

Safety and immunogenicity of *Haemophilus influenzae* type b conjugate vaccine (PedvaxHIB™) in 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. The choice of the Hib conjugate vaccine will be based on the evaluation of several Hib conjugate vaccines, after consideration of such factors as the ease of incorporation into the current vaccination schedule, cost, kinetics of antibody responses and safety. **Methods.** This study evaluated the safety and immunogenicity of Hib polysaccharide-*Neisseria meningitidis* outer membrane protein complex conjugate vaccine (PRP-OMPC) in Papua New Guinea. 95 children were recruited at Goroka Base Hospital, Eastern Highlands Province, and enrolled in the study. PRP-OMPC was administered at ages 2, 4 and 12 to 15 months. Blood was collected before each dose, one month after the second and booster doses, and at ages 18 and 24 months. Antibody to PRP (anti-PRP) was measured by radioimmunoassay. **Results.** PRP-OMPC was generally well tolerated. At successive sampling times from the prevaccination bleed through the 1-month post-booster bleed, geometric mean titres were 0.18, 1.45, 2.54, 1.03 and 8.05 µg/ml, respectively (n=60). The proportions of subjects with anti-PRP titres ≥1.0 µg/ml were 2%, 62%, 73%, 47% and 93%, respectively (n=60). Persistence of anti-PRP was ascertained in 41 subjects. The GMTs at 18 and 24 months were 3.42 and 2.0 µg/ml, respectively. **Conclusions.** PRP-OMPC was found to be immunogenic after the first dose and to elicit a robust booster response. Antibody titres persisted until age 24 months, at which time 100% of subjects had anti-PRP ≥0.15 µg/ml. These results are consistent with previous studies in US Native American infants and in Gambian infants.

Introduction

Approximately 4 million children worldwide die annually from pneumonia, the majority at a young age in developing countries (1). Bacterial pneumonia is common in children and is predominantly due to *Streptococcus pneumoniae* and *Haemophilus influenzae* (2). These two pathogens are also among the major aetiological agents of childhood bacterial

meningitis, which is associated with a high case fatality rate and neurological sequelae.

In Papua New Guinea (PNG), pneumonia is the most common cause of death and hospitalization among children aged less than 5 years (3). *S. pneumoniae* and *H. influenzae* (in approximately equal numbers) account for more than 80% of paediatric bacterial pneumonia; two-thirds of bacteraemic

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H. influenzae pneumonia are due to serotype b (Hib) (4,5). Hib has been isolated from 39% of purulent cerebrospinal fluid (CSF) samples collected from children at Goroka Base Hospital, Eastern Highlands Province (6). One-quarter of all invasive Hib disease in children aged less than 5 years who are admitted to Goroka Base Hospital occurs during the first 4 months of life, 50% before the age of 6 months, and more than 90% before the age of one year (7). Estimated incidence rates of Hib meningitis and all invasive Hib disease are approximately 60 per 100,000/annum and 500 per 100,000/annum, respectively, in children under the age of 5 years in Goroka District. The incidence of invasive Hib disease under the age of 1 year is estimated to be 2000 per 100,000/annum (8). These incidence rates are similar to those found in Aboriginal Australian children (9).

Several Hib conjugate vaccines have been shown to be immunogenic and efficacious in both developed and developing countries (10-13). Their routine use in many developed countries has resulted in a dramatic reduction in the incidence of invasive Hib disease and in upper respiratory tract carriage of Hib (14-18). Since the incidence of invasive Hib disease is high in PNG and multidrug-resistant strains of Hib have recently emerged, an efficacious Hib conjugate vaccine could greatly reduce the burden of invasive Hib disease in Papua New Guinean children (19). In view of environmental and genetic variation in antibody responses to Hib conjugate vaccines between populations, it is important to ensure the immunogenicity of a Hib conjugate vaccine in the target population before its introduction into a nationwide program. We assessed the immunogenicity and safety of the Hib conjugate vaccine PRP-OMPC, which is the first of several Hib conjugate vaccines to be evaluated in PNG as part of the development of a nationwide Hib vaccination program.

Methods

Site of study

The site is located in the Asaro Valley, Eastern Highlands Province, including Goroka town and nearby villages, situated between 1500 and 1900 metres above sea level.

Inhabitants of Goroka town are either wage earners or related to wage earners while people in rural areas are generally subsistence farmers, but earn cash through smallholder coffee production, employment on plantations and marketing of garden produce.

