein conjugate vaccine (PRP-T) had been done, the vaccine was effective in preventing Hib meningitis in British infants in an open study.[7] Two randomised trials of other Hib conjugate vaccines in American communities whose living conditions resemble those of developing countries produced differing results. Hib polysaccharide-*Neisseria meningitidis* group B outer membrane protein complex conjugate vaccine (PRP-OMPC) proved effective in Navajo Indians,[8] whereas Hib polysaccharide-diphtheria toxoid conjugate vaccine (PRP-D) did not in Alaskan natives.[9]

Before this study, no efficacy studies of any Hib

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vaccines had been done in developing countries, although an effectiveness study was done in Santiago, Chile, where PRP-T was shown to prevent invasive Hib disease.[12] However, the epidemiology of Hib disease in Santiago is more similar to that in the USA than to that in poorer developing countries. For example, the proportions of cases of Hib disease in infants younger than 12 months in Chile, the USA (before vaccination), and The Gambia were 60%, 51%, and 84%, respectively.[13,14] Furthermore, no studies have evaluated the efficacy of vaccination against Hib pneumonia, which is probably the most common manifestation of invasive Hib disease in developing countries. The study reported here was designed to assess the efficacy of an Hib conjugate given to infants in a poor developing country for the prevention of pneumonia and other invasive disease due to Hib. As selection of pneumonia cases for investigation is subjective, a double-blind study design was used.

Methods

The study was done in the Western Region of The Gambia, an area where the epidemiology of Hib disease has been well studied and where most cases of invasive Hib disease are likely to reach medical attention.[14,15] During the 3 years before the study, the incidence of invasive Hib disease in children under age 12 months living in the study area was estimated to be 274 per 100 000 children each year.[15] The study area, which is populated by about half of the one million people who live in The Gambia, comprises a mixture of urban and rural communities in one of the poorest countries in Africa. Despite this, the quality of health services in the area is fair and infant vaccination coverage high. In recent government surveys, over 85% of children aged 1 to 2 years had received their third DTP vaccination. The area is served by three hospitals with sufficient paediatric services to manage childhood meningitis, and several health centres and private hospitals that admit paediatric patients. Most children with pneumonia are treated as outpatients. Vaccines are given by staff of the 20 main health centres in the area, who also hold vaccination clinics in 43 smaller health centres.

Enrolment and vaccination

Vaccines used for the study were Hib polysaccharide-tetanus protein conjugate vaccine (PRP-T; ActHIB, Pasteur Mérieux, Lyon, France, lot numbers S2801 and S3044), 10 µg per dose, and diphtheria-tetanus-pertussis vaccine (DTP, Pasteur Mérieux, lot numbers G5801, J0102, and K5590). The batch number of all doses given was recorded. All children in the study received either PRP-T or dextrose placebo mixed with DTP at the time when they presented for their infant DTP vaccinations, which in The Gambia are recommended to be at ages 2 months, 3 months, and 4 months (table 1); although in practice they are generally given later than this. The combination of PRP-T with DTP has been shown to be safe and immunogenic in Gambian infants.[16] Study vaccine was packaged in vials labelled with a single digit number between 0 and 9; five of the codes were used for vials containing PRP-T and the other five for vials containing placebo. The code defining which numbers correspond to PRP-T was held by Pasteur Mérieux and by the Safety Monitoring Group.

Most Gambian infants make their first contact with the health services during the first 2 weeks of life when they are given a health card bearing a unique sequential number for the health centre at which they are registered, and they receive BCG, hepatitis B, and oral poliomyelitis vaccines. During the course of the study, mothers of infants who presented for this first visit were informed about the study and given an information sheet in English and a local language (Mandinka). When a mother and infant returned for the first DTP vaccination, at or after age 8 weeks, they were interviewed by project staff and given the opportunity to join the study. If the family chose to join, a label was fixed to the child's health card indicating the code for the vaccine allocated (0–9, according to the last digit of the health card number). The infant was then given the appropriate study vaccine, mixed with DTP vaccine, intramuscularly either into the anterolateral thigh or the deltoid muscle according to the usual practice at each health centre. Each vial of study vaccine bore two peel-off labels with the vaccine code. After the vaccine was administered one label was affixed to the child's health card and the date recorded, and the other was attached to the data sheet, which was returned to the main data entry room. When an infant returned for second and third doses of DTP the appropriate study vaccine was given again with DTP and the dose recorded in the same manner. Infants were ineligible to join the study if they had already received a dose of DTP from another health centre, or if they were over age 12 months at the time that they presented for their first dose of DTP. A detailed procedures manual was prepared as a guide for field workers, which included details of potential problems. It was stressed in the manual and during the training courses for staff that any mistakes that were recognised must be carefully recorded. During the first year of the study a randomly selected sample of 20% of enrolled infants was later visited at home where a detailed sociological questionnaire was completed.

Birth or soon after	2 months	3 months	4 months	9 months
BCG	DTP	DTP	DTP	Measles
OPV	OPV	OPV	OPV	OPV
Hepatitis B	Hepatitis B		Hepatitis B	Yellow fever

DTP=diphtheria-tetanus-pertussis; OPV=oral poliovaccine.

Table 1: The Expanded Programme of Immunisation schedule recommended by the Gambian government

Data sheets with demographic information from all infants enrolled and details of all immunisation contacts were submitted to the main data room where they were double-entered and verified. Data were cleaned and checked as they were entered and any irregularities investigated.

Case detection, bacteriology, and immunogenicity

Possible cases of invasive Hib disease were sought amongst study infants who presented to the three main hospitals in the study area—the Royal Victoria Hospital, Banjul; the Medical Research Council (MRC) Hospital, Fajara; and the Sibanor Mission Hospital, Sibanor. Health centres and other medical facilities in the area also identified cases of possible Hib disease who were either investigated at the facility, and specimens obtained from them transferred to the MRC laboratory, or they were referred to one of the three main hospitals for treatment and investigation.

Children in the study who were ill when they presented to one of the health facilities were referred directly to one of the study physicians or screened by an experienced nurse, who referred any children with signs of possible Hib disease to a physician. Rapid respiratory rate, fever, or signs of meningitis were general recommendations for referral. When a child was considered to possibly have Hib, a detailed history was taken and an examination done by the physician, usually one of the three paediatricians employed for the study (SU, AO, CO). Blood cultures and urine collection, for antigen detection, were done for all children with severe pneumonia, possible meningitis, or other possible invasive Hib disease, and many children with mild or moderate pneumonia. Lumbar punctures were done in possible cases of meningitis, and in cases of septic arthritis joint aspirations were done. Where possible (Royal Victoria Hospital, Banjul, and MRC Laboratories, Fajara) pneumonia was confirmed by chest radiography. At the end of the study, before the analysis, all radiographs were read by one of the five paediatricians working on the study but who were unaware to which study group the child belonged. Only radiographs showing definite radiological pneumonia were regarded as positive; minor changes were ignored. Percutaneous lung aspiration was done, as described previously,[17] in those patients who were clinically stable and had definite radiological consolidation adjacent to the chest wall.

All hospital admissions of study participants to the three main hospitals were recorded. Although the study was not designed to ascertain all deaths, verification of deaths of children in the study was sought by all means available. Deaths occurring at home were investigated by post mortem questionnaire completed by the family. Admissions and deaths were linked to the vaccination records of the children concerned.

