

## Safety and immunogenicity of two *Haemophilus influenzae* type b polysaccharide-tetanus toxoid conjugate vaccines (PRP-T) given with diphtheria-tetanus-pertussis vaccine to young Papua New Guinean children

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### SUMMARY

**Background.** In view of high mortality and morbidity from *Haemophilus influenzae* type b (Hib) in young Papua New Guinean children, the incorporation of a Hib conjugate vaccine into a nationwide immunization program would be of major public health benefit. **Methods.** We evaluated the safety and immunogenicity of a lyophilized and a liquid form of Hib polysaccharide-tetanus toxoid conjugate vaccines (PRP-T) given in the same syringe as diphtheria-tetanus-pertussis (DTP) vaccine to children in Goroka, Eastern Highlands Province. In Part 1 of the study 209 children were randomized to receive at ages 1, 2 and 3 months either DTP alone or a liquid formulation of DTP/PRP-T or lyophilized PRP-T dissolved in DTP suspension. A further 75 children were given the liquid DTP/PRP-T formulation at ages 2, 3 and 4 months (Part 2). 54 children aged 15-18 months were given a booster of the same preparation of PRP-T/DTP as they had received during Part 1. Blood for antibody assays was collected at enrolment, before (Part 1 only) and one month after the third dose, then just before and 3 weeks after the booster dose. **Results.** Follow-up to age of 12 months showed that PRP-T was safe with no evidence of impaired response to individual vaccine components when combined with DTP. Geometric mean titres (GMTs) of anti-PRP antibody before vaccination (n=64, mean age 41 days), after 2 doses (mean age 99 days) and after 3 doses (mean age 132 days) of the lyophilized formulation were 0.21, 1.48 and 5.04 µg/ml, respectively, with 58% and 89% having anti-PRP antibody titres ≥1.0 µg/ml after 2 and 3 doses, respectively. Anti-PRP antibody responses to the liquid Hib vaccine formulation were lower (GMT post-dose 3 = 0.48 µg/ml) than to the lyophilized formulation, but better responses were elicited from older children (Part 2; GMT post-dose 3 = 0.78 µg/ml, with 79% ≥0.15 µg/ml). Both PRP-T preparations elicited excellent booster responses suggesting that children are likely to be protected if exposed to Hib infection. **Conclusions.** Lyophilized PRP-T given together with DTP is safe and immunogenic when given to young infants. The liquid DTP/PRP-T formulation showed a lower immunogenicity than in earlier studies with this vaccine, which might have been due to exposure to low temperature during shipment or the younger age at immunization.

### Introduction

In many industrialized countries, the incidence of invasive *Haemophilus influenzae* type b (Hib) disease has fallen dramatically since protein-polysaccharide conjugate Hib

vaccines have been included in routine immunization programs (1,2). Unfortunately Hib remains an important cause of pneumonia and meningitis in developing countries. An estimated 900,000 children die annually from diseases due to *Haemophilus influenzae* (3). In

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The Gambia Hib-PRP-T conjugate vaccine (polyribosyl-ribitol phosphate polysaccharide conjugated to tetanus toxoid) mixed with diphtheria-tetanus-pertussis vaccine (DTP) and given at ages 2, 3 and 4 months was efficacious in preventing Hib pneumonia and Hib meningitis (4). Efficacy was 95% for prevention of all invasive Hib disease and 21% for radiologically defined pneumonia following 3 doses of vaccine; there was a 6% reduction in total mortality (4). Despite the demonstrated efficacy of this vaccine in young infants and the enormous burden of Hib disease in developing countries, very few low-income countries have included Hib vaccination into their routine immunization schedule; there are a number of reasons for this, including high cost of the vaccine and difficulties in delivering vaccine through routine health services.