Study population

Ethical approval to carry out the study was granted by the Medical Research Advisory Committee of PNG. From June 1992 to February 1994, healthy children aged 2 months receiving routine immunizations or treatment for minor ailments at the Family Health Clinic of Goroka Base Hospital were eligible for inclusion in the study if they resided permanently within one hour's drive of Goroka town. Children with severe congenital abnormalities, severe malnutrition or known history of Hib disease were not eligible for inclusion. Mothers were given a written explanation about the study to discuss with relatives at home. Eligible children of mothers who gave informed consent were enrolled in the study.

Vaccine

Vaccination with three different lots (Lot 1284T, Lot 0116W and Lot 1365W) of PRP-OMPC (PedvaxHIB™ [Haemophilus b Conjugate Vaccine (Meningococcal Protein Conjugate)], Merck & Co., Inc., Whitehouse Station, NJ, USA) enabled comparison of the safety and immunogenicity of different lots of vaccine. The vaccine and aluminium hydroxide diluent were stored at 2-8°C. Upon reconstitution with 0.7 ml of diluent just before vaccination, each 0.5 ml of vaccine contained 15 µg of Hib PRP (polyribosyl-ribitol phosphate, the capsular polysaccharide of Hib), 250 µg of *Neisseria meningitidis* OMPC (outer membrane protein complex, B11 strain), 225 µg of aluminium (as aluminium hydroxide), thimerosal (a mercury derivative) at 1:20,000 as a preservative, and 2.0 mg lactose in 0.9% sodium chloride.

Vaccination schedule and follow-up

A blood sample (5 ml) was collected by venepuncture before administering PRP-OMPC at ages 2, 4 and 12 to 15 months. PRP-

OMPC was injected intramuscularly in the left thigh. Other vaccines were administered at separate injection sites. Blood samples were also collected for assay 4 weeks after the second dose of PRP-OMPC, 4 weeks post-booster, and at approximately 18 and 24 months of age.

Hepatitis B, oral polio and measles vaccines were generally administered on the same day as PRP-OMPC, according to standard vaccination schedules used in PNG (20). Diphtheria-tetanus-pertussis vaccine (DTP) was administered on the day following the administration of PRP-OMPC so that the side-effects observed for PRP-OMPC would not be confounded by those associated with DTP (eg, fever and irritability). Children were examined for side-effects 2 and 24 hours after each vaccine dose.

We attempted to follow children up to the age of 2 years. If parents did not bring a child for vaccination or for a follow-up blood sample on the assigned day, a home visit was made on at least two occasions to urge parents to attend or to find out where the participants were currently living.

Laboratory methods

Blood samples were centrifuged and sera separated in Goroka. Sera were assayed for levels of antibody to PRP (anti-PRP) at Merck Research Laboratories (West Point, PA, USA). Anti-PRP was measured by a radioimmunoassay that uses radiolabeled PRP in a Farr-type procedure to detect total anti-PRP with a lower limit of detection of 0.125 µg/ml (21).

Data analysis

Data analysis was performed using Epi Info 6 (22). Geometric mean titres (GMTs) of anti-PRP, with 95% confidence limits, were calculated at each time point that serum samples were collected.

Results

A total of 95 subjects were enrolled (47 males, 48 females) and received a first dose of

PRP-OMPC. 91 subjects (96%) received a second dose of PRP-OMPC and 80 (84%) received the complete regimen of PRP-OMPC (2-dose primary series plus a booster dose). 3 of the 95 enrolled subjects were excluded from immunogenicity analysis because insufficient serum had been collected before the first dose of PRP-OMPC. Follow-up to age 24 months was difficult despite frequent home visits: 75% (69/92) and 60% (55/92) of subjects had blood collected at approximately 18 and 24 months of age, respectively. Only one parent refused to have further blood samples collected at age 12 months; others either had moved out of the area or chose not to come for further blood sampling. Anti-PRP titres were measured on 97% of all serum samples collected; the total number assayed at each bleeding time is shown in Figure 1. Antibody assays on complete series of serum samples up to and including ages 5, 13 to 16, 18 and 24 months were available on 74 (80%), 60 (65%), 54 (59%) and 41 (45%) of the subjects, respectively.

