THE NEW DTPw-HBV-Hib COMBINATION VACCINE CAN BE USED AT THE WHO SCHEDULE WITH A MONOVALENT DOSE OF HEPATITIS B VACCINE AT BIRTH

Lulu Bravo[1], Josefina Carlos[2], Salvacion Gatchalian[1], Charissa Borja-Tabora[1], Gyneth Bibera[2], Paul Willems[3], Assad Safary[3], Hans L Bock[3]

[1]University of the Philippines, Philippines General Hospital, Department of Pediatrics, Taft Avenue, Manila; [2]University of the East, Ramon Magsaysay Memorial Medical Center, Aurora Boulevard, Quezon City, Philippines; [3]SmithKline Beecham Biologicals, Rue de l'Institut, 89, B-1330 Rixensart, Belgium

Abstract. An open, randomized, clinical trial was conducted in order to assess the reactogenicity and immunogenicity of DTPw-HBV and Haemophilus influenzae type b (Hib) vaccines when given either as a mixed administration or as separate concomitant injections using the WHO schedule at 6, 10 and 14 weeks of age, following a dose of HBV at birth. There were no clinically relevant differences in the immune response to any component between the mixed and separate administrations. In fact the anti-tetanus GMTs were significantly higher (p=0.002) in mixed administration (3.9 IU/ml) compared with the separate administration (1.9 IU/ml). However although all subjects achieved anti-PRP titers ≥ 0.15 μg/ml, higher anti-PRP GMTs were seen in the group receiving the separate administration. Importantly, the addition of Hib did not adversely alter the reactogenicity profile of DTPw-HBV. This report which demonstrates that this novel combination can be used in WHO recommended schedule.

INTRODUCTION

Since 1974 the Expanded Program of Immunization (EPI) has brought about a dramatic reduction in the disease burden caused by diphtheria, pertussis, tetanus, poliomyelitis, measles and tuberculosis (Lee *et al,* 1997). Further to this success other diseases have been incorporated into the EPI, for example of newborns vaccination against hepatitis B was added in 1993 (WHO, 1993) and this year , the incorporation of Haemophilus influenzae b (Hib)Hib vaccination into routine infant immunization programs has been recommended, as appropriate to national capacities and priorities (WHO, 1998).

Consequently the number of mandatory childhood vaccines is likely to increase still further in the future. It is now widely accepted that combination vaccines offer the most practical and cost-effective means of achieving immunization goals (Hadler, 1994). Particularly in the developing world where compliance can be low, not only do combination vaccines give high coverage to established vaccines such as diphtheria-tetanus-pertussis (DTP), but they have the potential to offer the same degree of coverage to any additional components. For these reasons, the WHO has recommended the development of DTP-HBV combinations in order to facilitate the incorporation of hepatitis B vaccination into the EPI (WHO, 1992). Consequently, there are now a number of commercially available DTPw-HBV combinations (Aristegui *et al,* 1997; Usonis *et al,* 1996; Papaevangelou *et al,* 1995; Poovorawan *et al,* 1994). Therefore the addition of Hib to DTPw-HBV would allow the incorporation of Hib into childhood immunization programs.

In the developing countries, there is a higher incidence of Hib disease than in industrialized countries, and also the disease occurs in younger children with most cases occurring before 12 months of age and half of these before 6 months (Funkhouser *et al,* 1991; Bijlmer, 1994). As a result there is a need for infant vaccination. Furthermore infant immunization has already reduced the rates of invasive disease in the developed world (Campbell and Carter, 1993) and recent efficacy trials suggest this could also be achieved in the developing world (Mulholland *et al,* 1997; Lagos *et al,* 1996).

