EVALUATION OF AN ACELLULAR PERTUSSIS, DIPHTHERIA, TETANUS, INACTIVATED POLIOVIRUS, HIB-CONJUGATE COMBINED VACCINE (PENTAXIM™) AT 2, 4, AND 6 MONTHS OF AGE PLUS HEPATITIS B VACCINE AT BIRTH, 2, AND 6 MONTHS OF AGE IN INFANTS IN THAILAND

Usa Thisyakorn[1], Tawee Chotpitayasunondh[2], Chitsanu Pancharoen[1], Sunate Chuenkitmongkol[3] and Esteban Ortiz[4]

[1] King Chulalongkorn Memorial Hospital, Bangkok;
[2]Queen Sirikit National Institute of Child Health, Bangkok;
[3]Sanofi Pasteur, Bangkok, Thailand; [4]Sanofi Pasteur, Lyon, France

Abstract. The objective of this study was to evaluate the immunogenicity and safety of a pentavalent vaccine (Pentaxim™) containing diphtheria, tetanus, acellular pertussis, inactivated poliovirus, and Hib polysaccharide-conjugate (DTaP-IPV//PRP~T) antigens, in Thai children. One hundred eighty-six infants who had received a hepatitis B vaccine at birth were given a pentavalent vaccine at 2, 4 and 6 months of age and a hepatitis B vaccine concomitantly at 2 and 6 months of age. Immunogenicity was high for each vaccine antigen. The study vaccine was well tolerated and side effects were few. After the third dose, 100% of subjects had an anti-PRP ≥0.15 μg/ml and 96.5% ≥1.0 μg/ml; the anti-PRP GMT was 9.53 μg/ml. Seroprotective rates for diphtheria and tetanus (≥0.01 IU/ml) were 99.4% and 100%, respectively, and 100% for all three poliovirus types (≥8 1/dil U). The vaccine response rates to pertussis antigens (a 4-fold increase in antibody titer were 94.1% for PT and 93.0% for FHA. The DTaP-IPV//PRP~T vaccine given at 2, 4 and 6 months of age concomitantly with a monovalent hepatitis B vaccine, was well tolerated and highly immunogenic for primary immunization of infants in Thailand.

Key words: pentavalent combined vaccine, acellular pertussis, inactivated polio vaccine, Hib-conjugate vaccine, hepatitis B vaccine, immunogenicity, safety, Thailand

INTRODUCTION

Vaccines are cost-effective public health tools that have greatly reduced the burden of infectious diseases worldwide. The expanded program of immunization (EPI) initiated by the WHO in 1974 originally aimed to protect children against six diseases: tuberculosis, diphtheria, tetanus, whooping cough, poliomyelitis and measles. Since then other vaccines have been added to the EPI, including hepatitis B (HB) (WHO, 1992) and *Haemophilus influenzae* type b (Hib) (WHO, 1996).

Correspondence: Dr Sunate Chuenkitmongkol, Sanofi Pasteur, 87/2 CRC Tower, 23rd Fl., All Seasons Place, Wireless Road, Lumpini, Phatumwan, Bangkok 10330, Thailand.
E-mail: Sunate.chuenkitmongkol@sanofipasteur.com

Vaccines combining whole cell *Bordetella pertussis* antigens with diphtheria and tetanus toxoids (DTwP), first licensed in the 1940s, have been central to the EPI and now often include additional antigens to account for differences in disease burden among different countries. The past decade has seen a continuing increase in the number of vaccines licensed and recommended for use in infants and children, making development of combined vaccines of public health importance. Combining multiple individual antigens into a single injection can simplify vaccine administration programs. Combination vaccines also permit adding new vaccines to childhood schedules with an increased likelihood of achieving higher vaccination coverage than if they were given separately since there is less chance of missed doses. Combination vaccines can also reduce the direct and indirect costs of vaccination (Decker, 2001; Dodd, 2003; Kalies *et al*, 2006).

Hepatitis B vaccination was added to the Thai national immunization schedule in 1993 and is given at birth, 1 or 2 months of age and 6 months of age (WHO, 2007). Other vaccines not yet included in the national schedule, but for which a rationale exists are *Haemophilus influenzae* type b and inactivated polio vaccine (IPV). In Thailand, over 40% of all bacterial meningitis cases are caused by Hib, with over 90% of Hib meningitis cases occurring in children under the age of 2 years (Lolekha *et al*, 2001). The incidence of Hib meningitis has been estimated as between 3.8 and 8.8 per 100,000 person years, lower than in the US or Europe prior to implementation of routine Hib vaccination (Sunakorn 1996; Rerks-Ngarm *et al*, 2004; WHO, 2005), but this estimate, based on microbiologic case confirmation, probably does not include all cases and does not take the burden of Hib pneumonia into account (Sunakorn, 1996; Rerks-Ngarm *et al*, 2004). Nasopharyngeal Hib carriage in rural Thailand has been estimated at 7% in children younger than 5 years of age (Olsen *et al*, 2005).

