LONG-TERM IMMUNOGENICITY ASSESSMENT OF A DTaP-IPV//PRP-T VACCINE GIVEN AT 2, 4, 6 AND 18-19 MONTHS OF AGE, AND IMMUNOGENICITY AND SAFETY OF A DTaP-IPV VACCINE GIVEN AS A BOOSTER DOSE AT 4 TO 6 YEARS OF AGE IN THAI CHILDREN

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Abstract. Booster vaccination of infants aims to further reduce the burden of childhood infectious diseases. This study assessed the antibody persistence induced by a primary series vaccination at 2, 4, 6 months of age and a first booster at 18-19 months of age with a pentavalent diphtheria, tetanus, acellular pertussis, inactivated poliovirus, *Haemophilus influenzae* type b combined vaccine (DTaP-IPV//PRP-T) in 4-6 year-old Thai children (*N*=123). The safety and immunogenicity of a tetravalent acellular pertussis combined vaccine (containing the same DTaP-IPV antigens as the previous vaccine) given as a second booster at 4 to 6 years of age was also evaluated. Seroprotective antibody levels against diphtheria (≥0.01 IU/ml), tetanus (≥0.10 IU/ml), and polioviruses (≥8 1/dil) were maintained 4-6 years after primary-vaccination and first booster by ≥92.7% of children, and anti-pertussis antibodies ≥5 EU/ml were observed in the majority of children. The second booster with DTaP-IPV elicited a strong response for all antigens. GMT or GMC ratios for all antigens at the pre- and post-booster samples were from 4.7 to 52.5. Primary vaccination at 2, 4, 6 and a booster at 18-19 months of age with the DTaP-IPV//PRP-T vaccine induced satisfactory antibody persistence at 4-6 years of age. A second booster with DTaP-IPV induced a strong immune response and was well tolerated.

Keywords: pentavalent combined vaccine, tetravalent combined vaccine, acellular pertussis, booster vaccination, inactivated polio vaccine, Hib-conjugate vaccine, safety, immunogenicity

INTRODUCTION

The diphtheria, tetanus, and whole-cell pertussis (DTwP) vaccine was the first combined vaccine to include a pertussis antigen and has been routinely administered in many countries in combination with inactivated poliomyelitis virus vaccine (IPV) for more than 30 years. The reactogenic profile of whole-cell pertussis vaccines led vaccine manufacturers to develop acellular pertussis vaccines (aP) that contain purified, well characterized

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antigens that have improved tolerability and safety while maintaining the effectiveness of whole cell vaccines.

Poliomyelitis is caused by three serotypes of poliovirus (type 1, 2, and 3) and primarily affects children. Mass vaccination has led to three major regions of the world now declared polio-free, although global eradication has not yet been achieved (GPEI, 2011). Vaccine-associated paralytic poliomyelitis (VAPP) and vaccine-derived polioviruses (VDPV) are risks associated with the continued use of oral polio vaccine (OPV) (Kew *et al*, 2005). Outbreaks caused by circulating vaccine-derived poliovirus (cVDPV) have occurred worldwide (CDCP, 2007). Although both VAPP and VDPV are rare, once wild poliovirus transmission has been interrupted by OPV the only remaining poliomyelitis disease will be caused by OPV (WHO, 2006). The injectable inactivated poliovirus (IPV) vaccine is not associated with the risk of paralytic poliomyelitis, and is now widely used (CDCP, 2001; WHO, 2003; Bonnet and Dutta, 2008).