With respect to combination vaccines, an earlier report has shown that Hib can indeed be given in a single injection with DTPw-HBV at 1.5, 3 and 5 months of age, following a dose of hepatitis B vaccine at birth (Win *et al,* 1997). However in the

Correspondence: Dr Salvacion Gatchalian, University of the Philippines, Philippines General Hospital, Department of Pediatrics, Taft Avenue, Manila, Philippines. Tel: + 632-526 91 67; Fax: + 632-524 08 92

developing world many childhood vaccines are administered at 6, 10 and 14 weeks of age according to the WHO immunization schedule. As well as insuring that an adequate immune response can be achieved at each proposed schedule, combination vaccines must also be evaluated to insure there is no loss of immune response of any of the components or increase in reactogenicity. Therefore to take all these factors into account, we have evaluated the safety and immunogenicity of the pentavalent combination, DTPw-HBV-Hib, produced by the extemporaneous mixing of DTPw-HBV and Hib vaccrines using the WHO recommended schedule.

MATERIALS AND METHODS

Study design and participants

The study was an open randomized clinical trial and was conducted at the University of the East, Ramon Magsaysay Memorial Medical Center, Quezon City and the University of the Philippines, Manila. The study received local ethical approval and was conducted according to the Declaration of Helsinki and the Good Clinical Practice Guidelines in operation at the time. Prior to enrollment, the benefits and risks were explained to the children's parents or guardians in a language they clearly understood and then their written informed consent was obtained.

A total of 148 healthy male and female infants with an Apgar score ≥ 7 at birth were enrolled in the trial. All received hepatitis B vaccine (Engerix B) with in 72 hours of birth. At 6 to 8 weeks subjects were eligible to continue if they did not violate any of the exclusion criteria which were; participation in another trial; immunosuppressive and immunoglobulin therapy; any congenital defects, chronic illness, acute condition or systemic dysfunction (especially relating to the CNS); history of allergic reaction or immune disorder (including evidence of being born to an HIV seropositive mother). In addition during the trial, any adverse experience previously associated with whole cell *B. pertussis* vaccination led to exclusion from the trial (CDC, 1996).

Vaccines

Subjects were randomized in the order in which they enrolled between two groups. Both groups received hepatitis B at birth, then at 6, 10 and 14 weeks group 1 received DTPw-HBV extemporaneously mixed with Hib in the same syringe, and group 2 received the same vaccines as separate, concomitant injections. A single, 0.5ml dose of DTPw-HBV-Hib vaccine was prepared by resuspending the lyophilized Hib vaccine (10 μg of polyribose-ribitol phosphate (PRP) conjugated to ~ 30 μg tetanus toxoid) in the contents of one vial of DTP-HBV (7.5 Lf diphtheria toxoid, 3.25 Lf tetanus toxoid, 15 OU whole cell pertussis, 10 μg hepatitis B surface antigen (HBsAg), 0.63 mg of aluminum, (as aluminum salts), 25 μg thiomersal (as preservative), 150 mM sodium chloride and 25 μg of phenoxyethanol. For separate administration the Hib was reconstituted in 0.5 ml saline solution supplied by the manufacturer. Vaccines were administered in three doses at 6, 10 and 14 weeks of age. Injections were given in the anterolateral region of the thigh. All vaccines were manufactured by SmithKline Beecham Biologicals (Rixensart, Belgium).

Reactions

Diary cards were used by parents or guardians to record solicited local reactions (pain, redness and swelling) and general adverse experiences (fever, unusual crying, vomiting, diarrhea, loss of appetite, restlessness) on the day of vaccination and for three subsequent days. Fever (≥ 37.5°C) was assessed by measuring axillary temperature. Any solicited symptom that occurred between 4 and 30 days inclusive, post-vaccination was recorded as an unsolicited symptom. All other symptoms or reactions occurring within 30 days post-vaccination were recorded as unsolicited. The investigator recorded the outcome of all adverse experiences and assessed the relationship of unsolicited symptoms and general reactions to the vaccination.