The WHO has taken the position that continuing oral polio vaccine (OPV) use after interruption of wild virus transmission is incompatible with eradication of polio, since continuing use of OPV poses the risks of vaccine associated paralytic polio (VAPP), outbreaks caused by circulating vaccine derived polio viruses (cVDPV) and vaccine-derived poliovirus excretion by immunodeficient individuals (iVDPV) (WHO, 2006b). After interruption of wild poliovirus transmission, the only remaining source of poliomyelitis virus is the OPV. Cessation of the use of the OPV is followed by an indefinite period of risk for the emergence of cVDPV, rare cases of iVDPV and the emergence of orphan wild viruses not detected by routine surveillance (Bonnet and Dutta, 2008). The WHO position is that this risk may be managed with monovalent OPV (mOPV), but the use of the IPV is an alternative for countries that wish to continue polio vaccination in the "post eradication era" (WHO, 2006b). As of 2009, approximately 35 countries in North America, Western Europe, and Australia, New Zealand, South Korea, Hong Kong, Mexico, Turkey, South Africa and Russia, are using the IPV in their national immunization programs, mainly in combination vaccines. Other countries have shifted to sequential OPV/IPV schedules (Bonnet and Dutta, 2008; WHO, 2008a, 2009). The goal of the WHO Global Polio Eradication Initiative strategic plan for 2009-2013 is to "ensure that no child will ever again be paralyzed by either a wild or vaccine-derived poliovirus". The plan includes milestones for OPV use cessation as well as adoption of the IPV by

countries that choose to continue polio immunization (WHO, 2008b).

Acellular pertussis (aP) vaccines containing purified *B. pertussis* antigens that are better tolerated than the DTwP combination vaccines are used in many EPI countries, and have been evaluated in numerous clinical trials (Cherry, 1997; Hewlett and Cherry, 1997; Simondon *et al*, 1997; Edwards and Decker, 2008). Acellular pertussis combination vaccines have been widely adopted over the last 10 years, and are now included in national immunization programs in North America, most western European and some Asian countries, including Japan and South Korea; Australia, and more recently in Mexico, Turkey, and South Africa (Anonymous, 1997; CIG, 2006; IVS, 2009; WHO, 2009). The WHO position on pertussis vaccines is that "the best aP vaccines have shown similar protective efficacy as the best wP vaccines, and that all licensed vaccines have proved to be highly effective in controlling pertussis in infants and young children" (WHO, 2005).

Sanofi pasteur has developed an acellular pertussis combination vaccine (Pentaxim™, an AcXim family vaccine) including a liquid DTaP-IPV combination used to reconstitute a lyophilized Hib conjugate vaccine (PRP~T) at the time of injection. These antigens are well known as standalone vaccines. The PRP~T component is licensed worldwide, including in Thailand, under the trade name of ActHIB®. The IPV vaccine, as a standalone vaccine is also licensed worldwide, under the trade name of Imovax® Polio. Both of these vaccines are WHO qualified (WHO, 2009). The DTaP-IPV//PRP~T vaccine has been licensed since 1997 as Pentaxim™ or Pentavac™ in over 95 countries worldwide, including in Thailand, at the time of study initiation (Vidor and Plotkin, 2008).

As the recommended schedules in Thailand for Hib vaccination and DTP vaccination are the same (*ie*, at 2, 4, and 6 months), it would be more convenient and cause less distress for infants, as well as parents, to receive these vaccines in combination. This clinical study was designed to assess the immunogenicity, reactogenicity and safety of the DTaP-IPV//PRP~T combined Pentaxim vaccine as a three-dose primary vaccination at 2, 4, and 6 months of age concomitantly administered with the hepatitis B vaccine at 2 and 6 months of age in infants who received the first dose at birth.

MATERIALS AND METHODS

Study design

This open study enrolled infants at King Chulalongkorn Memorial Hospital and Queen Sirikit National Institute of Child Health, Bangkok, Thailand. The ethical review board at each center approved the study protocol. The study was conducted in compliance with Good Clinical Practice (GCP) and local regulations (Guidelines for Good Practice in the Conduct of Clinical Trials in Human Participants in Thailand). Written informed consent was obtained from the parents or legal representatives before enrollment.

Healthy, full-term (>37 weeks) infants who weighed ≥2.5 kg at birth and had received a first dose of hepatitis B vaccine within 24 hours of birth were eligible for inclusion at 2 months of age. Subjects were ineligible for the study if they had: an allergy to any of the vaccine components; received immunosuppressive therapy (*eg*, long-term systemic corticosteroids), immunoglobulins or other blood products; had a previous vaccination against DTP, *H. influenzae* type b (Hib) or poliomyelitis or vaccines other than Bacille Calmette-

Gurin (BCG) or a birth dose of HB required for enrolment; a chronic illness that could interfere with completing the trial; a history of diphtheria, tetanus, pertussis, poliomyelitis, Hib or hepatitis B infection; thrombocytopenia or a bleeding disorder contraindicating intramuscular (IM) vaccination; a history of seizures or a febrile illness (rectal temperature ≥38.0ºC or axillary temperature ≥37.4ºC) on the day of inclusion. Infants were enrolled at 2 months of age to receive the DTaP-IPV//PRP~T vaccine at 2, 4 and 6 months of age and the last two doses of hepatitis B vaccine at 2 and 6 months of age. The hepatitis B vaccine was not given as a study vaccine, but was administered according to the Thai national immunization schedule (WHO, 2007).