In Papua New Guinea (PNG) pneumonia is the commonest cause of death and reason for hospitalization in children (5). The overall case fatality rate (CFR) in children admitted to Goroka Hospital with pneumonia is 8% but children with bacteraemic pneumonia have 4 times the CFR as non-bacteraemic children. Hib accounts for approximately two-thirds of bacteraemic *Haemophilus influenzae* pneumonia, one-third of all bacteraemic pneumonia and 39-45% of bacterial meningitis in children in the highlands of PNG (6-8), while nonserotypable strains of *Haemophilus influenzae* have frequently been isolated from lung tissue in children with severe pneumonia, usually in conjunction with serotypable strains of *Haemophilus influenzae* or *Streptococcus pneumoniae* (9,10). Based on studies carried out at Goroka Hospital, estimated incidence rates of invasive Hib disease are in the order of 500 and 2000/100,000/annum in children aged under 5 and under 1 year, respectively (D. Lehmann, unpublished data). Half of childhood Hib disease occurs in the first six months of life and more than 90% during the first year of life (10). Immunization at a very young age is therefore essential, ideally coinciding with the routine schedule for DTP vaccine which, in PNG, is at ages 1, 2 and 3 months (11). To avoid several injections, DTP and Hib vaccines should be given simultaneously as a single injection.

The immunogenicity of the different components of DTP and conjugate Hib

vaccines has been investigated when given simultaneously either in separate syringes at different sites or together as a single injection (12-20). When given as a single injection most formulations require mixing a lyophilized form of a Hib-PRP-protein conjugate with DTP suspension though one study has shown good immunogenicity of both a lyophilized and a liquid form of PRP-T/DTP (Pasteur Mérieux, Lyon, France) (19). Results have varied between studies, but there has been no strong evidence of reduced immunogenicity to any components of the vaccines when administered simultaneously. Nevertheless, before introducing DTP/Hib combined vaccines in new settings, it is wise to investigate immune responses to all vaccine components since genetic, environmental and epidemiological factors as well as age of routine immunization may affect immune responses.

Swiss Serum and Vaccine Institute (SSVI) manufactured a combined vaccine in liquid form containing Hib-PRP conjugated to tetanus toxoid (PRP-T) and DTP. This product was safe and immunogenic in children immunized at 2, 4 and 6 months of age in Thailand (20).

In view of the enormous burden of Hib disease in PNG, an efficacious Hib vaccine could have a major impact on mortality and hospitalization. The first conjugate Hib vaccine evaluated in PNG was PedvaxHib™ (PRP-OMP), polyribosyl-ribitol phosphate conjugated to outer membrane protein of *Neisseria meningitidis* (21). This vaccine, given as a separate injection to DTP at ages 2, 4 and 12 months, was safe and immunogenic. In order to assist the PNG Department of Health in selecting a conjugate Hib vaccine to be incorporated into the routine childhood immunization program, the present study was carried out to assess safety and immunogenicity of PRP-T which was either in a solution with DTP or in lyophilized form to be mixed with DTP before administration.

## Methods

### Study population and immunization schedules

The study took place in the Asaro Valley, Eastern Highlands Province, where people live

either in villages or in Goroka town between 1500 and 1900 metres above sea level. There were three parts to the study: 1) 209 children enrolled at age 1-2 months and randomized to receive one of two different types of PRP-T in the same syringe as DTP (DiTePerHib, SSVI, Berne, Switzerland or TETRAct-HIB™, Pasteur Mérieux, Lyon, France) or to receive DTP alone (DiTePer Anatoxal™, SSVI, Berne, Switzerland); 2) 100 children enrolled at age 2 months who received DiTePerHib; 3) 54 children from Part 1 given a booster at age 15-18 months of the same preparation of PRP-T/DTP as they had received during the primary immunization series.

All parts of the study were approved by the Medical Research Advisory Committee of PNG.

### Vaccines

Each 0.5 ml dose of DTP (DiTePer Anatoxal™, lot number 131 990 204) contained 25 Lf of diphtheria toxoid, 10 Lf of tetanus toxoid and  $\geq 4$  IU killed *Bordetella pertussis*. The vaccine was adsorbed to aluminium phosphate 0.4% and contained 0.01% thiomersal.

Each 0.5 ml dose of DiTePerHib (in Parts 1 and 2 lot number 793007, Part 3 lot number 140995B) contained 25 Lf of diphtheria toxoid, 5 Lf of tetanus toxoid,  $\geq 4$  IU of killed *Bordetella pertussis*, 10  $\mu$ g PRP covalently coupled to approximately 10  $\mu$ g (equal to  $\sim 5$  Lf) of tetanus toxoid. The vaccine was in liquid form adsorbed to aluminium phosphate 0.4% and contained 0.01% thiomersal.