The mean ages of vaccination were as follows: first dose 8.5 weeks (range 4 to 13 weeks), second dose 16.3 weeks (range 13 to 26 weeks) and booster dose 52.3 weeks (range 48 to 61 weeks). 166 doses from Lot 0116W, 125 doses from Lot 1284T and 35 doses from Lot 1365W of PRP-OMPC were administered. The frequencies of local and systemic adverse reactions and anti-PRP responses were similar irrespective of whether immunization was with Lot 0116W, Lot 1284T or Lot 1365W. Accordingly, safety and immunogenicity data from all three lots were pooled for the remainder of the analyses.

Two deaths were reported among the study participants. One child who received 2 doses of PRP-OMPC died at age 9 months of severe pneumonia of unknown aetiology which was complicated by pleural effusion and heart failure. Another child who completed the 3-dose series of PRP-OMPC died at age 13 months from gastroenteritis with severe dehydration. Neither event was attributed to PRP-OMPC. Table 1 shows that the rates of systemic and local reactions following vaccination with PRP-OMPC were low.

The GMTs for all serum samples assayed at

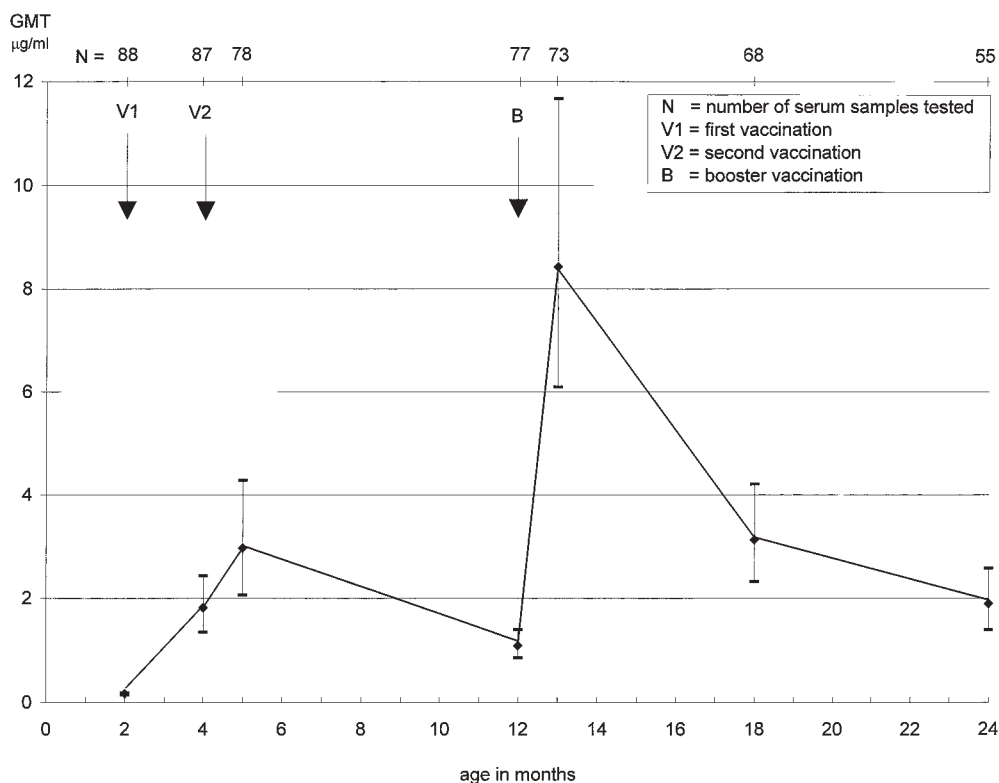


Figure 1. Geometric mean titres of anti-PRP (and 95% confidence limits) by age before and after first, second and booster doses of PRP-OMPC. Figure includes all available anti-PRP at each bleeding time.

TABLE 1

SYSTEMIC AND LOCAL REACTIONS 24 HOURS AFTER FIRST, SECOND AND BOOSTER DOSES OF PRP-OMPC

Reaction	Postvaccination 1 (N = 95)		Postvaccination 2 (N = 91)		Booster dose (N = 80)	
	No	%	No	%	No	%
<i>Systemic</i>						
Fever by history	2	2.1	4	4.4	4	5.0
Temp $\geq 37.5^{\circ}\text{C}^*$	0	0	1	1.3	1	1.5
Irritability	1	1.1	6	6.6	1	1.3
Prolonged crying	4	4.2	5	5.5	0	0
Drowsiness	0	0	1	1.1	0	0
Diarrhoea	2	2.1	4	4.4	0	0
<i>Local</i>						
Redness	3	3.2	2	2.2	2	2.5
Tenderness	0	0	2	2.2	5	6.3