Vaccines

The combined DTaP–IPV//PRP~T vaccine (Pentaxim, batch Z2044-1) was produced and supplied by Sanofi Pasteur, Lyon, France. Each 0.5 ml dose contained ≥30 IU [25 limit of flocculation (Lf) of diphtheria toxoid], ≥40 IU (10 Lf) of tetanus toxoid, 25 μg of PT, 25 μg of FHA, 40 D antigen units (DU) of IPV type-1 (Mahoney strain), 8 DU of IPV type-2 (MEF-1 strain), 32 DU of IPV type-3 (Saukett strain), and 10 μg of PRP~T. The lyophilized PRP~T component was reconstituted with the liquid DTaP-IPV vaccine immediately before injection. The recombinant hepatitis B vaccine (Recomvax B/Euvax B, LG Life Sciences, Seoul, the Republic of Korea) contained 10 μg of recombinant HBsAg. It is licensed and commercially available in Thailand. The DTaP-IPV//PRP~T and hepatitis B vaccines were administered by intramuscular injection into the right and left anterior thighs, respectively.

Serology

Blood samples for antibody determination were taken at 2 months of age, just before the first dose, and at 7 months of age, one month after the third dose, of the combination vaccine. All immunological assays were carried out at Sanofi Pasteur's central laboratory in Swiftwater, Pennsylvania, USA. Anti-tetanus toxoid antibody titers (IU/ml) were measured by enzyme-linked immunosorbent assay (ELISA) and compared to the WHO TE3 human standard. Anti-FHA and anti-PT antibody titers (EU/ml) were also assessed by ELISA. Anti-diphtheria antibody titers (IU/ml) were assessed by seroneutralization in Vero cell culture as compared to a WHO equine antitoxin standard. Anti-poliovirus antibody titers were assessed by microneutralization following a modified WHO standardized procedure (*WHO/EPI/GEN 93.9*) utilizing Vero cells and wild type viruses. The results were expressed as the highest reciprocal dilution of the test serum that inhibited the cytopathic effect of the challenge dose virus. Anti-*Haemophilus influenzae* type b polysaccharide (PRP~T) antibody titers (μg/ml) were performed by ELISA and compared to the Food and Drug Administration (FDA) human reference 1983. Anti-HBsAg antibody titers (mIU/ml) were measured using the VITROS ECi/ECiQ Immunodiagnostic System (Ortho-Clinical Diagnostics).

The pre-defined antibody levels for seroprotection were: anti-PRP ≥0.15 μg/ml and ≥1.0 μg/ml, anti-poliovirus ≥8 reciprocal dilution (1/dil), anti diphtheria ≥0.01 and ≥0.1, anti-tetanus ≥0.01 and ≥0.1 IU/ml and anti-HBsAg ≥10 mIU/ml. Since there are no accepted correlates of seroprotection for pertussis antibodies, the vaccine response to anti-pertussis antigens was defined as a ≥4-fold increase in anti-PT or anti-FHA antibody concentration after primary vaccination.

Reactogenicity and safety

Infants were monitored for 30 minutes

after each injection for immediate local or systemic reactions. Parents or legal guardians recorded solicited injection site reactions (tenderness, redness or swelling) and systemic events (axillary temperature ≥37.4ºC, drowsiness, irritability, abnormal crying, loss of appetite or vomiting) daily for 8 days following vaccination (days 0-7). Pain or tenderness was scored as severe if the infant cried when the limb was moved or if the pain appeared to prevent normal activity. For erythema and swelling, a diameter of less than 2.5 cm was graded as mild, from 2.5 to <5 cm as moderate and 5 cm or greater was graded as severe. Severe crying was defined as crying for more than 3 hours; severe irritability was described as "inconsolable;" severe loss of appetite required missing ≥3 feeds or refusing most foods. Severe drowsiness was defined as sleeping most of the time or being difficult to wake up. Fever was graded as severe if the axillary temperature was ≥39ºC. The date of onset, intensity and resolution of any unsolicited events were recorded for 30 days after each vaccination. Information regarding severe adverse events (SAEs) was collected and evaluated for relation to the study treatment throughout the trial. All subjects who received at least one dose of the study combination vaccine were included in the reactogenicity and safety evaluation.

Statistical analysis

The primary objective of the study was to determine the seroprotection rates for diphtheria, tetanus, polio types 1, 2, and 3 and PRP; and the seroconversion/vaccine response rates for PT and FHA in response to the DTaP-IPV//PRP~T combined vaccine, one month after the three dose primary vaccination. The secondary objectives were to determine the antibody titers in response to the study vaccine antigens one month after the three-dose primary vaccination and to evaluate the reactogenicity and safety of the study vaccine. Seroprotection and seroconversion/vaccine response rates were calculated with their 95% confidence intervals (CIs) by the Clopper-Pearson exact binomial method (Newcombe, 1998). Geometric mean antibody titers (GMTs) were calculated with 95% confidence intervals (CIs) using the normal approximation method. Reverse Cumulative Distribution Curves (RCDCs) were constructed for each antibody response after primary vaccination. For evaluation of reactogenicity, the numbers and percentage of subjects with a given symptom after each dose of vaccine were calculated.

The statistical analysis was descriptive; no hypothesis was tested. The sample size was chosen to give sufficient precision to allow a non-inferential comparison, based on CIs, with historical results of a previous study conducted in France on 212 infants with the same combined vaccine and administration schedule (Mallet *et al*, 1996). The sample size was thus set at 186 subjects to ensure 158 evaluable subjects assuming a 15% dropout rate.