Each 0.5 ml dose of TETRAct-HIB™ (Pasteur Mérieux, in Part 1 lot number K0550, Part 3 lot number M0349) consisted of 10  $\mu$ g lyophilized PRP covalently linked to 20  $\mu$ g of tetanus toxoid in a suspension containing  $>30$  IU diphtheria toxoid,  $>60$  IU tetanus toxoid,  $>4$  IU killed *Bordetella pertussis* adsorbed to aluminium hydroxide ( $<1.25$  mg aluminium) and containing 0.05 mg thiomersal.

### Part 1

Children aged 1-2 months attending either the Family Health Clinic (FHC) of Goroka Hospital or the clinic run by the PNG Institute

of Medical Research (PNGIMR) for routine immunization or for treatment of minor illnesses (eg sores or upper respiratory tract infections) were recruited between September 1994 and August 1995. Children were eligible for inclusion in the study if they were permanent residents of Goroka town or of the rural areas within an hour's drive from Goroka in the Asaro Valley and had no exclusion criteria (history of fever in the preceding 72 hours, history of hypersensitivity or convulsions, previous DTP vaccine, other vaccines given in preceding 2 weeks, serious congenital abnormality, temperature  $\geq 38^{\circ}\text{C}$  or severe malnutrition). The purpose of the study was explained verbally to mothers and an information sheet given to take home for discussion with other relatives. If a child was subsequently brought back to the clinic and found to be well on physical examination and the guardian gave informed consent, a child was enrolled and randomized to receive DiTePer Anatoxal™, DiTePerHib or TETRAct-HIB™. The randomization schedule was held by a principal investigator (DL) and known only to the nurse vaccinating the children until the code was broken when all sera had been analyzed. The DiTePerHib and DiTePer Anatoxal™ vaccines were formulated as slightly turbid suspensions and 0.5ml doses provided in ready-to-use syringes. The Pasteur Mérieux PRP-T (called ActHIB™) vaccine was lyophilized and dissolved in the 0.5 ml DTP suspension just before injection. Vaccines were given intramuscularly into the right deltoid. Second and third doses of vaccines were given after intervals of at least four weeks. 5 ml of blood was collected before the first immunization, just before giving the third dose (ie at least 4 weeks after the second dose) and again approximately 4 weeks after the third dose of vaccines.

To assess children for adverse events in a blinded manner, a nurse who had not given the vaccine examined children for adverse events. In addition, the preparation of vaccine for injection was done out of view of the child's guardian. Children were examined for adverse events 2 hours after each vaccination and visited again at home within 24 hours.

At each visit a physical examination was done, weight and length were measured and

other routine immunizations (BCG, hepatitis B, oral polio) given according to the standard immunization schedule used in PNG (11).

If a child did not return within one week of an assigned date of follow-up, a home visit was done to bring the child to the clinic. Mothers were also urged to bring their children to the PNGIMR clinic if they were sick. Children were followed up for illness until the age of 12 months and a record kept of all hospitalizations and deaths.

If children enrolled in the study were inadvertently given DTP at another baby clinic, they were subsequently excluded.

## Part 2

Children receiving DiTePerHib in Part 1 described above had a lower antibody response to PRP than expected from the earlier study in Thai children (20). One possible explanation was the younger age of immunization in PNG. To investigate this further, between October 1995 and June 1996, 100 more children were enrolled to receive DiTePerHib at approximately 2, 3 and 4 months of age. Insufficient vaccine was available to complete 3 doses of DiTePerHib. Hence 12 children were given TETRAct-HIB™ as a third dose and excluded from the analysis of this part of the study.

## Part 3

In order to determine whether there was any difference in booster responses to primary immunization series with either DiTePerHib or TETRAct-HIB™, children who had participated in Part 1 of the study and were aged 15-18 months between August and November 1996 were offered a booster of the same vaccine (though different lot numbers) as they had received in the primary immunization series (Part 1), ie either DiTePerHib or TETRAct-HIB™. 5 ml of blood was collected just before the booster dose and again 3 weeks later (ie earlier than in parts 1 and 2 of this study). Local and systemic reactions were recorded as described above.

## Laboratory methods

All sera were separated and stored at -20°C at PNGIMR until sent at -70°C to the SSVI

laboratories. Laboratory investigations at SSVI were carried out without knowledge of the identity or vaccination status of individuals.