Surveillance for invasive Hib disease in children under 5 years of age had been in place in the study area for 3 years before the beginning of the trial.[11] All specimens for bacterial detection were analysed at the MRC laboratories in Fajara. Venous blood (1–3 mL) was inoculated directly into a bottle of tryptic soy broth and brain-heart infusion containing sodium polyanethol sulphonate (liquoid; Becton Dickinson and Company Cockeysville, Maryland, USA). Bacteriology methods used were as described previously.[11]

Isolates from culture media were identified by standard methods, including X and V growth factor dependency determination. *H influenzae* isolates were serotyped by standard slide agglutination with type-specific antisera (Murex Diagnostics, Dartford, UK) and were shipped on chocolate agar slopes to the UK National Haemophilus Reference Laboratory, John Radcliffe Hospital, Oxford, for confirmation by capsular genotyping by PCR amplification of capsule-specific sequences.[11]

During the course of the study, immunogenicity of both batches of vaccine used were evaluated. Infants were enrolled into the immunogenicity studies if they presented for their third dose of vaccine at a specified health centre and their mother gave consent for blood samples to be taken at the time of the third dose and 1 month later. Samples were kept cool and centrifuged without delay. Serum was stored at −20°C until it was transported to France for analysis. PRP antibody concentrations were assayed by RIA in a masked fashion by the Pasteur Mérieux laboratories.[11]

Statistical analysis

Before the completion of the study an analytical plan was prepared by the investigators and approved by the International Steering Committee. The plan specified two primary endpoints for the calculation of vaccine efficacy: protection against proven Hib pneumonia after two or more doses of vaccine had been received, and protection against all invasive disease after three doses of vaccine had been received. The less rigorous endpoint for proven Hib pneumonia was chosen because of the anticipated difficulty in detecting cases in this category and the consequent lack of power for this endpoint. Plans were specified for determining the impact of the vaccine on pneumonia of undetermined aetiology. In the analytical plan, a child was deemed to have received one dose if 21 days had elapsed between the administration of the first dose and the child's presentation to hospital with possible Hib disease, or if 14 days had elapsed between the administration of the first dose and the onset of the child's illness. Second and third doses were considered to have been received if, after administration of the dose, 14 days had elapsed before hospital admission or 7 days had elapsed before the onset of illness.

Vaccine efficacy was calculated by comparing the numbers of events in each group, assuming that the denominators are the same.[10] The vaccine efficacy was checked using the actual number of child months at risk. Where vaccine failures after one or two doses could be divided into early and late failures the cut-off point was arbitrarily set, before the data were analysed, at 8 weeks from the last dose of vaccine, that being twice the recommended interval between doses. Comparisons between continuous variables were made using Student's *t* test, and 2×2 tables were analysed by the χ^2 or Fisher's exact test as appropriate. For the description of antibody concentrations, data were analysed by geometric means as logarithmic transformation normalised the data. CIs were calculated using exact methods. For the primary analyses, children who had accidentally received one dose of the wrong type of vaccine were regarded as partly vaccinated in the vaccine group.

	PRP-T recipients	Controls
Total population	21 490	21 358
Dose 1: median (IQR) age* (days)	78 (67–94)	78 (67–94)
Dose 2: median (IQR) age* (days)	124 (106–151)	124 (106–152)
Dose 3: median (IQR) age* (days)	169 (145–208)	170 (144–209)
Received one dose (%)	100	100
Received two doses (%)	93·5	94·9
Received three doses (%)	82·8	83·9
Population randomly sampled for detailed interview (n)	1353	1321
Mother had formal education (%)	25	25
Father had formal education (%)	28	29
Father farmer (%)	40	41
Polygamous families (%)	38	38
Electricity in house (%)	24	24
Mean distance from a health facility (km)	4·3	3·8
Mean number siblings alive	2·4	2·5
Mean number siblings dead	0·5	0·5
Mean mid upper-arm circumference (cm) at the time of enrolment	13·9	13·8

Table 2: **Demographic characteristics of PRP-T recipients and controls**

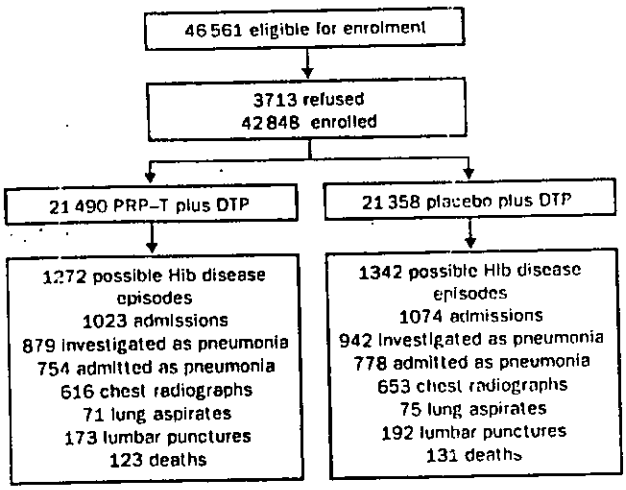
Ethical considerations

Individual informed consent was obtained from the families of infants enrolled in the main trial by the three-step process outlined above. A group discussion with mothers of newborn infants was followed by the distribution of an information sheet, which the mother was to bring when she brought the child for the first DTP vaccine, at which time an individual interview was conducted. If the family agreed that their infant should join the study, the information sheet was signed by the field worker involved and filed as a record of the consent. Demographic information was recorded for all refusals. Individual written informed consent was required for enrolment of an infant into the immunogenicity study and for lung aspiration. Specific consent was not sought for those investigations that were regarded as consistent with routine clinical care.

The study was approved by the Gambian Government MRC Ethical Committee, by the Secretariat Committee on Research Involving Human Subjects (SCRIHS) at the World Health Organization, Geneva, and by the Office for Protection from Research Risks (OPRR) of the United States Government. An international steering committee, comprising three recognised experts in the field and a representative of the Gambian Government, was formed to monitor and review the progress of the study and the development of the analytical plan. Rules for premature cessation of the study on safety grounds were agreed upon. A safety monitoring group was formed with two members located in Europe who had access to the study code. Their role was limited to monitoring and reviewing safety aspects of the trial. They received regular reports of deaths and hospital admissions from the investigators. They had the power to call a meeting of the International Steering Committee if they believed that on the basis of safety data conditions for stopping the trial existed. In addition to the above a local monitoring committee met regularly in The Gambia to monitor the day-to-day progress of the trial.

Results

Between March 8, 1993, and Oct 1, 1995, 42 848 infants were enrolled into the study, of whom 21 490 received the PRP-T mixed with DTP and 21 358 received DTP alone (figure). Study vaccines were available until March 1, 1996, for partly vaccinated children who presented for later doses. Of the 42 848 children enrolled, 40 366 (94%) received a second dose of vaccine and 35 713 (83%) received all three doses. The median ages of administration for doses one, two, and three were 11 weeks, 18 weeks, and 24 weeks, respectively, although some doses were given very late. The proportions of



Trial profile

*These figures differ from those in table 5 because those who received doses of the wrong vaccine were excluded from table 5.

infants receiving the second and third doses were similar in the vaccine and placebo groups (table 2). No attempt was made to encourage children to come for vaccination earlier than the time they would normally have presented for DTP vaccination. The characteristics of the two groups are summarised in table 2. There were no important differences between the groups with respect to sociological indicators that may affect the risk of Hib disease. 70 470 doses of vaccine were administered from the first batch and 48 457 from the second. Some children received doses from different batches. Some children received one dose of vaccine of the wrong number. 494 cases resulted in children receiving both vaccine and placebo; these children were regarded as partly vaccinated and included in the analysis. At all health centres mechanisms were in place to detect possible side-effects but no serious adverse effects were observed. 3713 families (8·0% of those asked) refused to allow their infants to join the study. The most common reason for refusal to join the study was fear of side-effects of an experimental vaccine.