Serology

Sera taken from infants at birth (week 0), prior to the first dose (week 6) and one month after the final dose (week 18), were stored at - 20°C until analyses could be performed at SmithKline Beecham Biologicals in a blinded fashion. Anti-HBs antibodies were determined using a commercial radioimmunoassay (AUSAB, Abbott), the assay cut-off was 10 mIU/ml (Hollinger *et al,* 1982). Anti-diphtheria and anti-tetanus antibody titers were

measured by ELISA, with an assay cut-off at 0.10 IU/ml. Anti-whole-cell-*B. pertussis* antibody titers (anti-*B. pertussis*) were measured by ELISA using an IgG EIA test kit (Labsystem, ICN-FLOW), the assay cut-off was 15 ELU/ml. The anti-PRP antibodies were measured using a radio-labeled antigen binding assay; assay cut-off was 0.15 μg/ml.

Seroconversion was defined as titers greater than assay cut-off. All assay cut-offs were considered to be seroprotective, except for *B. pertussis* for which there is no serological correlate of protection. A vaccine response against *B.pertussis* in initially seronegative subjects was defined as: the induction of antibody titers greater or equal to the assay cut-off value; and in initially seropositive as: a post-vaccination titer ≥ to the individual's pre-vaccination titer (taking into account the half-life (approximately 40 days) of maternal antibodies (Van Savage *et al,* 1990).

Statistical analysis

Geometric mean titers (GMTs) were calculated for all antigens (titers below the assay cut-off value were given an arbitrary value of one-half the assay cut-off). All statistical analyses were performed using SAS with a type 1 error of 5%. Fisher's exact test was used to compare seroconversion rates and the ANOVA test was used to compare GMTs between groups. The Fisher's exact test was used for the incidence of local reactions and general adverse reactions between groups.

RESULTS

A total of 148 subjects were enrolled in the trial and all but 8 completed the study. Three subjects were lost to follow-up; 2 violated the protocol; 2 subjects dropped-out following adverse reactions; and one subject dropped out following a non-serious event. Twenty-five subjects received Engerix B at birth but were eliminated from the subsequent analysis because they did not receive the study vaccine. Two subjects were eliminated from the reactogenicity and immunogenicity analysis because they received the wrong vaccine. A further 26 were eliminated from the immunogenicity analysis due to; violation of the demographic requirements (3 subjects); non compliance with the vaccination (10) or the blood sampling schedule (13).

Immunogenicity

A high proportion of subjects (36% and 33.3% in groups 1 and 2, respectively) were anti-HBs seropositive at birth, by 6 weeks of age (administration of 1st dose of study vaccines) the number of seropositive subjects had fallen by approximately one third. This is compatible with the natural decay of maternal antibodies. One month after the 3rd dose of the study vaccine 96.0% and 97.8 % of subjects in groups 1 and 2, respectively, had acquired seroprotective titers. No difference was seen between the two groups in seroconversion rates or GMTs post vaccination (Table 1).

All vaccines had achieved protective titers against tetanus and 94.0% and 93.3% of subjects in groups 1 and 2, respectively, had protective titers against diphtheria (Table 2). The anti-tetanus GMTs were significantly higher in group 1 compared with group 2 (3.9 *vs* 1.9 IU/ml respectively, $p = 0.002$). There was no significant difference in the anti-diphtheria GMTs between groups. All but 3 subjects in group 1, elicited a vaccine response to the *B. pertussis* component of the vaccine. All subjects had acquired anti-PRP titers ≥ 0.15 μg/ml, and all but one in group 1 had titers ≥ 1.0 μg/ml (data not shown). However, the GMT values in group 2 (15.4 μg/ml) were significantly higher ($p = 0.0015$) compared with those observed in group 1 (7.5 μg/ml).

Reactogenicity

There were no significant differences in the incidence of any local symptom reported between groups as shown in Table 3. Pain was the most commonly reported local reaction, occurring in 59.3% of group 1 and 51.4% and 20.8% of the DTPw-HBV and Hib vaccination sites respectively, in group 2. Fever (≥ 37.5°C) was the most commonly reported general symptom occurring in 49.4% and 64.7% of subjects in group 1 and 2, respectively, the difference between groups was significant ($p = 0.005$).