Antibodies against diphtheria toxin, tetanus toxin and pertussis toxin were measured by enzyme-linked immunosorbent assay (ELISA). Briefly, 100 µl of a 1.0 µg/ml tetanus toxoid or diphtheria toxin solution in 5 mM phosphate buffered saline (PBS), pH 7.2 or a 1.0 µg/ml of pertussis toxin solution in 0.1 M carbonate buffer, pH 9.6 was added to each well of a 96-well polystyrene plate (M129 A, Dynatec AG). The antigens were allowed to bind overnight at ambient temperature. The coating solution was decanted and 200 µl of a 2 mg/ml casein solution in PBS was added to each well and the wells were blocked at ambient temperature for 1 hour at 37°C. After the wells had been washed 3 times with PBS containing 0.05% Tween-20 (PBS-T), to each well was added 100 µl of serially diluted reference standards, control serum or test samples in PBS-TC diluent (1 mg/ml casein in PBS-T) and the antibody allowed to react for 3 hours at ambient temperature. The wells were washed 3 times, incubated for 2 hours at ambient temperature with 100 µl of a 1:2500 dilution of peroxidase-labelled goat anti-human IgG (γ-chain specific) (Kirkegaard & Perry, Gaithersburg, Maryland) in PBS-TC diluent and then washed 3 times with PBS-T. The wells were incubated with 100 µl of ABTS (2,2-azino-di [3-ethylbenzylthiazoline] sulphonate) substrate solution and the colour allowed to develop for 30 minutes at ambient temperature. The absorbance at 405 nm was determined using a 96-well microtitre plate reader. The specific IgG antibody concentrations of the control and test samples were extrapolated from the reference standard which was added to every plate. Antibodies to tetanus and diphtheria toxin are expressed in international units (IU)/ml, while anti-pertussis toxin IgG antibody is reported in µg/ml.

Anti-PRP antibody concentration was determined using a modification of the Farr assay (22). Briefly, 50 µl of a cocktail solution containing 5 ng of intrinsically labelled <sup>3</sup>H-PRP and ~10 nCi of <sup>36</sup>Cl per ml of saline was added to wells of a multiscreen plate (MAHVN4550, Millipore). 25 µl of various dilutions of reference standard, control serum

and unknown serum in fetal calf serum were added into triplicate wells and the antibody and antigen allowed to react overnight at 2-8°C. 75 µl of a cold saturated ammonium sulphate solution was added to the wells and precipitation was allowed to proceed for 2 hours at 2-8°C. The solution was drained from the plate using a vacuum manifold and the precipitate washed with cold ammonium sulphate solution. The filter was allowed to dry at ambient temperature, punched into a vial containing 250 µl of Solvable tissue and gel solubilizer (Canberra Packard) and the radioactivity counted in the presence of 3 ml of liquid scintillation cocktail. The anti-PRP antibody concentrations of the control and test samples were extrapolated from the reference standard and reported in µg/ml of total anti-PRP antibody.

Anti-*B. pertussis* agglutinin antibody was determined using *B. pertussis* strain 460 as the test agglutinin. In a U-bottomed 96-well microtitre plate, 50 µl of two-fold serially diluted control serum or test serum in saline was mixed with 50 µl of a ~20 US opacity units/ml *B. pertussis* suspension. Agglutination was allowed to take place at 37°C overnight. The plate was read using a black background with the light source from the bottom. The anti-*B. pertussis* agglutinin antibody titre is defined as the reciprocal of the highest serum dilution giving a thin sheet of cells with a slight button.

### Data analysis

There is considerable debate as to what antibodies confer protection against disease due to *B. pertussis* (23). However, it would appear that anti-pertussis toxin confers protection against disease and long-lasting immunity (23), while antibodies to pertussis agglutinins show a rough correlation with vaccine-derived immunity (24). Anti-diphtheria and anti-tetanus toxin levels  $\geq 0.1$  IU/ml were considered protective.

Chi-squared tests with continuity correction were used to compare groups of interest and the Kruskal-Wallis one-way analysis of variance to compare geometric mean antibody titres (GMT) between those who received DiTePerHib and those who received TETRAct-HIB™.