During the trial, 2256 children in the study were investigated on 2614 occasions by the study physicians for possible episodes of invasive Hib disease. 1821 episodes of pneumonia were investigated, from which 146 lung aspirates were done and 1269 chest radiographs were taken and read later in a blinded manner. There were ten cases of bone or joint infection and 365 lumbar punctures were done for possible meningitis.

During the course of the study there were 2097 hospital admissions involving 1879 study children (PRP-T vaccinees 913, controls 966). There were no significant differences between the groups in this regard and analysis of the reasons for admission did not reveal any unsuspected possible adverse effects. 254 deaths were documented in study children, 123 in PRP-T vaccine recipients and 131 in control recipients. The presumed causes of death were similar in the two groups. On the basis of other studies from the same community we believe that the number of deaths verified is only a proportion of the deaths that probably occurred amongst study children.

Doses	Pneumonia*		Meningitis		Other		All	
	PRP-T	Control	PRP-T	Control	PRP-T	Control	PRP-T	Control
0	0	1	3	1	0	0	3	2
1	2	4	3	4	0	1	5	9
2	0	5	0	4	1	1	1	10
3	0	5	1	12	0	2	1	19
Total	2	15	7	21	1	4	10	40
2 or 3	0	10	1	16	1	3	2	29

*Children with pneumonia which occurred in association with proven Hib meningitis were classified as meningitis.

Table 4: **Cases of confirmed invasive Hib disease by vaccination status and diagnosis**

Immunogenicity studies

Between June, 1993, and September, 1994, 391 infants who received the first batch of vaccine (lot number S2801) were enrolled at the time of their third dose and had a first blood sample drawn (median age 169 days, range 128–469 days), of whom 216 were located for taking the second blood sample (median time since last dose 35 days, range 21–108 days). Between December, 1994, and November, 1995, 109 infants who received the second batch of vaccine (lot number S3044) were enrolled at the time of their third dose (median age 164 days, range 116-281 days), of whom 48 had another blood sample taken (median time since last dose 35 days, range 28–147 days). PRP antibody concentrations for these two groups of infants are presented in table 3. There were no significant differences in immunogenicity between the two lots of vaccine used.

Vaccine efficacy

There were 50 episodes of culture-proven invasive Hib disease detected amongst study children between the start of enrolment in March, 1993, and the cessation of surveillance on March 1, 1996). These included 28 cases of meningitis, positive by blood (n=22) or cerebrospinal fluid (CSF; n=26) culture, 17 cases of pneumonia positive by culture of blood (n=16) or lung aspirate (n=2), three cases of septic arthritis positive by blood (n=2) or joint fluid (n=1) culture, and two cases of septicaemia positive by blood culture. Nine of the children with Hib disease died, all with meningitis (PRP-T vaccinees two, controls seven). All invasive isolates but one, which was lost, were confirmed by genotype analysis. One isolate from a child with pneumonia that was sent for genotype analysis as a type b was found to be genotypically a non-encapsulated *H influenzae*. The data from this child was excluded from the analysis. Th

	Vaccine lot number S2801						Vaccine lot number S3044					
	Geometric mean titre (μg/mL; 95% CI)		>0·15 μg/mL (%)		>1·0 μg/mL (%)		Geometric mean titre (μg/mL; 95% CI)		>0·15 μg/mL (%)		>1·0 μg/mL (%)	
	PRP-T	Control	PRP-T	Control	PRP-T	Control	PRP-T	Control	PRP-T	Control	PRP-T	Control
Before dose 3	3·20 (2·52–4·06)	0·08 (0·07–0·09)	188/202 (93)	23/182 (13)	154/202 (76)	5/182 (3)	4·04 (2·62–6·24)	0·08 (0·07–0·10)	49/51 (96)	3/42 (7)	38/51 (75)	1/42 (2)
After dose 3	6·59 (4·91–8·84)	0·08 (0·07–0·10)	92/95 (97)	11/99 (11)	87/95 (92)	4/99 (4)	5·44 (2·86–10·33)	0·10 (0·06–0·17)	25/26 (96)	1/17 (6)	23/26 (88)	1/17 (6)

Table 3: **PRP antibody concentrations in infants before and 1 month after receiving their third dose of PRP-T vaccine or placebo**

	All enrolled			Fully vaccinated		
	PRP-T	Control	Vaccine efficacy (%, 95% CI)	PRP-T	Control	Vaccine efficacy (%, 95% CI)
Cough with fast breathing or chest wall indrawing*	873	913	4·4 (−5·0, 12·9)	526	570	7·7 (−4·1, 18·2)
Diagnosed by physician as pneumonia and investigated	864	929	7·0 (−2·1, 15·3)	528	571	7·5 (−4·3, 18·0)
Lower chest wall indrawing†	436	466	6·5 (−6·8, 18·1)	259	286	9·4 (−7·5, 23·7)
Radiologically defined pneumonia	198	251	21·1 (4·6, 34·9)	132	170	22·4 (1·9, 38·6)
Lobar pneumonia or pneumonia with effusion	86	115	25·2 (0·24, 44·1)	61	72	15·3 (−20·8, 40·8)
Pneumonia with hypoxaemia (SaO_2 <90%)	36	40	10·0 (−44·7, 44·2)	18	28	35·7 (−20·5, 66·5)

Children who received a dose of the wrong type of vaccine were excluded.
*Defined by WHO as those cases with acute respiratory infection which require antibiotics; fast breathing is respiratory rate ≥50 if under age 12 months and ≥40 if over 12 months. †Regarded by WHO as requiring referral for admission.

Table 5: **Episodes of pneumonia in all children enrolled and in fully vaccinated children by pneumonia definition and vaccine group**

diagnoses and vaccination status of the children with confirmed Hib disease are summarised in table 4. With respect to the two primary endpoints in the trial, the efficacy of two or more doses of PRP-T for the prevention of Hib pneumonia was 100% (PRP-T vaccinees 0, controls ten [95% CI 55–100]), while the efficacy of three doses for the prevention of all invasive Hib disease was 95% (PRP-T vaccinees one, controls 19 [67–100]). There were two cases of Hib disease amongst children who had received two or more doses of vaccine. One was a child aged 14 months who developed blood and CSF culture-positive Hib meningitis 280 days after receiving the third dose of vaccine. The other case was a child aged 25 months who presented with kwashiorkor and fever 615 days after having received the second dose of vaccine; blood culture was positive for Hib, and serology positive for HIV type 1. This girl presented again 9 months later, again with Hib septicaemia and died in hospital.

Efficacy against all invasive disease after a single dose of vaccine was 44% (PRP-T vaccinees five, controls nine [95% CI −85, 85]). Amongst children who had received one dose only less than 56 days before their admission there were two cases of invasive disease in the vaccine group and seven in the control group. Thus, the short-term vaccine efficacy after one dose was 71% (CI −50, 97). Five children, who developed invasive Hib disease became ill within 7 days of a dose of study vaccine; three in the PRP-T group (two after the first dose and one after the second) and two in the control group.