Combination vaccines offer protection against multiple diseases with a single injection, increasing compliance with complex vaccination schedules. Use of combination vaccines increases vaccine coverage and the timeliness of vaccination, allowing a broader impact of vaccination benefits and better control of disease (Kalies *et al*, 2006). Sanofi Pasteur has developed several combination vaccines that include a liquid combination of D, T, aP, and IPV (DTaP-IPV) that can be administered alone, or used to reconstitute a *Haemophilus influenzae* type b (Hib) vaccine consisting of polyribosyl ribitol phosphate capsular polysaccharide conjugated to tetanus protein (PRP~T). These combined DTaP-IPV and DTaP-IPV//PRP~T vaccines have been licensed

since 1997 in more than 100 countries throughout the world with the AcXim family trade names Tetravac™/Tetraxim™ and Pentavac™/Pentaxim™, respectively. The approved indication for both is for a 3-dose primary vaccination series (during the 1st year of life) and/or booster vaccination (during the 2nd year of life). According to country recommendations, Tetravac™/Tetraxim™ is indicated for booster vaccination at 4 to 12 years of age. The IPV (Imovax™ Polio) and PRP-T (ActHIB ™) valences are WHO pre-approved as standalone vaccines (WHO, 2011).

To improve the duration of protection following primary vaccination, the World Health Organization (WHO) recommends a pertussis booster dose for children aged 1 to 6 years given at least 6 months after the last primary dose and preferably during the second year of life (WHO, 2010). The timing of this booster also provides an opportunity for catch-up vaccination and allows for the use of a combination vaccine containing pertussis, polio and Hib antigens. Completion of this schedule (primary series plus booster) is expected to ensure protection against pertussis for at least 6 years. The waning of vaccine-induced and naturally acquired immunity underlies this and other booster recommendations. A 3-dose primary immunization series with a booster dose in the second year is recommended in nearly every country in the Americas, Europe, the Middle East and Africa. Combination vaccines containing DTaP (full dose diphtheria antigen) are the standard of care for pre-school boosters. Pre-school booster doses are also widely recommended in the Americas, Central and Eastern Europe and the Middle East and an increasing number of countries now recommend adolescent and adult pertussis boosters (Guiso *et al*, 2011). When new vaccines and

additional doses are adopted by national immunization programs, a long-term follow-up of antibody persistence is important for epidemiological reasons and to determine the safety, immunogenicity, necessity, and timing of booster doses.

IPV immunization schedules vary widely among countries. Selected IPV schedules integrated into existing national immunization schedules range from four to seven doses, *eg*, at 2, 4, 6 - 18 months, 4 - 6 years (United States); 2, 4, 6, 12 - 18 months, 4-6 years (Canada); 2, 3, 4, 12 - 18 months, 6, 11, 16 years (France); 3, 5, 12 months, 6 years (Sweden); and 3, 4, 5, 12 months, 4, 9 years (Netherlands) (Plotkin and Vidor, 2009). According to expert opinion (Rennels, 2009), the possibility of exposure to either wild or vaccine-derived poliovirus is a risk in all parts of the world. Booster doses of IPV should be continued either until long-term antibody persistence has been demonstrated without additional doses after the second year of life, or until better world-wide control is achieved.

This study assessed the antibody persistence at 4-6 years of age, following a 3-dose primary series vaccination at 2, 4 and 6 months of age (Thisyakorn *et al*, 2010) and a booster vaccination at 18 to 19 months of age, with the DTaP-IPV//PRP-T vaccine (Pentaxim) (Chotpitayasunondh *et al*, 2012). We also report the immunogenicity and safety of the DTaP-IPV combined vaccine (Tetraxim) administered as a booster dose during the 4th to 6th year of life in the same group of children.

MATERIALS AND METHODS

Study design and participants

This Phase IV open study (Clinical-trials.gov NCT01031303) enrolled participants at King Chulalongkorn Memorial Hospital and Queen Sirikit National Insti-
tute of Child Health, Bangkok, Thailand to receive a booster dose of the DTaP-IPV vaccine. The institutional review committee at each center approved the study protocol. The study was conducted in accordance with the Edinburgh revision of the Declaration of Helsinki, 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 parents or legal representatives before inclusion in the study.

Eligible children were aged 4 to 6 years and had previously received a DTaP-IPV//PRP-T vaccine (Pentaxim, Sanofi Pasteur, Lyon, France) as a 3-dose primary series vaccination at 2, 4 and 6 months of age and a booster with the same vaccine at 18-19 months of age (ClinicalTrials.gov ID: NCT00255021) (Thisyakorn *et al*, 2010; Chotpitayasunondh *et al*, 2012).