## Results

### Part 1

A total of 71, 70 and 68 children were enrolled and received DiTePer Anatoxal™, DiTePerHib and TETRAct-HIB™, respectively. The mean age on entry to the study was 40.4, 41.3, 40.6 days for DTP, DiTePerHib and TETRAct-HIB™ groups, respectively; in these respective groups 28 (39%), 37 (53%) and 39 (57%) were boys. Evaluation of vaccine safety following a third dose of vaccine was completed on 69 (97%), 64 (91%) and 67 (99%) children who received DTP, DiTePerHib or TETRAct-HIB™, respectively (Table 1) and in these respective groups sufficient serum to complete immunogenicity studies was collected at each appointed time from 62 (87%), 61 (87%) and 64 (94%) children.

### Safety

Two children died. One infant who had had 2 doses of DiTePer Anatoxal™ died at age 3 months from pneumonia (*Streptococcus pneumoniae* type 4). Another infant completed 3 doses of TETRAct-HIB™ but died at age 8 months with a diagnosis of gastroenteritis and septicaemia, although blood culture was negative. Neither death was vaccine-related. No other serious adverse events occurred during the study.

Table 1 shows the adverse events experienced and collected by a nurse 24 hours after immunization. In all groups, mild adverse reactions were common and local reactions tended to be more common after the first dose than after subsequent doses. There was no significant difference in the number of reported adverse events between vaccine groups.

### Immunogenicity

After two doses of any of the vaccines, more than 97% of children had anti-diphtheria and anti-tetanus toxin antibodies  $\geq 0.1$  IU/ml and all children had anti-diphtheria and anti-tetanus toxin antibodies  $\geq 0.1$  IU/ml after 3 doses. 92%-97% had  $\geq 4$ -fold rises in anti-diphtheria and anti-tetanus toxin antibodies when compared with pre-immunization levels.

**TABLE 1**

LOCAL AND SYSTEMIC REACTIONS 24 HOURS AFTER FIRST, SECOND OR THIRD DOSE OF DiTePer ANATOXAL™, DiTePerHib AND TETRAC-T-HIB™ GIVEN AT AGES 1, 2 AND 3 MONTHS

	First dose		Second dose		Third dose	
	DiTePer Anatoxal™	TETRAC-T-HIB™	DiTePer Anatoxal™	DiTePerHib	DiTePer Anatoxal™	DiTePerHib
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Number seen	71	68	70	66	69	64
<b>Local</b>						
<i>At least one</i>	33 (46)	33 (49)	30 (43)	31 (47)	32 (46)	30 (47)
Tenderness	22 (31)	14 (21)	20 (29)	15 (23)	15 (22)	17 (27)
Swelling	17 (24)	20 (29)	19 (27)	22 (33)	14 (20)	16 (25)
Redness	26 (37)	27 (40)	24 (34)	17 (26)	23 (33)	20 (31)
<b>Systemic</b>						
<i>At least one</i>	47 (66)	35 (51)	38 (54)	46 (70)	36 (52)	36 (56)
Irritability	40 (56)	30 (44)	36 (51)	43 (65)	31 (45)	34 (53)
Drowsiness	22 (31)	15 (22)	15 (21)	22 (33)	18 (26)	15 (23)
Diarrhoea	3 (4)	4 (6)	3 (4)	2 (3)	5 (7)	4 (6)
Vomiting	6 (8)	3 (4)	4 (6)	4 (6)	4 (6)	6 (9)

**TABLE 2**

GEOMETRIC MEAN ANTIBODY TITRES (GMT) TO DIPHTHERIA, TETANUS AND PERTUSSIS TOXINS, PERTUSSIS AGGLUTININS AND HIB-PRP BEFORE IMMUNIZATION (PRE), ONE MONTH AFTER SECOND DOSE (POST 2) AND ONE MONTH AFTER THIRD DOSE (POST 3) OF DiTePer Anatoxal™, DiTePerHib AND TETRACt-HIB™ AND MEAN FOLD INCREASES (FI) IN ANTIBODY TITRES ONE MONTH AFTER THIRD DOSE COMPARED TO PRE-IMMUNIZATION LEVELS