RESULTS

A total of 186 infants were enrolled at the two study centers, 93 at the King Chulalongkorn Memorial Hospital and 93 at the Queen Sirikit National Institute of Child Health. The mean age at study entry was 1.9 months, the mean weight was 5.2 ± 0.7 kg, 56.5% of the subjects were male, 43.5% were female. All subjects received both the DTaP-IPV//PRP~T and hepatitis B vaccines on enrollment (Visit 1) and were included in the safety analysis. Of

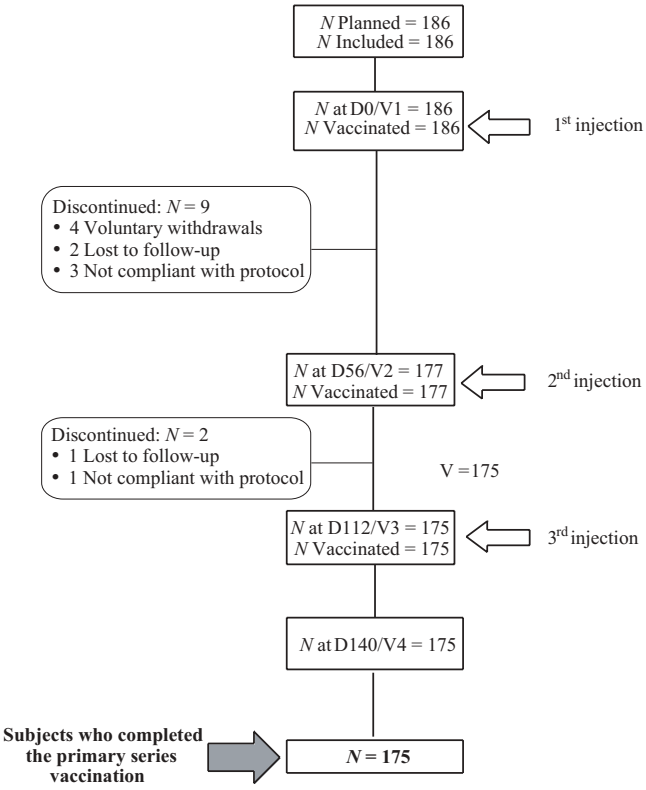

Fig 1–Subject disposition.

these, 175 subjects (94.1%) completed the primary vaccination series. Three of the 11 subjects who discontinued the study were lost to follow-up, four were withdrawn for protocol violations and parents voluntarily withdrew four. Two additional subjects were excluded from the immunogenicity analysis because of protocol violations, leaving 173 subjects (93%) who comprised the immunology analysis set. No infant withdrew because of an adverse event (AE). The subject disposition is summarized in Fig 1.

Immunogenicity

The seroprotection (SP) and seroconversion (SC) or vaccine response (VR) rates for the study vaccine and historical control study are summarized in Table 1. The immunogenicity was high for each vaccine antigen, and each SP and SC or VR rate was within or

Table 1
Seroprotection, vaccine response rates and geometric mean titers (GMT) one month after primary vaccination at 2, 4 and 6 months of age.

	Historical reference[a] Rate (95% CI)	DTaP-IPV//PRP~T + HB[b] Rate (95% CI)	GMT (95% CI)
Anti-diphtheria	100% (95.8 - 100)	99.4 (96.8 - 100.0)	0.12 (0.10 - 0.14)
Anti-tetanus	100% (95.9 - 100)	100.0 (97.9 - 100.0)	1.11 (1.01 - 1.21)
Anti-polio 1	97.1% (91.7 - 99.4)	100.0 (97.9 - 100.0)	1,207 (987.6 - 1,474.8)
Anti-polio 2	100% (96.5 - 100)	100.0 (97.9 - 100.0)	1,553 (1,284 - 1,880)
Anti-polio 3	99.0% (94.7 - 100)	100.0 (97.9 - 100.0)	3,004 (2,444 - 3,693)
Anti-PRP[c]	98.1% (93.2 - 99.8)	100.0 (97.9 - 100.0)	9.53 (7.91 - 11.5)
Anti-PRP[d]	89.3 (83.4 - 95.3)	96.5 (92.6 - 98.7)	-
Anti-PT	90.8% (83.3 - 95.7)	94.1 (89.4 - 97.1)	175.8 (158.6 - 194.9)
Anti-FHA	92.5% (85.1 - 96.9)	93.0 (88.1 - 96.3)	118.8 (108.2 - 130.4)
Anti-HBs	NA	100.0 (97.9 - 100.0)	1,562 (1,369 - 1,783)

[a]DTaP-IPV//PRP~T at 2, 4 and 6 months of age in 212 children in France
[b]HB vaccine at birth, 2 and 6 months of age; [c]≥0.15 μg/ml; [d]≥1.0 μg/ml; NA, not applicable

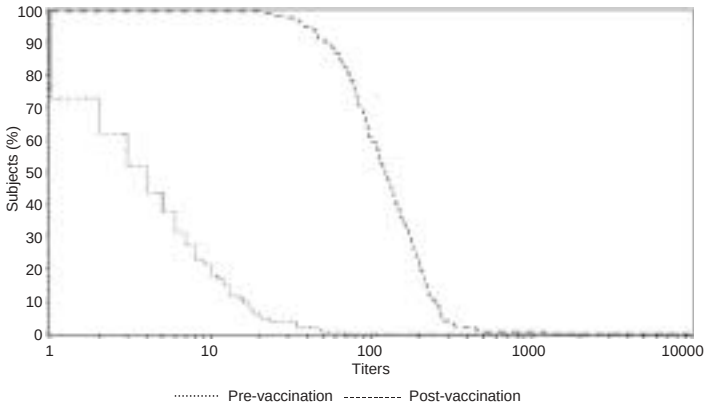

Fig 2–Anti-PT (ELISA, EU/ml) before and after the primary vaccination phase: reverse cumulative distribution curves.