An analysis was done to assess the impact of an Hib vaccination strategy on the overall rate of Hib disease beyond the day of administration of the first dose. By this analysis the vaccine efficacy for all invasive disease was 75% (PRP-T vaccinees ten, controls 40 [95% CI 49–89]), while for Hib pneumonia it was 87% (PRP-T vaccinees two, controls 15 [43–99]). The times between the previous dose and hospital admission for the ten children who received PRP-T vaccine and later developed invasive Hib disease were as follows: for those who had received only one dose: 2 days, 9 days, 9 days, 34 days, 49 days, 114 days, and 147 days; two doses, 13 days and 615 days; and three doses, 280 days. The four infants who were admitted less than 3 weeks after having received the first dose or less than 2 weeks after the second were regarded as not having received that dose for the purpose of the analysis.

Whilst the vaccine was shown to be efficacious in the prevention of proven Hib pneumonia, we were also interested to know whether the vaccine had an impact on overall rate of pneumonia as an indirect measure of the true incidence of Hib pneumonia in the community. Children who had received one dose of the wrong type of vaccine were excluded from this analysis. Amongst children in the study there were 449 episodes of definite pneumonia classified radiologically (PRP-T vaccinees 198, controls 251; p=0·012), of whom 13 died (PRP-T vaccinees five, controls eight). The numbers of episodes of acute lower respiratory tract infections categorised in different ways by vaccination group are presented in table 5. PRP-T vaccination reduced the overall incidence of acute lower respiratory infection regarded by WHO as requiring antibiotics by 4·4% (95% CI −5·0, 12·9). However, most of the cases prevented were cases of radiologically defined pneumonia, which were reduced by 21·1% (4·6–34·9) in PRP-T vaccinees, and by 25·2% (0·24–44·1) if only cases of lobar consolidation or consolidation with effusion, the features usually associated with Hib pneumonia,[21,22] are included.

Discussion

Murray and Lopez estimated that over 200 000 cases of Hib meningitis occur in the developing world every year.[23] The mortality and morbidity amongst those who get medical attention is substantial; a significant proportion die without getting any medical attention. Our study showed that PRP-T Hib conjugate vaccine was highly effective in preventing pneumonia and meningitis due to Hib after three doses given in infancy, and offered 75% protection from the time the first dose was given. As has been found in industrialised countries, good immunogenicity was associated with efficacy. Previous studies have shown that 12·7% of invasive Hib disease in The Gambia occurs in children who have not received their first dose of DTP[11] so, ignoring the herd effect, this study suggests that 66% of Hib disease could be prevented by the introduction of PRP-T with DTP into routine immunisation. This is likely to be an underestimate because herd effects resulting from diminished carriage will probably prevent many of the cases that would have occurred in children too young to be immunised effectively. During the course of this study we have shown that vaccination of infants with PRP-T reduces the probability of a child being an oropharyngeal carrier of Hib during the second year of life by 60%. Thus, the true impact of a Hib vaccination programme on the incidence of disease in a developing country will depend on the vaccine coverage rate and will not be known until we have the results of an impact study now underway in The Gambia.

The major obstacles to the introduction of Hib vaccines into developing countries are the cost of the vaccines and lack of information on the epidemiology of Hib disease for many parts of the world. At present the cost of the Hib vaccines is prohibitive for most developing countries. However, the introduction of a tiered pricing system and the entry of other manufacturers into the market is likely

to reduce the price substantially in the coming years, provided demand can be established. The burden of Hib disease in many developing countries has been underestimated because of technical difficulties surrounding the culture of the organism, particularly when community antibiotic use is prevalent. Where Hib disease has been studied it has been found to be responsible for a large proportion of meningitis cases in infancy, with incidence rates in the first year of life much higher than those that were seen in developed countries before the introduction of Hib vaccines. However, studies from Asia indicate that the rate of Hib disease there may be lower than elsewhere in the world.[11] More studies of Hib epidemiology in developing countries are needed, particularly from Asia.

An important finding of this study was the high degree of efficacy shown by the vaccine for the prevention of Hib pneumonia, and the significant reduction in the total number of radiological pneumonia cases seen in the vaccinated group. Because our study relied on children presenting to a health facility and because radiography was available at only two of the facilities, we could not have detected all cases of pneumonia. This does not affect the proportion of pneumonia cases in the two groups, but it gives a falsely low impression of the number of pneumonia cases in this population. Many doubted that the vaccine would be effective in preventing pneumonia because, unlike other manifestations of Hib disease, the pathogenesis of bacterial pneumonia does not necessarily involve blood-borne spread. In this respect our study supports the findings of a recent open study from Chile which also showed a reduction in Hib pneumonia rates in children receiving PRP-T.[12] Before our study it was believed widely, on the basis of hospital-based aetiology studies, that Hib was responsible for 10–15% of cases of severe pneumonia in Gambian infants and a similar proportion of pneumonia deaths.[1] The present study suggests that this may be an underestimate. If efficacy in fully vaccinated children is 100%, our data estimate the proportion of radiologically severe pneumonia cases due to Hib in Gambian children over age 2 months to be about 21%. Even this could be an underestimate because vaccine efficacy is probably less than 100% and herd effects may have reduced the incidence in the control group. Despite these biases, the study demonstrates that vaccine trials can provide more accurate information on the true aetiology of pneumonia than hospital-based aetiology studies.

We chose not to include a booster in the second year of life since most Hib disease in The Gambia occurs in the first year, and compliance for routine visits for vaccination in the second year of life is generally poor in African countries. The excellent vaccine efficacy demonstrated in this study indicates that, within the follow-up period of the study, late failures due to secondary failure of the vaccine do not appear to be a problem. However, caution must be expressed because the two vaccine failures observed after two or three doses had been given were seen 9 months after the third dose and 20 months after the second dose. Thus, there remains the possibility that more late cases will occur, and careful documentation of these cases will be required to establish whether these cases take the form seen in older cases of Hib disease in industrialised countries, with epiglottitis, and whether a booster dose in the second year of life may be needed.

In The Gambia, a developing country with high rates of invasive Hib disease occurring in very young children, PRP-T conjugate vaccine was efficacious in preventing both meningitis and pneumonia due to Hib. The vaccine trial proved to be a powerful tool for assessing the true burden of Hib disease in the community. Efforts to promote the introduction of Hib conjugate vaccines into developing countries will be strengthened by this information, and the remaining barriers to the large-scale introduction of the vaccines—cost and shortage of Hib epidemiology studies—should now be addressed aggressively. However, careful attention must be paid to both the costs and benefits of the introduction of Hib vaccines, particularly in countries whose Expanded Programme of Immunisation programmes are struggling to maintain coverage. Children in the developing world deserve to be protected against all serious, vaccine preventable diseases: measles, tuberculosis, poliomyelitis, pertussis, tetanus, diphtheria, hepatitis B, and Hib.