Of the 345 diary cards returned, there were only 37 (11%) reports of an unsolicited symptom which were considered to be related to the vaccination. The majority of cases (26) were local symptoms which extended past the 4 day follow-up period. All symptoms had resolved within the study period and did not require major medical intervention. There

Table 1

Percentage of seropositive subjects and anti-HBs geometric mean titers (GMTs).

	Group 1 DTP-HBV-Hib			Group2 DTP-HBV + Hib		
Timing	n/N %	%S+	GMT (95% CI)	n/N	% S+	GMT (95% CI)
Birth (week 0)	18/50	36	21.98 (11.10-43.52)	15/45	33.3	16.2 (9.06-30.49)
Pre (week 6)	12/48	25.0	13.31 (7.71-23.0)	11/42	26.2	11.05 (6.85-17.85)
Post (week 18)	48/50	96.0	139.66 (93.13-209.45)	44/45	97.8	186.96 (122.7-284.87)

N: number of individuals in the analysis.
Seropositive (S+) titers were defined as: ≥ 10mIU/ml.
GMT: Geometric mean titers given in IU/ml.

Birth (week 0) : blood sample taken within 72 hours of birth prior to administration of HBV.
Pre (week 6): blood sample taken prior to the administration of DTP-HBV and Hib vaccine(s).
Post (week 18): blood sample taken approximately one month after the administration of DTP-HBV and Hib vaccine(s).

ANOVA test p-values for post vaccination GMTs between groups (p = 0.32).
No statistical difference seen in seroconversion rates between groups.

Table 2

percentage of seropositive subjects and anti-diphtheria, anti-tetanus and anti-*B.pertussis* geometric mean titers (GMTs).

	Group 1 (N = 50*) DTP-HBV-Hib			Group 2 (n = 45) DTP-HBV + Hib		
Vaccine component	n	% S+	GMT (95 CI%)	n	% S+	GMT (95 CI%)
Anti-diphtheria	47	94.0	0.85 (0.63-1.14)	42	93.3	0.82 (0.59-1.14)
Anti-tetanus	50	100.0	3.93 (3.11-4.96)	45	100.0	1.91 (1.42-2.58)
Anti-*B. pertussis*	47	95.9	104.63 (81.77-133.88)	45	100.0	132.02 (111.79-155.91)
Anti-PRP	50	100.0	7.54 (5.49-10.34)	45	100.0	15.39 (11.39-20.80)

N: number of individuals in the analysis, * except for anti-*B. pertussis* in group 1 N= 49.
Seropositive titers (S+) were defined as: anti-diphtheria ≥ 0.10 IU/ml; anti-tetanus ≥ 0.10 IU/ml; anti-B.pertussis ≥ 15 EL U/ml; anti-PRP ≥ 0.15 µg/ml.
Geometric mean titers (GMTs) were given in: IU/ml for anti-diphtheria and anti-tetanus; EI U/ml for anti-*B. pertussis*.
ANOVA test p-values for GMTs for anti-diphtheria (p = 0.85), anti-tetanus (p = 0.0002), anti-*B. pertussis* (p = 0.25); anti-PRP (p = 0.0015).
No statistical difference seen in seroconversion rates between groups.

Table 3

The incidence of solicited local reactions and general adverse reactions.

	Group 1 (N = 172)	Group 2 (N = 173)	
	DTP-HBV-Hib (Right thigh)	DTP-HBV (Right thigh)	Hib (Left thigh)
Local symptoms	Total (%)	Total (%)	Total (%)
Pain	102 (59.3)	89 (51.4)	36 (20.8)
Redness	77 (44.8)	56 (32.4)	24 (13.9)
Swelling	85 (49.4)	91 (52.6)	40 (18.5)
General symptoms	DTP-HBV-Hib	DTP-HBV + Hib	
Diarrhoea	22 (12.8)	24 (13.9)	
Loss of appetite	26 (15.1)	28 (16.2)	
Restlessness	56 (32.6)	66 (38.2)	
Fever (≥ 37.5°C)	85 (49.4)	112 (64.7)	
Unusual crying	69 (40.1)	81 (46.8)	
Vomiting	14 (8.1)	13 (7.5)	

N: number analyzed for reactogenicity.
Comparison of the incidence of fever between groups 1 and 2 ($p = 0.005$).
There was no statistical difference in the comparison of any other individual symptom between groups.

were two reports of serious adverse reactions which were considered to be related to vaccination (severe local site reaction and febrile convulsion, and an episode of febrile convulsions) in both cases the vaccination course was discontinued (classified as drop-outs). However, following treatment both subjects made a full recovery without sequelae.