The objectives of the present study were to measure antibody persistence just before administering the DTaP-IPV booster, to calculate seroprotection SP rates (diphtheria, tetanus, poliovirus) and seroconversion/vaccine response rates (PT and FHA) 1 month after the booster dose, and to evaluate the reactogenicity and safety of the DTaP-IPV booster vaccine.

Children were excluded if they had participated in a clinical trial during the preceding 4 weeks or planned to participate in another clinical trial during the conduct of this one; had congenital or acquired immunodeficiency; were receiving immunosuppressive therapy; had systemic hypersensitivity to any of the vaccine components or history of a life threatening reaction to the trial vaccine or a vaccine containing the same substances; had a chronic illness that could interfere with trial completion; had received or planned to receive blood or blood prod-

ucts during the trial; had received any vaccines during the preceding 4 weeks (except oral polio vaccine during a National Immunization Days campaign); had a history of diphtheria, tetanus, pertussis, poliomyelitis infection or systemic illness including hepatitis B, hepatitis C and/or HIV infection; had a bleeding disorder contraindicating intramuscular vaccination, had a history of neurological diseases or seizures; were febrile (temperature ≥38°C) or had an acute illness on the day of inclusion; or had a serious or severe reaction after a previous dose of any vaccine containing a pertussis antigen.

Vaccine

The DTaP-IPV combined vaccine (Tetraxim™, batch D0502-1) 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 g of pertussis toxoid (PT), 25 g of filamentous hemagglutinin (FHA), 40 D antigen units (DU) of poliovirus type 1 (Mahoney), 8 DU of poliovirus type 2 (MEF-1), 32 DU of poliovirus type 3 (Saukett) adsorbed onto 0.30 mg of aluminum hydroxide. The vaccine antigens were the same as those included in the primary and booster vaccinations with the DTaP-IPV//PRP~T combined vaccine and described in previous publications (Plotkin *et al*, 2011). The vaccine was administered intramuscularly into the anterolateral aspect of the right upper thigh.

Serology

Blood samples for antibody determination were taken just before and 1 month after the booster dose. The immunological assays were performed at Sanofi Pasteur's Global Clinical Immunology Laboratory in Swiftwater, Pennsylvania, USA. Anti-tetanus toxoid (IU/ml), anti-

FHA and anti-PT antibody (EU/ml) were measured by enzyme linked immunosorbent assay (ELISA). Anti-diphtheria antibody titers (IU/ml) and anti-poliovirus antibody titers (1/dil) were assessed by seroneutralization. The methods used for antibody determinations were the same as for the previous primary vaccination study (Thisyakorn *et al*, 2010). Pre-defined seroprotection (SP) levels were: anti-PRP ≥0.15 and 1.0 g/ml; anti-polio ≥8 (1/dil); anti-diphtheria and anti-tetanus ≥0.01 and 0.1 IU/ml. Seroconversion (SC) rates to pertussis antigens were assessed using ≥4-fold increases in antibody concentration from pre- to post-booster vaccination.

Reactogenicity and safety

Infants were monitored for 30 minutes after injection for immediate local or systemic reactions. In the week following vaccination, parents or legal guardians recorded the onset, duration and grade (1-3) of solicited injection site (pain, redness and swelling) and systemic [fever (axillary temperature ≥38°C), headache, malaise, and myalgia] reactions on diary cards. For injection site reactions, Grade 3 for pain was defined as preventing normal activity and for erythema and swelling, a diameter <2.5 cm was Grade 1, 2.5 to <5 cm was Grade 2 and ≥5 cm was Grade 3. For systemic reactions, Grade 3 headache/malaise/myalgia was defined as preventing daily activity and Grade 3 fever was defined as axillary temperature ≥39°C. Unsolicited adverse events were recorded for 30 days after vaccination and serious adverse events (SAEs) were assessed and reported throughout the trial.