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Antibody responses and symptoms after DTP and either tetanus or diphtheria *Haemophilus influenzae* type B conjugate vaccines given for primary immunisation by separate or mixed injection

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The safety and immunogenicity of two conjugate Haemophilus influenzae *type B (Hib) vaccines administered either mixed with, or in separate limbs to, a whole-cell DTP vaccine, was compared in infants vaccinated at 2, 3 and 4 months of age. Antibody titres to purified polyribosylribitol phosphate, diphtheria, and to pertussis antigens between infants who received the Hib and DPT vaccines in separate limbs or in the same limbs were similar (P>0.1) while antibody titres to tetanus toxoid were higher in the later group (P<0.05). This study demonstrated that both Hib vaccines can be mixed with whole-cell DTP vaccine without reducing immunogenicity of either vaccine or increasing the incidence of adverse reactions.*

Keywords: *Haemophilus influenzae* conjugate vaccine

Conjugate *Haemophilus influenzae* (Hib) vaccines have resulted in a sharp fall in the incidence of this disease in the UK since their introduction into the primary immunisation schedule in October 1992[1]. Two Hib vaccines [(purified polyribosylribitol phosphate conjugated with tetanus toxoid (PRP–T) or combined oligosaccharide conjugate (HbOC)] were licensed to be administered in separate limbs to the whole-cell diphtheria–tetanus toxoids–pertussis (DTP) vaccine. The license was restricted to separate limbs because the safety and immunogenicity of combining the two preparations when they were given at 2, 3 and 4 months of age was not known. Previous studies comparing these Hib vaccines, given separately or mixed with DTP vaccine, had involved only older infants and had been conducted in other countries using different DTP vaccines. The administration of Hib and DTP vaccines would, however, be easier and more convenient if they were administered together in the same syringe.

Our aim was to determine if the safety and immunogenicity of mixing the Hib and DTP vaccines, for administration in a single injection, was similar to administering the vaccines in separate limbs for the UK primary immunisation schedule.

MATERIALS AND METHODS

The study population for the mixing group was infants aged 8–12 weeks of age who were eligible for routine primary immunisation with DTP and polio vaccines in Gloucester health district. Infants were recruited by trained study nurses who obtained written informed consent from parents. Participants were immunised according to the standard UK schedule at 2, 3 and 4 months of age. Infants were excluded from the study on the basis of recommendations for childhood vaccination published by the Department of Health[2]. Between May 1992 and February 1993, infants were recruited into an open, nonrandom study to receive DTP vaccine with either HbOC (n=75) or PRP–T (n=100) vaccines in the same syringe.

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Table 1 Antibody titres to pertussis, diphtheria, tetanus and *Haemophilus* and number with protective antibody titres after separate and mixed vaccinations

Antibody	PRP–T Separate GMT (95% CI)	PRP–T Mixed GMT (95% CI)	HbOC Separate GMT (95% CI)	HbOC Mixed GMT (95% CI)
Number	91	41	48	51
FHA	4027[a] (3392, 4781)	4477 (3589, 5585)	3404 (2561, 4524)	4732 (3861, 5796)
PT	367[a] (263, 513)	615 (364, 1038)	695 (459, 1053)	736 (522, 1037)
Agg2+3	22 335[a] (17 386, 28 563)	25 061 (13 812, 45 469)	30 761 (20 493, 46 174)	33 729 (26 673, 42 650)
Number	67	74	78	87
Diphtheria	4.54 (3.86, 5.34)	4.60 (3.89, 5.44)	4.78 (4.00, 5.69)	4.44 (3.81, 5.17)
≥0.1 IU ml^{-1}	67	74	78	87
Number	66	74	77	88
Tetanus[b]	0.65 (0.54, 0.77)	1.19 (0.98, 1.44)	0.96 (0.83, 1.11)	1.42 (1.23, 1.65)
≥0.1 IU ml^{-1}	66	74	77	88
Number	69	74	80	86
PRP	4.50 (3.21, 6.32)	3.39 (2.48, 4.66)	2.79 (1.93, 4.04)	3.63 (2.72, 4.85)
≥0.15 IU ml^{-1}	69	73	77	86

GMT=geometric mean titre, CI=confidence interval, FHA=filamentous haemagglutinin, PT=pertussis toxin, Agg2+3=agglutinogens 2 and 3, PRP=polyribosylribitolphosphate. Protective titres for antibodies were ≥0.1 IU ml^{-1} for diphtheria and tetanus and ≥0.15 IU ml^{-1}. [a]Data obtained from the North Hertfordshire infants. [b]$P<0.05$ for difference between titres obtained from infants given Hib and DTP vaccines separately or mixed in the same syringe (Mann–Whitney test)

The group of infants selected for comparison were infants who received DTP and Hib vaccines given at the same visit, but in separate limbs. These infants were recruited from two previous studies carried out between November 1990 and February 1992 in the Gloucester and North Hertfordshire health districts. In Gloucester 83 infants received HbOC and 81 received PRP–T. In North Hertfordshire, 95 infants received PRP–T. In both studies whole-cell DTP was administered at the time, but in the opposite limb. Infants in the mixing group (1992–1993) are compared throughout this study to the 83 (HbOC) and 81 (PRP–T) infants from the initial Gloucester study (1990–1992), with three exceptions. For these three exceptions an additional group of 95 infants from North Hertfordshire, who received PRP–T in separate limbs from DTP, was used for information on the size of the local reaction from DTP alone, on temperatures and on antibodies to the three pertussis antigens (pertussis toxin (PT), filamentous haemagglutinin (FHA) and agglutinogens 2 and 3). Comparison with the 95 infants from North Hertfordshire was necessary because the thermometers used in the initial Gloucester studies (1990–1992) were different from those used in the mixing study (1992–1993). In addition there was insufficient sera available from the initial Gloucester studies (1990–1992) to test for the three pertussis antigens.

Two Hib conjugate vaccines were used: PRP–T (Pasteur-Merieux, Lyon France) conjugated to tetanus toxoid and HbOC (Lederle-Praxis Biologicals, Pearl River, USA) conjugated to diphtheria CRM_{197}. The Wellcome (Biotech, Beckenham, UK) DPT whole-cell vaccine in 0.6 mg of $Al(OH)_3$ was used in all infants. Each infant also received oral polio vaccine.

The follow-up procedures were identical at each study site. Post-vaccination systemic symptoms (irritability, reduced feeding, inconsolable crying, disturbed night, less feeding, vomiting), the size of local reactions and twice-daily axillary or rectal temperatures were recorded by parents in a 7-day health diary following each dose of vaccine. Temperatures in the group of infants given DTP vaccine and HbOC separately were not included in the analysis as the thermometers used for this group were from a different manufacturer with a different calibration from those used at the other study sites. Study nurses made home visits at 24 h and 7 days after each immunisation to complete a standard symptom questionnaire and to measure local reactions and measure temperatures.

Blood samples were obtained by either venepuncture or heel prick from each infant before the first vaccine dose and 6–8 weeks after the third dose. Sera were tested for anticapsular *Haemophilus influenzae* type b antibody, antibody to diphtheria toxoid, tetanus toxoid and antibody to pertussis antigens (PT, FHA and agglutinogens 2 and 3) as described previously[3,4]. The same laboratories tested samples from children in both study sites.

Record cards and laboratory results were returned to the PHLS Communicable Disease Surveillance Centre. Children were grouped according to whether they had received vaccines separately or mixed in the same syringe. Post-vaccination geometric mean concentrations with 95% confidence intervals were calculated for each antibody. The presence of a statistical difference between categorical variables was determined using a Fisher's exact test and a Mann–Whitney test for numerical variables.