DISCUSSION

The benefits of combination vaccines are clear and as a result their development has been endorsed by a number of world heath bodies (WHO, 1992; SAGE, 1996). However, possible interference between components must be assessed before routine use. In this respect most studies show that any changes in titers are small and that there are wide variations between reports. It is therefore hard to attach clinical significance to such observations. Similarly, in this study the pertussis and diphtheria responses were unaffected by the combination administration and in fact a significantly higher anti-tetanus GMTs were seen in the group receiving the mixed administration. This observation has also been reported in an earlier study involving these vaccines (Win *et al,* 1997). Although it is possible that the local increase in tetanus concentration in the combination administration could account for the observation, such speculation is beyond the scope of this paper.

Although reports of a reduced anti-PRP response following combination with DTPa have raised concerns (Eskola, 1996), this effect seems to be less marked with DTPw-based combinations (Eskola, 1996). This is probably due to the adjuvant effect of the Pw component (Vogel *et al,* 1987). Certainly in this study a high anti-PRP response (≥ 7μg/ml) was observed in both groups. And although there was a significance difference in the GMTs between the groups it is unlikely to be of any clinical relevance given the magnitude of the anti-PRP response. In addition there was no appreciable difference in the anti-PRP response between the combined and separate administration in an earlier report which used the same vaccines as in this trial (Win *et al,* 1997) in fact the response in both the combined and separate administrations (6.1 μg/ml and 5.7 μg/ml respectively) was similar to the value reported here for the combined administration. In addition, due to

the ability of Hib conjugate vaccines to induce immunological memory in infants at an early age (Granoff, 1993), the use of absolute titers to predict protection afforded by Hib conjugate vaccines has recently been questioned. Furthermore it is this priming capacity which allows these vaccines to be used at an early age and in accelerated schedule such as 8, 12 and 16 weeks (Booy *et al,* 1993, 1997). Recently, immunological memory has been demonstrated using a DTPw-Hib combination at this schedule (Goldblatt *et al* 1998).

The interference of maternal antibodies in the development of immune response in very young infants has also been of concern in the past. As mentioned earlier an adequate anti-diphtheria, anti-tetanus, anti-pertussis and anti-PRP responses were seen. The anti-HBs GMTs are comparable with those reported elsewhere for infants of this age (Poovorawan *et al,* 1990) and the seroconversion rates fulfilled the WHO requirements. Although there was evidence of the presence maternal antibodies at birth, the GMT values in both groups showed a marked increase (greater than 10 fold) by the end of the vaccination course, thereby demonstrating a vaccine response in the infants. Also a monovalent dose of HBV is recommended for regions of high endemicity, such as the Philippines, and was given here. In this respect, the benefits of neonatal vaccination programs in other countries are already evident (Chunsuttiwat *et al,* 1997; Chen *et al,* 1996; Chainuvati, 1994; Lolekha, 1989).

In keeping with the criteria for developing combination vaccines, the addition of Hib to DTPw-HBV did not alter the reactogenicity profile, in fact values were similar to those reported for DTPw vaccines (Cody *et al,* 1981). Having demonstrated that the DTPw-HBV-Hib combination elicits a good immune response and is well tolerated, it is worth considering the economic advantages of this new combination. The majority of costs associated with any vaccination program are due to storage and transport (Hadler, 1994), therefore the addition of Hib will not results in a significant increase in cost compared with DTPw-HBV vaccination. In fact this could be considered as a saving, because the cost of vaccination with a monovalent Hib vaccine will not be incurred. Furthermore the cost-benefits of monovalent Hib vaccination in developing countries have already been demonstrated (Clemens *et al,* 1996; Levine *et al,* 1993). Therefore taking into consideration the recent inclusion of Hib into the EPI, the pentavalent combination DTPw-HBV-Hib offers an attractive option to health care providers.