Statistical analysis

Seroprotection and SC rates were calculated with 95% confidence intervals (CI) using the exact binomial method (Newcombe, 1998b). Geometric mean

titers (GMTs: polio 1, 2, 3) or geometric mean concentrations (GMCs: D, T, PT, FHA) were calculated with 95% CIs using normal approximation (Newcombe, 1998a). Reverse Cumulative Distribution Curves (RCDCs) were derived for antibody concentrations and titers before and after booster vaccination. Post- and pre-booster GMTs (or GMCs) and GMT (or GMC) ratios (GMRs: post-/pre-booster) with 95% CIs were calculated. For evaluation of reactogenicity, the number and percentage (with 95% CI) of subjects reporting a given symptom after vaccination were calculated. All subjects who received the booster vaccine were included in the safety evaluation. The post-booster immunogenicity analysis set included subjects given the booster vaccination, and with sera obtained according to protocol. The statistical analysis was descriptive; no hypothesis was tested.

RESULTS

Subjects

A total of 123 participants were included at the two study centers from December 2009 to May 2010. All had received a primary series vaccination and booster at 18-19 months of age with the DTaP-IPV-PRP~T vaccine. Forty-one participants received OPV during a National Immunization Days campaign either before (n=8) or after (n=33) the booster vaccine in the present study. Immunogenicity (Immunogenicity Analysis Set) and safety (Safety Analysis Set) data were available from all 123 participants. The median age was 4.1 years and there were more male (59.3%) than female (40.7%) subjects. All 123 participants completed this study.

Immunogenicity

The SP/SC rates, GMCs, GMTs and GMRs with 95% CIs for each vaccine an-

tigen are shown in Table 1. Just before the booster dose, 99.2% of the children had protective concentrations of antibodies to tetanus ($\geq$0.10 IU/ml), 100% had anti-poliovirus titers $\geq$8 l/dil, and 92.7% had anti-diphtheria concentrations $\geq$0.01 IU/ml. However, antibody concentrations and titers had decreased since the first booster, such that 60.2% of participants had anti-diphtheria concentrations $\geq$0.10 IU/ml and anti-PT and -FHA concentrations were 10.9 and 24.3 EU/ml, respectively. Prior to the second booster, 84.6, 56.9, and 22.0% of participants had anti-PT concentrations and 90.2, 81.1, and 48.4% of subjects had anti-FHA concentrations $\geq$5 EU/ml, $\geq$10 EU/ml, and $\geq$25 EU/ml, respectively (not included in Table 1).

Following booster vaccination, SP rates for diphtheria and tetanus ($\geq$0.1 IU/ml) and polioviruses ($\geq$8 1/dil) were 100% for both participants who received OPV and those who had not. At least 92.6% of participants had seroconverted ($\geq$4-fold increase in antibody titer) against the PT and FHA antigens. GMCs increased strongly following booster administration, from 10.9 to 190 EU/ml for anti-PT and from 24.3 to 356 EU/ml for anti-FHA (GMR=17.4 and 14.6, respectively). Strong increases were also observed for the other antigens. Comparisons of 95% CI for GMTs or GMCs after booster vaccination did not indicate any differences between participants who had received OPV compared with those who had not (Table 2). Reverse cumulative distribution curves showed large, linear increases in antibody titers from pre- to post-booster vaccination for anti-PT and anti-FHA (Fig 1).

Reactogenicity and safety

No immediate AEs were reported. Of the 123 vaccinated subjects, 107 (87.0%) experienced at least 1 solicited reaction and 40 (32.5%) experienced at least 1

Table 1

Immunogenicity: seroprotection, seroconversion rates, GMCs, GMTs and GMRs pre- and post-booster administration at 4 to 6 years of age.