	DiTePer Anatoxal™			DiTePerHib (Part 1)			TETRAct-Hib™			DiTePerHib (Part 2)		
	Pre	Post 2	Post 3	FI	Pre	Post 2	Post 3	FI	Pre	Post 2	Post 3	FI
Number tested	62	62	62	-	61	61	61	-	64	64	64	-
Mean age (days)	40	99	132	-	41	103	131	-	41	99	132	-
Anti-diphtheria toxin IU/ml	0.06	1.6	2.0	33.1	0.08	1.8	3.5	45.6	0.07	1.2	2.2	32.1
Anti-tetanus toxin IU/ml	1.8	0.9	3.5	1.9	1.9	1.3	5.0	2.7	1.5	1.3	3.4	2.2
Anti-pertussis toxin µg/ml	0.8	0.5	0.7	0.9	0.7	0.8	1.6	2.3	0.6	0.8	2.4	3.8
Anti-pertussis agglutinin titre	10.6	121.0	309.5	29.3	11.3	139.3	312.8	27.6	12.1	64.9	287.2	23.8
Anti-PRP antibody µg/ml	0.2	0.1	0.1	0.4	0.2	0.3	0.5	2.5	0.2	1.5	5.0	24.4
% ≥0.15 µg/ml	55%	24%	13%	-	44%	62%	66%	-	45%	88%	97%	-
% ≥1.0 µg/ml	13%	2%	0%	-	7%	13%	31%	-	13%	58%	89%	-

A  $\geq 4$ -fold titre rise over the pre-immune level of anti-pertussis toxin was reached by 41% of the infants receiving DiTePerHib and by 53% receiving TETRAAct-HIB™ compared with 16% in those receiving DiTePer Anatoxal™. The GMT of anti-pertussis toxin was also significantly lower in those who had received DTP alone (one month after third dose 0.71  $\mu\text{g/ml}$ , 95% confidence limits 0.54, 0.92) than in those who received either DiTePerHib (post-dose 3 1.64  $\mu\text{g/ml}$ , 95% confidence limits 1.13, 2.38) or TETRAAct-HIB™ (post-dose 3 2.35  $\mu\text{g/ml}$ , 95% confidence limits 1.61, 3.43). More than 92% in all groups had a  $\geq 4$ -fold titre rise of anti-pertussis agglutinin antibodies when comparing titres before immunization with titres one month after the third dose. GMTs of anti-pertussis agglutinin antibodies were comparable for the tetravalent formulations and the trivalent DiTePer Anatoxal™.

GMTs of anti-PRP antibody before immunization, one month after the second dose and one month after the third dose are shown in Table 2 and Figure 1. After a single dose of Hib vaccine, anti-PRP antibody titres were higher in those who had conjugate Hib vaccine than in those who had received only DTP.

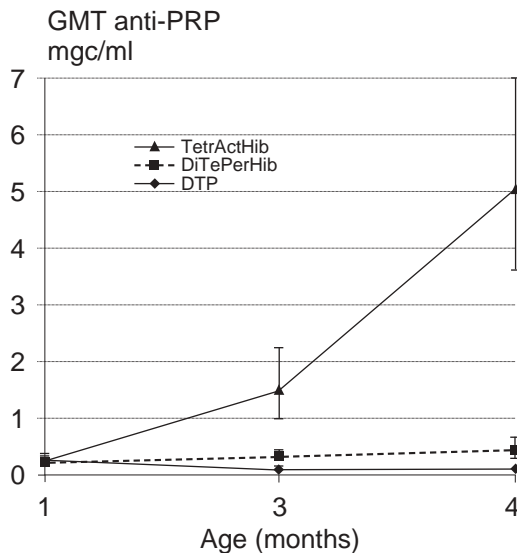


Figure 1. Geometric mean antibody titres (GMT) and 95% confidence limits of anti-PRP antibody at approximately 1, 3 and 4 months of age before dose 1, one month after dose 2 and one month after dose 3 in children who received DTP alone or DiTePerHib or TETRAAct-HIB™ vaccines.

Post-immunization anti-PRP antibodies were significantly higher in children who received TETRAAct-HIB™ than in those who received DiTePerHib ( $p < 0.0001$ ). 88% of children given TETRAAct-HIB™ had anti-PRP antibody titres  $\geq 0.15 \mu\text{g/ml}$  after two doses and 97% one month after the third dose. Equivalent figures for anti-PRP antibody titres  $\geq 1.0 \mu\text{g/ml}$  were 58% and 89%, respectively. Among children who received DiTePerHib, 66% had anti-PRP antibody titres  $\geq 0.15 \mu\text{g/ml}$  after 3 doses and 31% had anti-PRP antibody titres  $\geq 1.0 \mu\text{g/ml}$  after 3 doses.