* Temperatures were measured in 84, 77 and 65 of the children who had first, second and booster doses, respectively

the various bleeding times before and after PRP-OMPC vaccination through the age of 2 years are shown in Figure 1. It has been usually regarded that immediate protection is provided by an anti-PRP antibody level of at least 0.15 µg/ml and long-term protection by 1.0 µg/ml. Käyhty et al. (23) in Finland found that an anti-PRP level exceeding 0.15 µg/ml derived from natural infection correlated with protection, whereas, after vaccination, the level of antibody that predicted protection was close to 1.0 µg/ml. Both these levels are therefore relevant, even if their exact significance is not known. Table 2 presents the GMTs and the proportion of subjects with anti-PRP titres ≥ 0.15 µg/ml and ≥ 1.0 µg/ml in the 60 children who received all 3 doses of PRP-OMPC and had a complete series of serum samples assayed through the one-month post-booster sample. No significant differences in GMTs were found between an analysis of all available anti-PRP results at each bleeding time (Figure 1) and an analysis that was restricted to subjects with a complete series of anti-PRP results through the post-booster bleed at 13 to 16 months (Table 2). This was also the case for the analysis at the 18-month and 24-month time points. Our study did not demonstrate an effect of age at first immunization on the anti-PRP responses to the first, second and booster doses (data not shown).

PRP-OMPC elicited a post-dose 1 and a post-dose 2 anti-PRP response (Table 2): 88% of children had anti-PRP titres ≥ 0.15 µg/ml and 62% had anti-PRP titres ≥ 1.0 µg/ml post dose 1 and 90% of children had anti-PRP titres ≥ 0.15 µg/ml and 73% had anti-PRP titres ≥ 1.0 µg/ml post dose 2. Anti-PRP titres declined between 5 and 12 to 15 months of age, but subjects showed a marked booster response to PRP-OMPC given at 12 to 15 months of age. At age 24 months, the GMT was still 1.9 µg/ml (Figure 1) and 100% and 69% of infants had anti-PRP titres ≥ 0.15 µg/ml and ≥ 1.0 µg/ml, respectively.

Table 3 presents the fold differences in GMTs between successive assay time points. There was an 8-fold rise in anti-PRP titres following the first dose and a small additional increase following the second dose. Anti-PRP titres decreased by more than 50% between 5 and 12 to 15 months of age, but rose 8-fold following the booster dose.

Discussion

We have demonstrated that a primary series of PRP-OMPC administered at 2 and 4 months of age is highly immunogenic and generally well tolerated in Papua New Guinean infants. A booster dose administered at 12 to 15 months of age elicited a robust booster response, after which antibody titres remained relatively high through the age of 24 months. To our knowledge, this was the first study in a developing country in which a booster of PRP-OMPC was given at age 12 to 15 months and in which antibody titres were measured to 24 months of age. This is an important and neglected aspect of the immune response to vaccination, the purpose of which, after all, is to protect children from subsequent infection. The ability to make a rapid and effective immune response to natural boosting by infection is the most critical outcome of immunization, and yet the effect of boosting is rarely studied explicitly.

The anti-PRP titres found in this study are comparable to those found in US Native American populations and Gambian children (Tables 4, 5), although antibody responses in PNG more closely resemble responses observed in US Native American children than responses in Gambian children (24-26). The less robust response in Gambian children may be due to genetic or environmental factors, including malaria, which is not endemic in our study area. The GMT of anti-PRP in Papua New Guinean children appears to be higher than in Navajo or Alaskan children before a booster at age 12 months (Table 4). This might be due to more frequent environmental boosting in PNG where very poor hygienic conditions result in higher transmission rates of both Hib and other cross-reacting bacteria (eg, pneumococcus type 6) than in US Navajo and US Alaskan Native populations (18,27). GMTs of anti-PRP were similar at age 24 months in Papua New Guinean children (Table 4) to those in Navajo children who had the same vaccination schedule; Calandra et al. reported a GMT of 1.41 µg/ml (95% confidence limits = 0.71, 2.78 µg/ml) at 22 to 28 months of age, approximately one year after a booster dose (28).