RESULTS

One hundred and sixty-four infants received DTP and Hib vaccines in separate limbs (81 received PRP–T and 83 received HbOC) of which 156 (95%) completed the study. Six were withdrawn because of local reactions (all PRP–T) and two because of systemic symptoms (one each for PRP–T, HbOC). One hundred and seventy-five infants received DTP and Hib vaccines in the same limb (75 received PRP–T and 100 received HbOC), of which 174 (99.4%) infants completed the study. One infant was withdrawn from the trial at the mother's request.

There was no significant difference in the antibody titres to polyribosylribitolphosphate (PRP), diphtheria, and pertussis FHA, PT and agglutinogens 2 and 3 between infants who received the Hib and DPT vaccines

Table 2 Local reactions after separate and mixed injections of DTP and Hib vaccines at 2, 3 and 4 months

	Injection sites PRP–T (%)	PRP–T /DTP (%)	*P* value[a]	DTP[b] (%)	*P* value[c]	HbOC/DTP (%)	HbOC (%)
Dose 1							
Redness ≥2.5 cm	10/81 (12)	5/75 (7)	0.08	14/95 (15)	0.2	10/100 (10)	0/83
Swelling ≥2.5 cm	6/81 (7)	6/75 (8)	0.03	11/95 (12)	0.2	7/99 (7)	0/83
Dose 2							
Redness ≥2.5 cm	7/76 (9)	4/75 (5)	0.11	11/93 (12)	0.5	11/99 (11)	0/81
Swelling ≥2.5 cm	3/76 (4)	3/76 (4)	0.04	12/93 (13)	0.4	11/99 (11)	0/81
Dose 3							
Redness ≥2.5 cm	1/73 (1)	4/75 (5)	0.14	25/93 (27)	0.008	12/99 (12)	2/82 (2)
Swelling ≥2.5 cm	1/73 (1)	6/74 (8)	0.0006	22/93 (24)	0.02	11/97 (11)	0/82

[a]*P* value for the difference between redness or swelling at the injection site of mixed Hib and DTP vaccines compared to the injection site of the DTP vaccine alone. [b]Data obtained from The North Hertfordshire infants. Fisher's exact test used. [c]*P* value <0.05 for the difference between redness or swelling at the injection site of mixed Hib and DTP vaccines compared to the injection site of PRP–T or HbOC given separately

Table 3 Febrile reactions after separate and mixed injections of DTP and Hib vaccines

Temperatures	PRP–T and DTP Separate[a]	Mixed	*P* value[a]	HbOC and DTP Separate	Mixed
Dose 1					
≥38°C	3/95 (3)	7/69 (10)	0.07	NA	12/95 (13)
≥39°C	0/95	0/69	1.0	NA	0/95
Group mean (°C)	37.57	37.54			37.54
Dose 2					
≥38°C	14/90 (16)	11/69 (10)	0.6	NA	20/92 (22)
≥39°C	0/90	0/69	1.0	NA	1/92 (1)
Group mean (°C)	37.65	37.62			37.67
Dose 3					
≥38°C	22/93 (24)	11/69 (16)	0.16	NA	21/86 (24)
≥39°C	0/93	0/69	1.0	NA	0/86
Group mean (°C)	37.78	37.65			37.71

[a]Data obtained from the North Hertfordshire infants. Fisher's exact test used. [b]*P* value for the difference between temperature of infants who received PRP–T and DTP separately or mixed in the same syringe

in the same limb or separately (*Table 1*) ($P>0.1$). In contrast antibody titres to tetanus were significantly greater in those who received Hib and DPT vaccines in the same limb compared to separately in different limbs ($P<0.05$). Post-vaccination antibody titres for diphtheria and tetanus were greater than 0.1 IU l^{-1} (lower limit of antibody titre considered to be protective) for all infants. Protective titres for pertussis antibodies are not known, although similar titres were obtained in infants who received their vaccines as separate or mixed injections. One infant who received PRP–T mixed with DTP and failed to develop an antibody titre of ≥ 0.15 IU ml^{-1} to PRP.

The local reactions (erythema and swelling) are shown in *Table 2*. These were no greater at the injection site of the mixed Hib and DTP vaccines than at the injection site of DTP vaccine alone. Local reactions were greater at the injection site of PRP–T given separately compared to the site where HbOC was given separately but for the first and second doses only ($P<0.05$). The local reactions at the site of both Hib vaccines lasted for less than 2 days in all infants and became less frequent with successive vaccinations. In contrast the local reactions at the site of the DTP vaccine lasted for more than 7 days in some infants and became more frequent with successive vaccinations.

The incidence of one or more systemic symptoms was similar in infants who received either Hib vaccine given separately or mixed in the same syringe ($P>0.05$). Temperatures over 38 or 39°C were no more frequent in infants who received DTP and PRP–T vaccines given in the same limb than in those receiving them in separate limbs (*Table 3*, $P>0.05$).

DISCUSSION

Our study is the first to compare the immunogenicity and reactogenicity of PRP–T and HbOC Hib vaccines either given separately from or mixed in the same syringe with Wellcome whole-cell DTP vaccine, in infants immunised at 2, 3 and 4 months of age. Previous studies have involved only older children[5,14]. Our results indicate that administration of Wellcome whole-cell DTP vaccine mixed with either Merieux PRP–T or Lederle HbOC vaccine as a single injection at 2, 3 and 4 months of age is both safe and effective. This conclusion is supported by the results of laboratory assays of reactogenicity and potency conducted by the National Institute of Biological Standards and Control using mixed Hib/DTP vaccine preparations[15].

The immunogenicity and reactogenicity of HbOC given mixed with a whole-cell DTP vaccine has been assessed in two studies in infants immunised at 2, 4 and 6 months of age[12,13]. These studies, using a different schedule and whole-cell vaccine to our study, found that the immunogenicity and reactogenicity were similar for infants given the vaccines separately or mixed in the same syringe[12,13].

Five studies have compared the immunogenicity and reactogenicity of PRP–T given either mixed with a whole-cell DTP vaccine or concurrently, but in separate limbs in infants immunised at 2, 4 and 6 months of age[5–11]. Similar or greater antibody responses to tetanus, diphtheria, pertussis and PRP were found in three other studies and ours (mixed versus separate injections), despite different whole-cell DTP vaccines being used[5,7,9–11]. Some reduction in antibody responses was seen in two studies using the Merieux and Connaught DTP vaccines, although others have reported similar responses using these vaccines[5,6,8,14]. Both systemic and local reactions were similar at the site of the combined DTP/PRP–T vaccine compared to the site of the DTP vaccine alone[5–8]. In those studies where reactions were greater in infants who received the vaccine mixed with whole-cell DTP vaccine, the difference was usually small[7,9,11].

Our results indicate that Wellcome whole-cell DTP vaccine can be administered in the same syringe with either PRP–T or HbOC vaccine as a single injection when a 2, 3 and 4 month schedule is used. This finding should be of use to those countries participating in the WHO expanded programme on immunisation, that uses an identical schedule to the UK with the exception of the Hib vaccines.