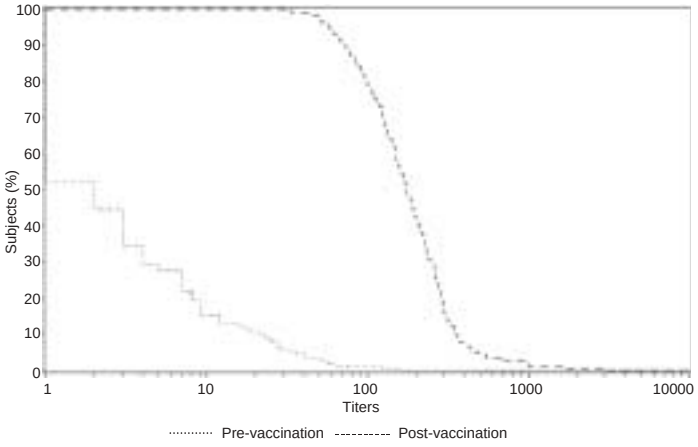

Fig 3–Anti-FHA (ELISA, EU/ml) after the primary vaccination phase: reverse cumulative distribution curves.

above the 95% CI for the corresponding rate observed in the historical control study. After the third dose, anti-PRP ≥0.15 μg/ml was observed in 100% of subjects and 96.5% of subjects had anti-PRP ≥ 1.0 μg/ml. The seroprotection rates for diphtheria (≥ 0.01 IU/ml), tetanus (≥0.01 IU/ml) and polio (≥8 1/dil) were between 99 and 100%. Vaccine response rates for PT and FHA (≥4-fold increase in antibody titers) were 94.1% and 93.0%, and ≥2-fold increases occurred in 97.6% and 97.7% of subjects, respectively. Anti-PT and anti-FHA titers ≥25 EU/ml were observed in 100% and 98.8% of subjects, respectively. GMTs of anti-diphtheria, tetanus, polio, PRP and HBsAg antibodies observed one month following the three-dose primary immunization series (Table 3) were all within their expected ranges. As there were no accepted protective titers for anti-PT and FHA antibodies, the post- to pre-vaccination GMT ratio (GMTR), 61.96 (49.59-77.41) for PT and 31.84 (26.08-38.86) for FHA were also used to demonstrate the immune response to these pertussis antigens in the study vaccine. The RCDCs also showed strong, linear increases in antibody titers for anti-PT and anti-FHA antibodies (EU/ml) from pre- to post-primary vaccination (Figs 2 and 3).

Reactogenicity and safety

The occurrence of solicited adverse reactions during the 8 days following vaccination is presented in Table 2 as a percentage of vaccine doses given. Overall, 66.7% of subjects (124/186) experienced at least one solicited injection site reaction and 68.8% (128/186) experienced at least one solicited systemic reaction. Erythema and tenderness were the most frequent solicited injection site reactions, reported in 29.2 and 27.1% of the combined vaccine doses and 29.4 and 29.6% of the hepatitis B vaccine doses, respectively,

Table 2
Solicited local adverse reactions and systemic events occurring within 8 days (days 0-7) of any dose of vaccine.

		DTaP-IPV//PRP~T *N* = 538 doses % (95% CI)	Hepatitis B *N* = 361 doses % (95% CI)
Injection site reactions	Any	43.9 (39.6-48.2)	43.5 (38.3-48.8)
	Severe	0.2 (0.0-1.0)	0.6 (0.1-2.0)
Tenderness	Any	27.1 (23.4-31.1)	29.6 (25.0-34.6)
	Severe	0.2 (0.0-1.0)	0.0 (0.0-1.0)
Erythema	Any	29.2 (25.4-33.2)	29.4 (24.7-34.4)
	Severe	0.0 (0.0-0.7)	0.0 (0.0-1.0)
Swelling	Any	13.8 (11.0-17.0)	11.9 (8.8-15.7)
	Severe	0.0 (0.0-0.7)	0.6 (0.1-2.0)
Systemic events	Any	45.4 (41.1-49.7)	
	Severe	1.3 (0.5-2.7)	
Fever	Any	11.5 (9.0-14.5)	
	Severe	0.9 (0.3-2.2)	
Vomiting	Any	14.7 (11.8-18.0)	
	Severe	0.0 (0.0-0.7)	
Crying abnormal	Any	34.9 (30.9-39.1)	
	Severe	0.2 (0.0-1.0)	
Drowsiness	Any	13.2 (10.5-16.4)	
	Severe	0.0 (0.0-0.7)	
Appetite loss	Any	13.6 (10.8-16.8)	
	Severe	0.2 (0.0-1.0)	
Irritability	Any	32.2 (28.2-36.3)	
	Severe	0.4 (0.0-1.3)	

Pain "severe", child cries when injected limb is moved or the movement of the injected limb is reduced; Redness/Swelling "severe", longest diameter was ≥5 cm; Fever "severe", axillary temperature ≥ 39ºC; Irritability "severe", child "inconsolable"; Drowsiness "severe", Sleeping most of the time or difficulty to wake up; Appetite lost "severe", refuses ≥ 3 feeds/meals or refuses most feeds/meals; Crying abnormal "severe", more than 3 hours; Vomiting "severe", ≥6 episodes per 24 hours or requiring parenteral hydration.

followed by swelling after 13.8% and 11.9% of doses, respectively. Most solicited injection site reactions were mild in intensity, occurred within 3 days after vaccine injection, and resolved within 1 to 3 days. Severe solicited injection site reactions were infrequent, reported after ≥0.6% of doses regardless of the vaccine given. Abnormal crying and irritability were the most frequently reported solicited systemic reactions (34.9% and 32.2%, respectively), followed by vomiting (14.7%) appetite loss (13.6%), drowsiness (13.2%) and fever (11.5%). Most solicited systemic reactions were mild in intensity and occurred within 3 days of vaccination. Severe solicited systemic reactions occurred in ≥0.9% of vaccine doses.