An antibody response to the first dose of

TABLE 2

GEOMETRIC MEAN TITRES (GMTs) OF ANTI-PRP AND PERCENTAGES OF SUBJECTS WITH ANTI-PRP TITRES ≥ 0.15 $\mu\text{g}/\text{ml}$ OR ≥ 1.0 $\mu\text{g}/\text{ml}$ IN 60 CHILDREN WHO HAD ALL THREE DOSES OF PRP-OMPC (AT APPROXIMATELY 2, 4 AND 12 MONTHS OF AGE) AND ALL SERUM SAMPLES TESTED AT APPROXIMATELY 2, 4, 5, 12-15 AND 13-16 MONTHS OF AGE

Age (months)	Time	GMT $\mu\text{g}/\text{ml}$	GMT (95% confidence limits)	≥ 0.15 $\mu\text{g}/\text{ml}$ (95% confidence limits)	≥ 1.0 $\mu\text{g}/\text{ml}$ (95% confidence limits)
2	Prevaccination	0.18	(0.15, 0.20)	31.7 (19.9, 43.5)	1.7 (0, 5.0)
4	Two months postvaccination 1	1.45	(1.03, 2.10)	88.3 (80.5, 96.4)	61.7 (49.4, 74.0)
5	One month postvaccination 2	2.54	(1.67, 3.84)	90.0 (82.2, 97.8)	73.3 (62.1, 84.5)
12-15	Pre-booster	1.03	(0.78, 1.37)	96.7 (94.4, 100)	46.7 (34.1, 59.2)
13-16	One month post-booster	8.05	(5.66, 11.45)	100	93.3 (87.0, 99.6)

TABLE 3

FOLD DIFFERENCES IN GMTs OF ANTI-PRP BEFORE AND AFTER FIRST, SECOND AND BOOSTER DOSES OF PRP-OMPC

Interval	Number of paired samples	Fold difference in GMT	(95% confidence limits)
Prevaccination 1 to 2 months postvaccination 1	60	8.3	(5.8, 12.0)
Postvaccination 1 to 1 month postvaccination 2	60	1.7	(1.1, 2.6)
Postvaccination 2 to pre-booster	60	0.4	(0.3, 0.6)
Pre-booster to 1 month post-booster	60	7.8	(5.4, 12.2)
Post-booster to 18-month bleed	54	0.3	(0.3, 0.4)
18-month bleed to 24-month bleed	41	0.6	(0.4, 0.9)

TABLE 4

COMPARISON OF ANTI-PRP RESPONSES TO PRP-OMP (GMTs, µG/ML) IN US NAVAJO, PAPUA NEW GUINEAN, GAMBIAN AND US ALASKAN NATIVE CHILDREN

Age (months)	Time	Geometric mean titres (95% confidence limits)			
		Navajo ^a (N = 28)	PNG (N = 41) [*]	The Gambia ^b (N = 56)	Alaska ^c (N = 44)
2	Prevaccination	0.23 (0.16, 0.35)	0.18 (0.15, 0.19)	0.16 (0.14, 0.18)	0.16
4	Before 2nd dose	1.63 (0.96, 2.78)	1.55 (1.06, 2.26)	0.82 (0.57, 1.18)	1.37
5	One month post 2nd dose	2.51 (1.41, 4.45)	2.88 (1.73, 4.80)	1.59 (1.01, 2.49)	2.71
12-15	Pre-booster	0.53 (0.31, 0.93)	1.06 (0.74, 1.51)	-	0.53 [†]
13-16	Post-booster	8.38 (4.86, 14.46)	10.57 (7.04, 15.86)	-	
18		-	3.42 (2.35, 4.97)	-	
24		-	2.00 (1.42, 2.79)	-	

^a Reference 24

^b Reference 26

^c Reference 25

^{*} 41 children who had complete series of serum samples tested

[†] Serum samples collected 9-12 months of age

TABLE 5

COMPARISON OF PERCENTAGE OF SUBJECTS WITH ANTI-PRP LEVELS ≥1 µG/ML IN US NAVAJO, PAPUA NEW GUINEAN, GAMBIAN AND US ALASKAN NATIVE CHILDREN BEFORE AND AFTER PRP-OMP

Age (months)	Time	CHILDREN BEFORE AND AFTER PRP-OMP		
		Navajo ^a (N = 28)	PNG (N = 60) [†]	The Gambia ^{b,*} (N = 56)
2	Prevaccination	11	2	4
4	Before 2nd dose	50	62	52
5	One month post 2nd dose	78	73	60
12-15	Pre-booster	29	47	-
13-16	Post-booster	89	93	-

^a Reference 24

^b Reference 26

^c Reference 25

^{*} > 1µg/ml

[†] 60 children who had complete series of serum samples tested up to age 13-16 months