## Part 2

Of the 100 children enrolled to receive DiTePerHib at 2, 3 and 4 months of age, 12 were given TETRAAct-HIB™ as a third dose, 10 were lost to follow-up, 1 caucasian child was excluded and 2 children were inadvertently given DTP at another baby clinic, leaving 75 children with complete immunogenicity data for analysis. The mean age when this group had the first dose of DiTePerHib was 63 days (range 50-90 days). There was no difference in the number of adverse events in these older children compared to children immunized at a younger age. Responses to all components of the vaccine were higher than in children who had received the same vaccine at a younger age in Part 1 of this study and, for vaccine components other than Hib-PRP, responses were higher than those attained in children immunized with TETRAAct-HIB™ at a younger age (Table 2). The GMT of anti-PRP antibody one month after the third dose was 0.78  $\mu\text{g/ml}$  (95% confidence limits 0.51, 1.18), an 11-fold rise from pre-immunization levels. 63% of this older group of children given DiTePerHib attained a  $\geq 4$ -fold rise in anti-PRP antibody titres; 79% and 37% had anti-PRP antibody titres  $\geq 0.15$  and  $\geq 1.0 \mu\text{g/ml}$ , respectively, one month after a third dose (Table 2).

## Part 3

One way of assessing the effectiveness of conjugate vaccines is to examine the antibody response to a booster dose. 2 of the 54 children enrolled in this part of the study had incomplete serum sample collection. 26 children in each of the Hib vaccine groups were given a booster at 15-18 months of age

(mean age approximately 17 months in both groups). The GMT of anti-PRP rose from 0.34 µg/ml before a booster of DiTePerHib to 7.91 µg/ml 3 weeks after the booster dose, a 23-fold rise. Equivalent figures for the 26 who received TETRAct-HIB™ were 0.72 µg/ml pre-booster and 38.5 µg/ml 3 weeks post-booster, a 54-fold rise. After the booster dose 96% and 100% of children in the DiTePerHib and TETRAct-HIB™ groups, respectively, had anti-PRP antibody titres  $\geq 0.15$  µg/ml; equivalent figures for antibody titres  $\geq 1.0$  µg/ml were 89% and 96%, for DiTePerHib and TETRAct-HIB™ groups, respectively. These results indicate a good booster response to both vaccines.

### Discussion

In this study we found that PRP-T combined with DTP was well-tolerated and resulted in no serious side-effects up to age 12 months. There was no evidence of impaired response to individual vaccine components when PRP-T was combined with DTP. Anti-pertussis toxin antibody titres were higher among children who received either of the combined DTP/PRP-T vaccines than in those who received DTP alone, suggesting an adjuvant effect of PRP-T.

The antibody responses to TETRAct-HIB™ were consistent with those found in Gambian children immunized with the same vaccine at ages 2, 3 and 4 months. In our study children who received TETRAct-HIB™ had better anti-PRP antibody responses than those who received DiTePerHib. The poorer response in children given DiTePerHib was not consistent with earlier immunogenicity studies of the same vaccine in Thai and Indonesian children (20,25). In Thailand, at age 7 months, one month after a third dose of DiTePerHib, the GMT of anti-PRP was 5.6 µg/ml and 89% of children had anti-PRP antibody titres  $\geq 1.0$  µg/ml; equivalent figures for Indonesian children were 6.1 µg/ml and 88%, respectively. Possible explanations for the lower immunogenicity of DiTePerHib in the present study include 1) inappropriate storage temperature of the vaccine or 2) the young age at which Papua New Guinean babies were immunized or 3) differences in the epidemiology of Hib disease (eg high

incidence of disease and upper respiratory tract carriage from a very young age in PNG).

The DiTePerHib vaccine may have been exposed to too low a temperature during shipment from Switzerland to PNG. No record of temperature is available for the first large shipment of vaccines. A low temperature might not have affected the TETRAct-HIB™ vaccine which was packaged and was in lyophilized form. By contrast the DiTePerHib vaccines were in syringes and not packaged and may have been exposed to lower temperatures near ice packs. At PNGIMR, vaccines were at all times in a cold room where temperatures remained at 2-8°C throughout the study period. Just before a dose of vaccine was to be given it was carried from the cold room to the Institute's clinic one minute's walk away in an insulated box where it was kept cool but not frozen.