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Clinical and laboratory observations

Immunogenicity and safety of *Haemophilus influenzae* type b–tetanus protein conjugate vaccine alone or mixed with diphtheria-tetanus-pertussis vaccine in infants

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Haemophilus capsular polysaccharide–tetanus toxoid conjugate (PRP-T) and diphtheria-tetanus-pertussis (DTP) vaccines were administered in a single syringe (group 1) or separate syringes (group 2) to 284 infants at 2, 4, and 6 months of age. Group 1 infants had a slightly greater incidence of local reactions. Systemic reactions were similar. The geometric mean titers of polyribosylribitol phosphate (PRP) serum antibody concentrations after the third dose of PRP-T vaccine were 4.8 and 4.3 μg/ml for groups 1 and 2, respectively. Antibody responses to DTP antigens were also similar. The immunogenicity and safety of the PRP-T and DTP vaccines are equivalent when the vaccines are administered in separate syringes or the same syringe to infants. (J PEDIATR 1994;124:323-7)

The number of vaccines currently recommended or under development for administration to infants at routine visits is steadily increasing. Three vaccines (diphtheria-tetanus-pertussis, *Haemophilus influenzae* type b, and hepatitis B) may be administered at separate sites intramuscularly at 2, 4, and 6 months of age.[1, 2] The administration of two or more of these vaccines in the same syringe would be advantageous for the infants, families, and health care workers. Previous studies have examined the immunogenicity and safety of administering Hib polyribosylribitol phosphate

Hib	*Haemophilus influenzae* type b
PRP	Polyribosylribitol phosphate (Hib capsular polysaccharide)
PRP-T	PRP–tetanus toxoid protein conjugate
DTP	Diphtheria-tetanus-pertussis
PRP-D	PRP–diphtheria toxoid conjugate
FHA	Filamentous hemagglutinin
CHO	Chinese hamster ovary
CRM	Cross-reacting material

diphtheria toxoid conjugate alone or in the same syringe with DTP or acellular DTP vaccine as well as PRP–tetanus toxoid protein conjugate alone or mixed with DTP.[3-5] The purpose of this study was to determine the immunogenicity

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and reactogenicity of the PRP-T vaccine when administered simultaneously with DTP vaccine in the same syringe or separate syringes to infants in the United States.

METHODS

Study population infants. Healthy infants between 6 and 12 weeks of age were eligible for enrollment. Patients were recruited predominantly from private practices in each of four cities (Houston, Tex., Portland, Ore., Chicago, Ill., and Washington, D.C.). Exclusion criteria included (1) premature birth (≤36 weeks of gestation); (2) prior immunization with DTP or Hib vaccine; (3) developmental delay or neurologic disorder, febrile seizures, or family history of epilepsy or neurologic disorders; (4) uncorrected congenital cardiac anomaly, major organ system defect, immunodeficiency or receipt of immunosuppressive medications or blood or blood products, including immunoglobulins; (5) known or suspected allergy to components of the vaccine; (6) acute febrile (≥38° C) illness at time of vaccination; and (7) infant or parent known to be seropositive for human immunodeficiency virus.

Vaccines. The PRP-T vaccine (from Pasteur Merieux Serums et Vaccine, France) is composed of 10 μg of purified Hib capsular polysaccharide covalently coupled to 20 μg tetanus toxoid. A single lot (S2189) was used in the study. The PRP-T vaccine was lyophilized in single-dose vials.

The DTP vaccine was supplied by Connaught Laboratories, Inc., and contained 6.7 Lf units of diphtheria toxoid, 5.0 Lf units of tetanus toxoid, and approximately 4.0 units of pertussis vaccine, with ≤0.25 mg aluminum potassium sulfate and 0.01% thimerosal, per 0.5 ml dose. The same lot of DTP vaccine (OD21090) was used throughout the study.

Vaccination protocols. After consent was obtained, children were randomly selected separately at each site to receive either PRP-T combined with DTP vaccine in the same syringe intramuscularly in the right leg (group 1) or PRP-T vaccine in the right leg and DTP vaccine in the left leg (group 2) at 2, 4, and 6 months of age. The PRP-T vaccine was reconstituted with either 0.5 ml DTP vaccine (group 1) or 0.5 ml of 0.4% sodium chloride (group 2) just before administration. Oral trivalent polio vaccine was also administered at the 2- and 4-month visits. Blood specimens for antibody determinations were drawn before the first immunization at 6 to 12 weeks of age, before the 6-month immunization, and 1 month later. Sera were separated and stored at −20° C until assayed.

Monitoring for adverse reactions. Each child was observed for 15 minutes after each immunization. Parents recorded on standard forms the rectal temperature and systemic and local reactions at 6, 24, 48, and 72 hours after the injection(s). Parents also were contacted by study personnel at 3 and 14 days after each immunization, and the questions on the standard forms were reviewed at that time. Local reactions were recorded for each leg separately and included erythema, swelling, tenderness, and hardness. For this analysis, only objective measures such as temperature, vomiting, and seizures are reported for the systemic reactions. Acetaminophen or other antipyretics were not administered unless fever was present.

Antibody assays. Sera were shipped frozen to Connaught Laboratories, Swiftwater, Pa., where the following antibody assays were performed:

1. PRP antibody. The Farr type of radioimmunoassay was performed with intrinsically labeled tritiated PRP as the test antigen. The PRP antibody concentration was expressed as micrograms per milliliter. Reference sera (lot No. 1983) were provided by the U.S. Food and Drug Administration Center for Biologic Evaluation and Research (CBER). The lower limit of detectable antibody is 0.06 μg/ml.
2. Diphtheria antitoxin antibodies. A vero cell protection assay was performed, with results reported as units per milliliter by comparison with U.S. Standard Diphtheria Antitoxin (CBER).[6]
3. Tetanus antitoxin antibody. Results of an indirect enzyme-linked immunosorbent assay were reported as equivalents per milliliter by comparison with pooled sera assigned a value of 1.0 equivalent per milliliter.
4. Petussis antibodies.
   a. Antibody to lymphocytosis promoting factor (pertussis toxin) or filamentous hemagglutinin by indirect enzyme-linked immunosorbent assay. The results are expressed as enzyme immunoassay (EIA) units per milliliter by comparison with a convalescent antiserum of assigned unitage (U.S. Reference Pertussis Antiserum (Human) lot 3, provided by Dr. C. Manclark, CBER).
   b. Titers of antibody to lymphocytosis promoting factor were also determined by a neutralization assay in which Chinese hamster ovary cells were used. Results are reported as the reciprocal of the highest dilution of test sera that provided complete protection from the clustering effects of pertussis toxin.

Statistical analysis. Comparisons between groups were performed by analysis of variance and Student *t* test on log-transformed serologic antibody levels (geometric mean titers). The chi-square test or Fisher Exact Test was employed to analyze dichotomous variables. Sera with no detectable PRP antibody were assigned a value of 0.06 μg/ml for analysis. Similarly, nondetectable levels of anti-

Table I. Summary of local and systemic reactions 6 to 72 hours after dose 1

	At 6 hr		At 24 hr		At 48 hr		At 72 hr	
	Combined vaccine	Separate injections	Combined vaccine	Separate injections	Combined vaccine	Separate injections	Combined vaccine	Separate injections
Subjects (No.)	154	152	154	152	154	152	154	151
Any local reaction*	64	56	41	34	18	13	10	11
Erythema								
Diameter <1 inch	36	28	17	15	2	1	1	1
Diameter >1 inch	12‡	5	3	1	0	1	1	1
Swelling	29	28	18	11	5	3	3	2
Tenderness	42	34	19†	9	4	2	2	0
Hardness	36†	23	27	21	16	11	9	10
Fever ≥38° C	21	26	2	4	1	0	2	1
Vomiting	3	3	7‡	2	3	2	5‡	1

Reactions are reported as percentages.
*Reaction at DTP site of those receiving DTP and PRP-T vaccines in separate injections.
†Groups significantly different at $p < 0.01$.
‡Groups significantly different at $p < 0.05$.

body for the DTP antigens were assigned the lowest detectable values for analysis.