ACKNOWLEDGEMENTS

The authors wish to thank Miranda Crichton for help in preparation of this manuscript. Funding for this study was provided by SmithKline Beecham Biologicals.

REFERENCES

Aristegui J, Garrote E, Gonzalez A, Arrate J-P, Peze A, Vandepapelière. Immune response to a combined hepatitis B, diphtheria, tetanus and whole-cell pertussis vaccine administered to infants at 2, 4 and 6 months of age. *Vaccine* 1997; 15: 7-9.

Bijlmer HA. Epidemiology of *Haemophilus influenzae* invasive disease in developing countries and intervention strategies. In: Ellis RW, Granoff DM, eds. Development and uses of *Haemophilus* b conjugate vaccines. New York: Marcel Dekker, 1994: 247-64.

Booy R, Hodgson S, Griffiths H, Chapel HM, Moxon RE. Antibody persistence after accelerated immunization against *Haemophilus influenzae* type b. *Br Med J* 1993; 306: 971-2.

Booy R, Heath PT, Slack MPE, Begg N, Moxon ER. Vaccine failures after primary immunization with *Haemophilus influenzae* type b conjugate vaccine without booster. *Lancet* 1997; 349: 1197-202.

Campbell H, Carter H. Rationale use of *Haemo- philus influenzae* type b vaccine. *Drugs* 1993; 46: 378-83.

Centers for Disease Control (CDC). US Department of Health and Human Services. Update. Vaccine side effects, adverse reactions, contraindications, and precautions. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR* 1996; 45 (RR12): 1-35.

Chainuvati T. Perspective of viral hepatitis in Thailand. In: Viral hepatitis and liver disease. Proceedings of the International Symposium on viral hepatitis and liver disease: molecules today, more cures tomorrow. Tokyo: Springer-Verlag. 1994: pp 403-5.

Chen HL, Chang NH, Ni YH, Lee PI, Lee CY, Chen DS. Seroepidemiology of hepatitis B virus infection in children. Ten years of mass vaccination programs in Taiwan. *JAMA* 1996;. 276: 906-8.

Chunsuttiwat S, Biggs BA, Maynard J, *et al.* Integration of hepatitis B vaccination into the expanded programme on immunization in Chon Buri and Chiang Mai provinces, Thailand. *Vaccine* 1997;15: 769-77.

Clemens J, Brenner R, Rao M, Tafari N, Lowe C. Evaluating new vaccines for developing countries: efficacy or effectiveness? *JAMA* 1996; 275: 390-7.

Cody CL, Barraff LJ, Cherry JD, Marcy SM, Manclark CR. Nature and rates of adverse reactions associated with DTP and DT immunizations in infants and children. *Pediatrics* 1981; 68:650-60.

Eskola J. Analysis of *Haemophilus influenzae* type b conjugate and diphtheria-tetanus-pertussis combination vaccines. *J Infect Dis* 1996;174 (suppl 3): S302-5.

Funkhouser A, Steinhoff MC, Ward J. *Haemophilus influenzae* disease and immunization in developing countries. *Rev Infect Dis* 1991; 13: S542-54.

Goldblatt D, Miller E, McCloskey N, Cartwright K. Immunological response to conjugate vaccines in infants: follow up study. *Br Med J* 1998; 316: 1570-1.

Granoff DM, Holmes JS, Osterholm MT, *et al.* Induction of immunologic memory in infants primed with *Haemophilus influenzae* type b conjugate vaccines. *J Infect Dis* 1993; 168: 663-71.

Hadler SC. Cost benefit of combining antigens. *Biologicals* 1994; 22: 415-8.