A total of 118 subjects (63.4%) reported at least one unsolicited event. One experienced induration of mild severity that began two days after the first injection with DTaP-IPV//PRP~T and resolved without treatment after 6 days. The remaining unsolicited events were systemic. The most frequently reported were upper respiratory tract infection in 62 subjects, pyrexia in 42 subjects, and diarrhea in 9 subjects. Ten subjects (5.4%) reported a total of 14 SAEs, but none of these led to withdrawal from the study. None of the SAEs reported were considered by the investigator to be related to vaccination, and none were fatal. Most of the SAEs were infectious diseases commonly observed during infancy, such as viral infection, urinary tract infection, bronchitis or gastroenteritis.

DISCUSSION

This study evaluated the immunogenicity, reactogenicity and safety of a DTaP-IPV//PRP~T combination vaccine given to infants as a primary series at 2, 4 and 6 months of age. The infants were also given a monovalent recombinant hepatitis B vaccine at birth, 2 and 6 months of age following the national vaccination schedule of Thailand. The results were compared with a historical control group given the same vaccine at 2, 4 and 6 months of age in a clinical trial conducted in France, where the vaccine has been in routine use for several years (Mallet *et al*, 1996; IVS, 2009). No inferential comparison was done with the results observed in the historical control study, but the mean seroprotection and vaccine response rates and their 95% CI were compared with those observed in the French study; no clinically significant differences in response were seen.

The seroprotection and vaccine response rates observed in this study were also similar to those seen in previous studies evaluating the same vaccine for primary vaccination in infants in a number of European countries, Chile, Turkey and the Philippines (Carlsson *et al*, 1998; Lagos *et al*, 1998; Kanra *et al*, 2000; Mallet *et al*, 2000; Capeding *et al*, 2008). The high immunogenicity of the inactivated poliovirus antigens was of particular interest because of the anticipated worldwide cessation of the use of oral polio vaccine (OPV) and the IPV vaccination options for maintaining population immunity against poliomyelitis (WHO, 2006b). The inclusion of the IPV in a DTaP combination vaccine ensures high vaccination coverage while avoiding the additional infections required if IPV were added to the schedule as a separate vaccine. In recent years, the vaccination coverage in Thailand for 3 doses of DTP has been 98% (WHO, 2007).

An anti-PRP antibody concentration of ≥0.15 μg/ml was observed in 100% of subjects and 96.5% had an anti-PRP antibody concentration ≥1.0 μg/ml. The anti-PRP GMT was 9.53 μg/ml. Our results are consistent with a previous study in Thailand conducted with the same PRP~T included in the study vaccine (ActHib Sanofi Pasteur, Lyon, France) given either concomitantly or combined with a DTwP vaccine (D.T.COQ™, Sanofi Pasteur, Lyon, France). In that study, 100% of subjects had an anti-PRP antibody concentration of ≥0.15 μg/ml and 98.5% had a concentration ≥1.0 μg/ml after primary vaccination (Lolekha *et al*, 2001).

As there are no recognized serological correlates of protection for pertussis, this study used the classical criteria for evaluating vaccine response against pertussis antigens: a 4-fold increase from pre- to post-vaccination anti-PT and FHA antibody titers. A 4-fold increase in anti-PT and anti-FHA antibody titers was obtained

in 94.1% and 93.0% of subjects, respectively. Anti-PT or anti-FHA antibody titers ≥25 EU/ml were seen in 100% and 98.8% infants, respectively. In addition, strong increases in GMTs were observed from pre- to post-primary vaccination. These results are in line with anti-PT and anti-FHA responses to this 2-component acellular pertussis vaccine reported in 36 clinical trials conducted in 17 countries in Europe, North and South America, Africa and Asia that have included nearly 10,000 subjects (Vidor and Plotkin, 2008).

The DTaP-IPV//PRP~T study vaccine has been in routine use in several European countries since 1997. The National Surveillance Program in Sweden has documented the long-term impact of this vaccine on pertussis incidence (Olin *et al*, 1999; Hessel *et al*, 2004; Gustafsson *et al*, 2006; SIIDC, 2006). With 3-dose vaccination coverage of >98%, the overall incidence of culture or PCR confirmed that pertussis cases declined from 113-150/100,000 in 1993-1995 to 11-16/100,000 in 2001-2004 (Gustafsson *et al*, 2006). The reported average annual incidence of confirmed cases was 14 per 100,000 person-years in children born during the 10 year period following adoption of the acellular pertussis vaccine (SIIDC, 2006). In a cohort of children who received only this vaccine, the reported incidence of laboratory confirmed pertussis cases was 13 per 100,000 person-years; a 91% decrease in incidence was seen after 2 doses and a 95% reduction was reported after the third dose compared to no dose (Hessel *et al*, 2004; SIIDC, 2006). The long term surveillance data thus provides evidence of effective control of pertussis disease in routine use, and this acellular pertussis vaccine has protected children for 5 to 6 years after the third dose (Gustafsson *et al*, 2006). Thus, the immunogenicity results together with the surveillance data, suggest the study vaccine would be expected to achieve high protection against pertussis and four other vaccine preventable childhood diseases.