[‡] Serum samples collected at 9-12 months of age

vaccine is desirable in populations such as US Native American and Australian Aboriginal and Torres Strait Islander populations where invasive Hib disease occurs at very young ages (24,25,29). PRP-OMPC was recommended for use in Australian Aboriginal and Torres Strait Islander populations because this vaccine demonstrated a marked immunogenic response following the first dose (at 2 months of age) in US Native American infants. Papua New Guinean infants also showed a good antibody response following the first dose of PRP-OMPC (62% had anti-PRP ≥ 1.0 $\mu\text{g/ml}$, GMT = 1.45 $\mu\text{g/ml}$). Studies of other Hib conjugate vaccines have shown that Hib polysaccharide conjugated to tetanus toxoid (PRP-T) is immunogenic at a young age after several doses (30,31). In The Gambia, after a second dose of PRP-T, the GMT of anti-PRP was similar at age 4 months to the GMT of anti-PRP after a single dose of PRP-OMPC in PNG (30). In both the Philippines and The Gambia, at age 5 months (one month after a third dose of PRP-T) GMTs were higher and more children had an anti-PRP titre greater than 1.0 $\mu\text{g/ml}$ than after 2 doses of PRP-OMPC in PNG (30,31).

A robust antibody response to a Hib conjugate vaccine at one month of age is attractive for PNG since Papua New Guinean children are at high risk of invasive Hib disease in early infancy and are routinely given other childhood vaccines at one month of age. Although the first vaccination with PRP-OMPC was administered at 2 months of age in our study, data from The Gambia suggest no difference in response to PRP-OMPC if the primary series of two doses is started at one month rather than two months of age (26).

A booster of PRP-OMPC is recommended at 12-15 months (28,29). This may be logistically difficult in developing countries since other routine vaccinations are generally not scheduled at this age. As suggested by Mulholland et al. a booster dose of PRP-OMPC vaccine could be given concurrently with measles vaccine which is frequently offered at 9 months of age (32). But a booster dose of PRP-OMPC might be of limited value in PNG since more than 90% of invasive Hib disease occurs in the first year of life, and practically all invasive Hib disease occurs

before the age of 18 months. The excellent booster dose response at age 12 to 15 months in our study also suggests that children would probably respond to natural infection with a vigorous rise in anti-PRP titre as a result of immunologic priming during the primary vaccination series. However, other data argue in favour of a booster dose administration. In the Protective Efficacy Trial for PRP-OMPC conducted in US Navajo children, the one vaccine failure occurred in a 15.5-month-old child who developed osteomyelitis due to Hib after receiving two doses of PRP-OMPC in the first year of life and who had an antibody titre of 0.14 $\mu\text{g/ml}$ at one year of age (11). A booster dose given to toddlers might also assist in eradicating Hib carriage in the upper respiratory tract, thus inducing herd immunity and protecting younger unvaccinated siblings.

Evaluation of another Hib conjugate vaccine is currently underway in PNG. The PNG Department of Health will select a Hib conjugate vaccine for inclusion in the routine vaccination schedules based on such factors as the kinetics of antibody responses, frequency of side-effects, ease of incorporation into the current vaccination program, the feasibility of simultaneous administration of a Hib conjugate with DTP, and cost.

In Australia there has been a dramatic reduction in the incidence of invasive Hib disease following the introduction of Hib conjugate vaccines. The overall Hib conjugate vaccine efficacy in adequately vaccinated children is in the order of 90%. Some vaccine failures have occurred in the general Australian population and at a proportionally higher rate in Aboriginal and Torres Strait Islander children (16). Whichever Hib conjugate vaccine is selected for use in PNG, ongoing surveillance, particularly for vaccine failures, will be extremely important in monitoring the effectiveness of a national vaccination program.