Hollinger FB, Adam E, Heiberg D, Melnick JL. Response to hepatitis B vaccine in a young adult population. In: Szmuness W, Alter HJ, Maynard JE, eds. Viral hepatitis and liver disease. Proceedings of the 1981 International Symposium. Philadelphia: Franklin Institute Press, 1982; pp 451-66.

Lagos R, Horwitz I, Toro J, *et al.* Large scale, post licensure, 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: 216-22.

Lee JW, Aylward B, Hull HF, Batson A, Birmingham E, Lloyd J. In: Levine MM, Woodrow GC, Kraper JB, Cobon GS, eds. New Generation vaccines. 2nd ed. New York: Dekker, 1997; 79-88.

Levine OS, Ortiz E, Cintreras R, *et al.* Cost-benefit analysis for the use of *Haemophilus influenzae* type b conjugate vaccines in Santiago, Chile. *Am J Epidemiol* 1993; 173: 1221-8.

Lolekha S. Strategies for control of hepatitis in Thailand. In: Proceedings of critical issues for control of hepatitis B, Asia Pacific Congress Series. *Excerpta Medica* 1989: pp26-9.

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

Papaevangelou G, Karvelis E, Alexiou D, *et al.* Evaluation of a combined tetravalent diphtheria, tetanus, whole-cell pertussis and hepatitis B candidate vaccine administered to healthy infants according to a three-dose vaccination schedule. *Vaccine* 1995; 13: 175-8.

Poovorawan Y, Theamboonlers A, Sanpavant S, *et al.* The immunogenicity and reactogenicity of combined tetravalent diphtheria, tetanus, pertussis, and hepatitis B vaccine in infants. In: Nishioka K, Suzuki H, Mishiro S, Oda T, eds. Viral hepatitis and liver disease. Proceedings of the International Symposium on viral hepatitis and liver disease: Molecules today, more cures tomorrow. Tokyo: Springer-Verlag, 1994; pp 526-9.

Poovorawan Y, Sanpavat S, Pongpunlert W, *et al.* Immunogenicity of a yeast-derived hepatitis B vaccine in neonates. In: Progress in Hepatitis B immunization: La vaccination contre l'Hépatite B. Proceedings of a symposium organised by the Université François Rabelais de Tours, Palais des Congrès, Paris, France May 3-5, 1989. INSERM/John Libbey Eurotext. 1990; 34: 371-7.

Special Advisory Group of Experts (SAGE) Report. *Weekly Epidemiol Rec* 1996; 71: 263-4.

Usonis V, Bakasenas V, Taylor D, Vandepapelière P. Immunogenicity and reactogenicity of a combined DTPw-hepatitis B vaccine in Lithuanian Infants. *Eur J Pediatr* 1996; 155: 189-93.

Van Savage J, Decker M, Edwards K, Sell S, Karzon D. Natural history of pertussis antibody in the infant and effect on vaccine response. *J Infect Dis* 1990; 161:487-92.

Vogel FR, Leclerc C, Schultze MP, *et al.* Modulation of carrier-induced epitopic suppression by *Bordetella pertussis* components and muramyl peptide. *Cell Immunol* 1987; 107: 40-51.

Win KM, Aye M, Htay-Htay H, Safray A, Bock H. Comparison of separate and mixed administration of DTP-HBV and Hib vaccines: immunogenicity and reactogenicity profiles. *Int J Infect Dis* 1997; 2: 79-84.

World Health Orgainsation. Global perspectives on hepatitis. Newsletter of the International Task Force on Hepatitis B Immunization and Program for Appropriate Technology in Health. *PATH* 1993; 4: 1-8.

World Health Organization. Informal discussion on quadrivalent diphtheria-tetanus-pertussis-Hepatitis B vaccine. Final report. Geneva, World Health Organization (WHO) Global Programme for Vaccines and Immunization: The WHO position paper on *Haemophilus influenzae* type b conjugate vaccines. *Weekly Epidemiol Rec* 1992; 73: 64-8.