The DTaP-IPV vaccine had a low reactogenicity, as seen by the rate of occurrence of solicited injection site reactions and systemic events. Tenderness, erythema or swelling at the injection site were reported in 13.8 to 29.2% of combination vaccine doses. The incidence of solicited systemic adverse events was also low. Fever (axillary temperature ≥37.4ºC) was observed in 11.5% of doses administered. Other solicited systemic adverse events (drowsiness, vomiting or appetite loss) were observed in approximately 13 to 15% of doses given and crying and irritability in 32 to 35%. Most solicited events occurred within 4 days of vaccination, lasted less than 3 days and were of mild severity. The incidence of severe solicited adverse reactions was very low, following less than 0.9% of doses given. No subject withdrew because of an adverse event.

Randomized controlled trials have shown the incidence of relatively frequent side effects (such as fever, erythema, swelling or drowsiness) to be lower with the aP than the wP vaccines (Edwards and Decker, 2008). The results of this study are consistent with the low reactogenicity documented for aP based combination vaccines. The generally mild nature and short duration of reported local reactions and systemic events and the very low occurrence of severe events indicate the study vaccines were well tolerated.

In summary, the DTaP-IPV//PRP~T combined vaccine (Pentaxim) was highly immunogenic for all antigens when administered to infants in Thailand following a 2, 4 and 6 months of age schedule. The seroprotection and vaccine response rates are consistent with the historical data

obtained with this vaccine used with the same vaccination schedule in France, a country where the vaccine is in routine use, as well as other studies in various countries using a number of different vaccination schedules. The inclusion of IPV and PRP~T antigen should make it easier to successfully incorporate these vaccines into the national schedule. The vaccine is also easily compatible with hepatitis B vaccination at birth, 2 and 6 months of age and is well tolerated in infants when given concomitantly with a monovalent hepatitis B vaccine.

ACKNOWLEDGEMENTS

The authors would like to thank Clement Weinberger of Le Stylo Medical Communications for his assistance with the preparation of this manuscript.

REFERENCES

Anonymous. Pertussis vaccination: Use of acellular pertussis vaccines among infants and young children. Recommendations of the Advisory Committee on Immunization Practices (ACIP) - *MMWR Recommend Rep* 1997; 46(RR-7): 1-25.

Bonnet M-C, Dutta A. World wide experience with inactivated poliovirus vaccine. *Vaccine* 2008; 26: 4978-83.

Capeding RM, Cadorna-Carlos J, Book-Montellano M, Ortiz E. Immunogenicity and safety of a DTaP-IPV//PRP~T combination vaccine at 6, 10, 14 weeks of age (EPI schedule) and concomitant Hepatitis B vaccination at birth, 6, 14 or 6, 10, 14 weeks of age. *WHO Bull* 2008; 86: 443-51.

Carlsson RM, Claesson BA, Selstam U, *et al*. Safety and immunogenicity of a combined diphtheria, tetanus, acellular pertussis-inactivated polio vaccine- Haemophilus influenzae type b vaccine administered at 2-4-6-13 or 3-5-12 months of age. *Pediatr Infect Dis J* 1998; 17: 1026-33.

Cherry J. Comparative efficacy of acellular pertussis vaccines: an analysis of recent trials. *Pediatr Infect Dis J* 1997; 16(suppl4): S90-6.

Canadian Immunization Guide (CIG). 7th ed. 2006 Part 3. Recommended Immunization Public Health Agency of Canada. 2006. [Cited 2009 Jan 30]. Available from: URL: http://www.phac-aspc.gc.ca/publicat/cig-gci/p03-01-eng.php

Decker MD. Principles of pediatric combination vaccines and practical issues related to use in clinical practice. *Pediatr Infect Dis J* 2001; 20(11 suppl): S10-18.

Dodd D. Benefits of combination vaccines: effective vaccination on a simplified schedule. *Am J Managed Care* 2003; 9 (1 suppl) S6-12.

Edwards KM, Decker M. Pertussis vaccines. In: Plotkin SA, Orenstein WA, eds. Vaccines. 5th ed. Philadelphia PA: Saunders, 2008; 21: 467-517.

Gustafsson L, Hessel L, Storsaeter J, Olin P. Long-term follow-up of Swedish children vaccinated with acellular pertussis vaccines at 3, 5, and 12 months of age indicates the need for a booster dose at 5 to 7 years of age. *Pediatrics* 2006; 118: 978-84.

Hessel L, Mast CH, Teyssou R. Effectiveness of the 2-component acellular pertussis combination vaccine 5 years experience of routine practice in Sweden [Abstract 18.023]. *Int J Infect Dis* 2004; 8 (suppl1): S75.

Hewlett EL, Cherry JD. New and improved vaccines against pertussis. In: Levine MM, Woodrow GC, Kaper JB, Cobon GS eds. New generation vaccines. 2nd ed. New York, NY: Marcel Dekker, 1997: 387-416.

Institut Vielle Sanitaire (IVS). Vaccination schedule and recommendations from the "Haut conseil de la santé publique" in France. *Bull Èpidémiol Hebdomad* 2009; 16-17:145-75. [Cited 2009 Sept 21]. Available from : URL: http://www.invs.sante.fr/beh/2009/16_17/beh_16_17_2009.pdf

Kalies H, Grote V, Verstraeten T, Hessel L,

Schmitt HJ, von Kries R. The use of combination vaccines has improved timeliness of vaccination in children. *Pediatr Infect Dis J* 2006; 25: 507-12.