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REFERENCES

- 1 **Garenne M, Ronsmans C, Campbell H.** The magnitude of mortality from acute respiratory infections in children under 5 years in developing countries. *World Health Stat Q* 1992;45:180-191.
- 2 **Berman S.** Epidemiology of acute respiratory infections in children of developing countries. *Rev Infect Dis* 1991;13(Suppl 6):S454-S462.
- 3 **Papua New Guinea Department of Health.** National Health Plan 1996 - 2000. Port Moresby: Department of Health, 1996.
- 4 **Lehmann D.** Epidemiology of acute respiratory infections, especially those due to *Haemophilus influenzae*, in Papua New Guinean children. *J Infect Dis* 1992;165(Suppl 1):S20-S25.
- 5 **Barker J, Gratten M, Riley ID, Lehmann D, Montgomery J, Kajoi M, Gratten M, Smith D, Marshall TF deC, Alpers MP.** Pneumonia in children in the Eastern Highlands of Papua New Guinea: a bacteriologic study in patients selected by standard clinical criteria. *J Infect Dis* 1989;159:348-352.
- 6 **Gratten M, Barker J, Shann F, Gerega G, Montgomery J, Kajoi M, Lupiwa T.** The aetiology of purulent meningitis in highland children: a bacteriological study. *PNG Med J* 1985;28:233-240.
- 7 **Funkhouser A, Steinhoff MC, Ward J.** *Haemophilus influenzae* disease and immunization in developing countries. *Rev Infect Dis* 1991;13(Suppl 6):S542-S554.
- 8 **Lehmann D, Yeka W, Rongap T, Javati A, Saleu G, Clegg A, Michael A, Lupiwa T, Omena M, Alpers MP.** Aetiology and clinical signs of bacterial meningitis in children admitted to Goroka Base Hospital, Papua New Guinea, 1989-1992. *Ann Trop Paediatr* 1999;19:in press.
- 9 **Hanna JN.** The epidemiology of invasive *Haemophilus influenzae* infections in children under five years of age in the Northern Territory: a three-year study. *Med J Aust* 1990;152:234-240.
- 10 **Eskola J, Käyhty H, Takala AK, Peltola H, Rönöberg PR, Kela E, Pekkanen E, McVerry PH, Mäkelä PH.** A randomized, prospective field trial of a conjugate vaccine in the protection of infants and young children against invasive *Haemophilus influenzae* type b disease. *N Engl J Med* 1990;323:1381-1387.
- 11 **Santosham M, Wolff M, Reid R, Hohenboken M, Bateman M, Goepf J, Cortese M, Sack D, Hill J, Newcomer W.** The efficacy in Navajo infants of conjugate vaccine consisting of *Haemophilus influenzae* type b polysaccharide and *Neisseria meningitidis* outer-membrane protein complex. *N Engl J Med* 1991;324:1767-1772.
- 12 **Black SB, Shinefield HR, Fireman B, Hiatt R, Polen M, Vittinghoff E.** Efficacy in infancy of oligosaccharide conjugate *Haemophilus influenzae* type b (HbOC) vaccine in a United States population of 61,080 children. *Pediatr Infect Dis J* 1991;10:97-104.
- 13 **Mulholland K, Hilton S, Adegbola R, Usen S, Oparaugo A, Omosigho C, Weber M, Palmer A, Schneider G, Jobe K, Lahai G, Jaffar S, Secka O, Lin K, Ethévenaux C, Greenwood B.** Randomised trial of *Haemophilus influenzae* type-b tetanus protein conjugate vaccine for prevention of pneumonia and meningitis in Gambian infants. *Lancet* 1997;349:1191-1197.
- 14 **Harrison LH, Tajkowski C, Croll J, Reid R, Hu D, Breneman G, Weatherholtz RC, Santosham M.** Postlicensure effectiveness of the *Haemophilus influenzae* type b polysaccharide-*Neisseria meningitidis* outer-membrane protein complex conjugate vaccine among Navajo children. *J Pediatr* 1994;125:571-576.
- 15 **Lagos R, Horwitz I, Toro J, San Martin O, Abrego P, Bustamante C, Wasserman SS, Levine OS, Levine MM.** Large scale, postlicensure, selective vaccination of Chilean infants with PRP-T conjugate vaccine: practicality and effectiveness in preventing invasive *Haemophilus influenzae* type b infections. *Pediatr Infect Dis J* 1996;15:216-222.
- 16 **Herceg A.** The decline of *Haemophilus influenzae* type b disease in Australia. *Commun Dis Intell* 1997;21:173-176.
- 17 **Takala AK, Eskola J, Leinonen M, Käyhty H, Nissinen A, Pekkanen E, Mäkelä PH.** Reduction of oropharyngeal carriage of *Haemophilus influenzae* type b (Hib) in children immunized with an Hib conjugate vaccine. *J Infect Dis* 1991;164:982-986.
- 18 **Takala AK, Santosham M, Almeida-Hill J, Wolff M, Newcomer W, Reid R, Käyhty H, Esko E, Mäkelä PH.** Vaccination with *Haemophilus influenzae* type b meningococcal protein conjugate vaccine reduces oropharyngeal carriage of *Haemophilus influenzae* type b among American Indian children. *Pediatr Infect Dis J* 1993;12:593-599.
- 19 **Michael A, Combs B, Gratten M, Montgomery J, Lupiwa T, Mgone J, Lehmann D.** Emergence of multiresistant *Haemophilus influenzae* type b in Papua New Guinea. Abstract in Program and Abstracts of the International Conference on Acute Respiratory Infections, Canberra, 7-10 Jul 1997:81.
- 20 **Papua New Guinea Department of Health.** Standard Treatment of Common Illnesses of Children in Papua New Guinea. A Manual for Nurses, Health Extension Officers and Doctors, 6th edition. Port Moresby: Department of Health, 1993.
- 21 **Ward JI, Greenberg DP, Anderson PW, Burkart KS, Christenson PD, Gordon LK, Käyhty H, Kuo JSC, Vella P.** Variable quantitation of *Haemophilus influenzae* type b