RESULTS

Of 306 children enrolled, 284 completed the study without any protocol violations and are the subjects of the antibody analysis. For all sites combined there were no differences with respect to age at enrollment, sex, or racial origin between the two study groups.

In general, local reactions were reported more commonly after the first dose than after the second or third dose at 6 hours. The significant differences for local reactions between the combined-vaccine and DTP-alone sites were for erythema (>2.5 cm diameter) 6 hours after the first and second doses, hardness 6 hours after all three doses and 24 hours after the second dose, and swelling 48 hours after the second dose. At each of these times the combined vaccine had the higher rate of local reaction (Table I).

The systemic reactions observed in the two groups were also very comparable. Except for more vomiting at 24 hours after the first and second doses ($p < 0.05$) and at 72 hours after the first dose ($p < 0.05$) for the combined-vaccine groups, there were no differences in systemic reactions (Table I). No seizures were reported for any of the children.

Antibody responses to the antigens contained in the DTP vaccine were very similar in the two study groups after both the second and third doses. The PRP antibody levels were higher in the separately injected group after the second dose but were equivalent in the two groups 1 month after the third dose (Table II). The percentage of children with PRP antibody concentrations >1.0 μg/ml was virtually identical in each group after three doses of vaccine (group 1, 86%; group 2, 85%).

DISCUSSION

Several studies have examined the safety and immunogenicity of PRP-conjugate vaccines when administered at separate sites or combined in the same syringe with DTP vaccine. Eskola et al.[7] noted no effect of PRP-D vaccine on antibody responses to diphtheria toxoid, tetanus toxoid, or polioviruses in 50 children studied. Kovel et al.[3] compared the immunogenicity of PRP-D vaccine and an acellular DTP vaccine administered separately or in the same syringe to 18-month-old children; no differences were noted for systemic or local reactions or for the proportion of children responding to the various antigens.

Two studies have focused on the safety and immunogenicity of PRP-T vaccine administered separately or in the same syringe with DTP vaccine. In the study conducted in Chile, the addition of PRP-T to DTP vaccine (prepared by Institut Merieux and containing ≥4 IU [15 opacity units] of killed *Bordetella pertussis*) increased the occurrence of febrile reactions after the first and second doses in comparison with DTP vaccine alone.[5] Local reactions were not affected. The PRP antibody titers were higher and a greater of proportion of children achieved levels >1.0 μg/ml when PRP-T vaccine was administered separately than when it was combined in the same syringe with DTP vaccine. These investigators also observed that the concurrent administration of PRP-T vaccine with DTP vaccine either in the same syringe or at a separate site was associated with significantly lower levels of antibody to pertussis agglutinin and toxin than when DTP was administered without PRP-T af-

Table II. Antibody responses to PRP and DTP antigens in children receiving DTP and PRP-T vaccines in separate or combined injections

	Before dose 1		At 2 mo after dose 2		At 1 mo after dose 3	
	Combined vaccine	Separate injections	Combined vaccine	Separate injections	Combined vaccine	Separate injections
Anti-PRP						
Subjects (No.)	135	129	135	128	135	128
GMT	0.12	0.14	0.41	0.68[a]	4.26	4.83
>1.0 μg/ml (%)	3.0	2.3	26.2	38.4[a]	85.1	86.2
>0.15 μg/ml (%)	31.1	40.3	72.3	76.1	97.9	96.4
Diphtheria antitoxin						
Subjects (No.)	134	129	132	128	134	128
GMT	0.101	0.105	0.05	0.058	0.406	0.372
Fourfold rise (%)			16.7	21.9	55.2	58.6
Tetanus antitoxin						
Subjects (No.)	135	129	134	127	134	128
GMT	0.037	0.034	0.033	0.038	0.148	0.158
Fourfold rise (%)			16.4	21.3	57.8	51.6
Antibody response to pertussis						
Anti-LPF (IgG ELISA)						
Subjects (No.)	135	129	134	127	134	128
GMT	4.34	4.25	2.09	2.09	2.95	2.93
Anti-LPF (CHO cell)						
Subjects (No.)	134	129	132	127	134	128
GMT	10.52	13.99[a]	5.23	5.58	6.34	6.00
Anti-FHA (IgG ELISA)						
Subjects (No.)	135	129	134	127	134	128
GMT	7.47	7.86	3.03	3.20	5.44	5.44

GMT, Geometric mean titer; *LPF*, lymphocytosis promoting factor; *ELISA*, enzyme-linked immunosorbent assay; *CHO*, Chinese hamster ovary; *FHA*, filamentous hemagglutinin.
[a]Groups significantly different at $p < 0.05$.

ter the primary three dose series.[8] The implications of this observation are not certain because there is no direct correlation between pertussis antibody values and protection against whooping cough.[9] A similar effect of coadministration of PRP-T on antibody response to DTP (prepared by Institut Merieux) was found in a study conducted in Israel.[4] However, in another study of DTP vaccine prepared by Connaught Laboratories, PRF-T vaccine when given separately or in the same syringe did not have any effects on antibody levels to DTP antigens compared with DTP vaccine given alone.[10] Furthermore, Rennels et al.[11] did not note any interference of an Hib conjugate vaccine in the serologic responses to whole-cell or acellular pertussis vaccines.

We have found that local reactions occur more frequently in children receiving the combined PRP-T and DTP vaccine than with DTP vaccine alone, predominantly at 6 hours after administration; otherwise there was little difference in local or systemic reactions. Furthermore, except for PRP antibody levels at 2 months after the second series of immunization, there were no differences in the antibody response to PRP, diphtheria toxin, tetanus toxin, and pertussis antigens between the two groups. Similar findings for effects on adverse reactions and immunogenicity have been reported for the oligosaccharide-CRM_{197} or the Hib–*Neisseria meningitidis* group B outer-membrane-protein conjugate vaccines when combined in the same syringe or administered at separate sites with DTP vaccine.[12, 13]

The antibody concentrations to pertussis antigens in our study were lower than those in the other PRP-T/DTP studies with the DTP vaccine prepared by Institut Merieux. Assays were similar for these antibody measurements but were performed in different laboratories. The DTP vaccine that we employed is not identical to the Institut Merieux vaccine administered in the first two PRP-T studies; this, in part, may explain the differences in these antibody levels. Differences in antibody response to various whole-cell pertussis vaccines have been reported.[14] The antibody levels to pertussis antigens after administration of the DTP vaccine manufactured by Connaught Laboratories were similar for our study and the second study in Chile.

We conclude that the administration of PRP-T and DTP

vaccines either at separate sites or in the same syringe at 2, 4, and 6 months of age appears to be equally safe and immunogenic. The U.S. Food and Drug Administration has approved a combined DTP and oligo-CRM_{197} Hib conjugate vaccine for children 2 months to 5 years of age. Our study provides data supporting a similar combination with PRP-T.

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