Kanra G, Selier T, Yurdakök K, *et al*. Immunogencity study of a combined diphtheria, tetanus, acellular pertussis, inactivated poliomyelitis vaccine used to reconstitute a freeze-dried *Haemophilus influenzae* type b vaccine (DTacP-IPV// PRP-T) administered simultaneously with a hepatitis B vaccine at two, three and four months of life. *Vaccine* 2000; 18: 947-54.

Lagos R, Kotloff, KL Hoffenbach A, *et al*. Clinical acceptability and immunogenicity of a pentavalent parenteral combination vaccine containing diphtheria, tetanus, acellular pertussis, inactivated poliomyelitis and Haemophilus influenzae type b conjugate antigens in two-, four- and six month-old Chilean infants. *Pediatr Infect Dis J* 1998; 17: 294-304.

Lolekha S, Hiranchote A, Simasathien S. Safety and immunogenicity of combined or associated administration of PRP-T vaccine with diphtheria, tetanus and pertussis vaccine in Thai children. *J Trop Pediatr* 2001; 47: 24-9.

Mallet E, Fabre P, Pines E, *et al*. Immunogenicity and safety of a new liquid hexavalent combined vaccine compared with separate administration of reference licensed vaccines in infants. *Pediatr Infect Dis J* 2000; 19: 1119-27.

Mallet E, Hoffenbach A, Salomon H, Blondeau C, Fritzell B. Primary immunization with combined, acellular DTaP-IPV-Act-HIB vaccine given at 2-3-4 or 2-4-6 months of age [Abstract]. Elsinore, Denmark: The 14th European Societies for Paediatric Infectious Diseases, 1996.

Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. *Stat Med* 1998; 17: 857-72.

Olin P, Hallander H. Marked decline in pertussis followed reintroduction of pertussis vaccination in Sweden. *Euro Surveill* 1999; 4: 128-9.

Olsen SJ, Dejsirilert S, Sangsuk L, Chunsutiwat S, Dowell SF. Frequent *Haemophilus influenzae* type B colonization in rural Thailand. *Pediatr Infect Dis J* 2005; 24: 739-42.

Rerks-Ngarm S, Treleaven SC, Chunsuttiwat S, *et al*. Prospective population-based incidence of *Haemophilus influenzae* type b meningitis in Thailand. *Vaccine* 2004; 25; 22: 975-83.

Swedish Institute for Infectious Disease Control (SIIDC). Pertussis surveillance in Sweden with enhanced follow-up of cohorts immunized with acellular pertussis vaccines. 2006; Appendix 2 SP-MSD. [Cited 2009 Jul 28]. Available from: URL: http://www.smitt skyddsinstitutet.se/upload/PDF-filer/seven-year-report-app-2-SP-MSD.pdf

Simondon F, Preziosi MP, Yam A, *et al*. A randomized double-blind trial comparing a two-component acellular to a whole-cell pertussis vaccine in Senegal. *Vaccine* 1997; 15: 1606-12.

Sunakorn P. Current situation of *Hemophilus infuenzae b* diseases in Thailand. *Comm Dis J* 1996; 22: 301-7.

Vidor E, Plotkin SA. Immunogenicity of a two-component (PT & FHA) acellular pertussis vaccine in various combinations. *Hum Vaccines* 2008; 4: 328-40.

World Health Organization (WHO). Vaccine preventable diseases monitoring system: Country profiles. 1996. [Cited 2009 Jul 15]. Available from: URL: http://www.who.int/vaccines/globalsummary/immunization/countryprofileselect.cfm

World Health Organization (WHO). United Nations prequalified vaccines (WHO list of vaccines for purchase by UN agencies as of September 2009). 2009 [Cited 2009 Jul 29]. Available from: URL: http://www.who.int/immunization_standards/vaccine_quality/pq_suppliers/en/index.html

World Health Organization (WHO). Report of

the 21st Meeting of the European Regional Certification Commission for Poliomyelitis Eradication: Copenhagen, Denmark, 9-11 June 2008a. [Cited 2009 Jul 22]. Available from: URL: http://www.who.int/immunization/sage/5_EURO_Polio_RCC_Report.pdf

WHO Global Polio Eradication Initiative – Strategic Plan 2009-2013 Framework for the new GPEI Strategic Plan 2009-2013. 2008b. [Cited 2009 Jul 22]. Available from: URL: http://www.polioeradication.org/content/publications/PolioStrategicPlan09-13_Framework.pdf

World Health Organization (WHO). Thailand 2007 EPI Fact Sheet. 2007. [Cited 2009 Jul 26]. Available from: URL: http://www.searo.who.int/vaccine/LinkFiles/EPI2007/Thailand07.pdf

World Health Organization. The WHO position paper on *Haemophilus influenzae* type b conjugate vaccines. *Weekly Epidemiol Rec* 2006a; 81: 445-52.

World Health Organization (WHO). Inactivated poliovirus vaccine following oral poliovirus vaccine cessation. *Weekly Epidemiol Rec* 2006b; 81: 137-44.

World Health Organization (WHO). Pertussis vaccines. WHO position paper. *Weekly Epidemiol Rec* 2005; 80: 31-9.

World Health Organization. Expanded programme on immunization. Global advisory group-part 1. *Weekly Epidemiol Rec* 1992; 67: 11-5.