- anticapsular antibody by radioantigen binding assay. *J Clin Microbiol* 1988;26:72-78.
- 22 **Dean AG, Dean JA, Coulombier D, Brendel KA.** Epi Info, version 6: a Word Processing, Database, and Statistics Program for Epidemiology on Microcomputers. Atlanta, GA: Centers for Disease Control and Prevention, 1994.
- 23 **Käyhty H, Peltola H, Karanko V, Mäkelä PH.** The protective level of serum antibodies to the capsular polysaccharide of *Haemophilus influenzae* type b. *J Infect Dis* 1983;147:1100.
- 24 **Santosham M, Hill J, Wolff M, Reid R, Lukacs L, Ahonkhai V.** Safety and immunogenicity of a *Haemophilus influenzae* type b conjugate vaccine in a high risk American Indian population. *Pediatr Infect Dis J* 1991;10:113-117.
- 25 **Bulkow LR, Wainwright RB, Letson GW, Chang SJ, Ward JI.** Comparative immunogenicity of four *Haemophilus influenzae* type b conjugate vaccines in Alaskan Native infants. *Pediatr Infect Dis J* 1993;12:484-492.
- 26 **Campbell H, Byass P, Ahonkhai VI, Vella PP, Greenwood BM.** Serologic responses to an *Haemophilus influenzae* type b polysaccharide-*Neisseria meningitidis* outer membrane protein conjugate vaccine in very young Gambian infants. *Pediatrics* 1990;86:102-107.
- 27 **Montgomery JM, Lehmann D, Smith T, Michael A, Joseph B, Lupiwa T, Coakley C, Spooner V, Best B, Riley ID, Alpers MP.** Bacterial colonization of the upper respiratory tract and its association with acute lower respiratory tract infections in highland children of Papua New Guinea. *Rev Infect Dis* 1990;12(Suppl 8):S1006-S1016.
- 28 **Calandra GB, Lukacs LJ, Jonas LC, Santosham M, Ward JI, Greenberg DP, Daum RS, Matthews H, Vella PP, Ryan JL.** Anti-PRP antibody levels after a primary series of PRP-OMPC and persistence of antibody titres following primary and booster doses. *Vaccine* 1993;11(Suppl 1):S58-S62.
- 29 **National Health and Medical Research Council.** The Australian Immunisation Handbook, 6th edition. Canberra: Australian Government Publishing Service, 1997:102-109.
- 30 **Mulholland EK, Hoestermann A, Ward JI, Maine N, Ethévenaux C, Greenwood BM.** The use of *Haemophilus influenzae* type b-tetanus toxoid conjugate vaccine mixed with diphtheria-tetanus-pertussis vaccine in Gambian infants. *Vaccine* 1996;14:905-909.
- 31 **Capeding MRZ, Nohynek H, Pascual LG, Käyhty H, Sombrero LT, Eskola J, Ruutu P.** The immunogenicity of three *Haemophilus influenzae* type b conjugate vaccines after a primary vaccination series in Philippine infants. *Am J Trop Med Hyg* 1996;55:516-520.
- 32 **Mulholland EK, Todd J, Rowe M, Campbell H, Byass P, Vella PP, Ahonkhai VI, Greenwood BM.** Persistence of antibody at 18 months following vaccination of young Gambian infants with PRP-OMPC *Haemophilus influenzae* type b conjugate vaccine. *Ann Trop Paediatr* 1993;13:153-158.