Sometimes, after prolonged storage of DiTePerHib (and DiTePer Anatoxal™), flocculation can be seen in the suspension but breaks down on gentle shaking. Some doses of DiTePerHib which were hand-carried back to SSVI in Switzerland at recommended temperature were flocculated but could not be properly suspended on gentle shaking. On the other hand, the vaccine potency of these samples assessed in the mouse model was high, though these results may be of limited value since mice produce a lot of low-affinity antibodies. Since completion of this study, the stability of DiTePerHib has been improved by lowering the pH of the liquid formulation from 7.2 to 6.0.

Papua New Guinean children were immunized at a younger age than in earlier studies (mean age of entry in Part 1 was 41 days and in Part 2 was 63 days compared to 99 days in Indonesia and approximately 2 months in Thailand) (20,25). Furthermore, in the PNG study, the time interval between doses in the primary schedule was approximately one month compared to 2 months in the studies in Indonesia and Thailand. Antibody titres after a third dose of vaccine were measured at approximately 7 months of age in Thailand and Indonesia compared to approximately 4 and 5 months in Parts 1 and 2 of the PNG study, respectively. One would expect higher

antibody titres in older children and possibly with longer interval between doses. In the older group of children in Part 2 of the PNG study, there was a better response to PRP-T than in those in Part 1, but the response was still poorer than in the Indonesian and Thai studies. Data from Thailand and Indonesia following only 1 or 2 doses of vaccine are not available and hence we are unable to compare antibody titres in children aged less than 6 months in the three countries.

It should be noted that antibody concentration is not the sole criterion on which to base immunogenicity; ability to prime for immunological memory as well as quality of antibody (eg avidity or opsonizing, bactericidal or neutralizing capacity) are also important and these vary between vaccines (26). It is therefore interesting to note that despite poor anti-PRP antibody responses to a primary schedule of DiTePerHib in this study, there was a very good response to a booster dose of DiTePerHib (23-fold rise in anti-PRP titres resulting in high anti-PRP antibody titres), suggesting that these children are likely to be protected if exposed to Hib infection.

Prior to the present study, the immunogenicity of Hib polysaccharide-*Neisseria meningitidis* outer membrane protein complex conjugate vaccine (PRP-OMPC) given at 2, 4 and 12 months of age was evaluated in PNG (21). Following 2 doses of PRP-OMPC, the GMT of anti-PRP antibody at age 5 months was 2.54 µg/ml and 73% of children had anti-PRP titres ≥1.0 µg/ml. This compares with a GMT of 5.0 µg/ml and 89% with anti-PRP ≥1.0 µg/ml at age 4 months following 3 doses of TETRAct-HIB™. Even at age 3 months (ie one month after 2 doses of TETRAct-HIB™), the GMT of anti-PRP was the same (1.5 µg/ml) as that found in serum collected in 4-month-old children two months after the first dose of PRP-OMPC. PRP-OMPC has been recommended for prevention of Hib disease in populations such as Indigenous North American and Australian babies who also suffer from Hib disease at a young age. However, this study shows that early immunization with PRP-T, coinciding with the standard recommended schedule for DTP in PNG, is as immunogenic as a single dose of PRP-OMPC given at the recommended age of 2 months.

Hib disease remains a major cause of morbidity and mortality in PNG. Of growing concern is the emergence of invasive disease caused by chloramphenicol-resistant strains of Hib in PNG (Trevor Duke and Audrey Michael, personal communication). Economic constraints limit the use of cephalosporins as alternative treatment to chloramphenicol. Possible ways of reducing the cost of immunization against Hib disease are being explored globally. Lower doses of PRP-T in the conjugate vaccine appear to be immunogenic (27). To reduce childhood mortality and morbidity as well as national expenditure on inpatient care in PNG, ways of improving vaccine delivery throughout the country need to be addressed urgently and conjugate Hib vaccines should be included in the routine immunization program as soon as possible with mechanisms in place to monitor adverse events and overall mortality in children.

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