| | DTaP-IPV (N=123) | | | | |
| | Pre-booster | | Post-booster | | |
	SP or SC % (95% CI)	GMC or GMT	SP or SC % (95% CI)	GMC or GMT	GMR (95% CI)
Anti-Diphteria ≥0.01 IU/ml	92.7 (86.6-96.6)	0.15 (0.109-0.204)	100.0 (97.0-100.0)	7.81 (6.47-9.43)	52.5 (42.5-64.9)
Anti-Diphteria ≥0.1 IU/ml	60.2 (50.9-68.9)	-	100.0 (97.0-100.0)	-	11.6 (10.0-13.5)
Anti-Tetanus ≥0.01 IU/ml	100.0 (97.0-100.0)	0.65 (0.556-0.769)	100.0 (97.0-100.0)	7.72 (6.83-8.72)	
Anti-Tetanus ≥0.1 IU/ml	99.2 (95.5-100.0)	-	100.0 (97.0-100.0)	-	
Anti-Polio 1 ≥8 1/dil All	100.0 (97.0-100.0)	583 (467-729)	100.0 (97.0-100.0)	3,013 (2,631-3,450)	5.14 (4.08-6.46)
received OPV	100.0 (71.5-100.0)	563 (296-1,070)	100.0 (71.5-100.0)	2,719 (1,752-4,221)	4.83 (1.91-12.2)
did not receive OPV	100.0 (96.7-100.0)	586 (461-744)	100.0 (96.8-100.0)	3,043 (2,634-3,516)	5.17 (4.07-6.57)
Anti-Polio 2 ≥8 1/dil) All	100.0 (97.0-100.0)	714 (566-900)	100.0 (97.0-100.0)	3,430 (2,969-3,963)	4.77 (3.82-5.96)
received OPV	100.0 (71.5-100.0)	875 (406-1,885)	100.0 (71.5-100.0)	3,285 (1,835-5,881)	3.76 (1.79-7.89)
did not receive OPV	100.0 (96.7-100.0)	700 (547-895)	100.0 (96.8-100.0)	3,444 (2,961-4,006)	4.88 (3.85-6.19)
Anti-Polio 3 ≥8 1/dil All	100.0 (97.0-100.0)	481 (374-619)	100.0 (97.0-100.0)	3,837 (3,261-4,515)	8.26 (6.33-10.8)
received OPV	100.0 (71.5-100.0)	724 (315-1,666)	100.0 (71.5-100.0)	2,896 (1,758-4,772)	4.00 (1.55-10.3)
did not receive OPV	100.0 (96.7-100.0)	462 (354-603)	100.0 (96.7-100.0)	3,945 (3,318-4,692)	8.88 (6.72-11.7)
Anti-PT (EU/ml) ≥4-fold increase	-	10.9 (9.11-13.1)	97.6 (93.0-99.5)	190 (168-216)	17.4 (15.1-20.0)
Anti-PT (EU/ml) ≥2-fold increase	-	-	99.2 (95.6-100.0)	-	
Anti-FHA (EU/ml) ≥4-fold increase	-	24.3 (19.7-29.9)	92.6 (86.5-96.6)	356 (314-405)	14.6 (12.3-17.3)
Anti-FHA (EU/ml) ≥2-fold increase	-	-	99.2 (95.5-100.0)	-	

Study population: DTaP-IPV vaccine at 4 to 6 years of age. All had been primed at 2, 4, 6 months; and boosted at 18-19 months, of age with DTaP-IPV//PRP-T vaccine.

SP, seroprotection; SC, seroconversion; OPV, oral polio vaccine; GMT, geometric mean titer, GMC, geometric mean concentration, GMR, Post-to Pre-booster GMC or GMT ratio

Table 2
Participants reporting at least one solicited reaction in the week after vaccine injection.

Participants with at least one:	Intensity	DTaP-IPV (*N*=123)		
		n/M	*%*	(95% CI)
Injection site reaction	Any	101/123	82.1	(74.2-88.4)
	Grade 3	6/123	4.9	(1.8-10.3)
Pain	Any	93/123	75.6	(67.0-82.9)
	Grade 3	1/123	0.8	(0.0-4.4)
Erythema	Any	60/123	48.8	(39.7-58.0)
	Grade 3	5/123	4.1	(1.3-9.2)
Swelling	Any	45/123	36.6	(28.1-45.7)
	Grade 3	3/123	2.4	(0.5-7.0)
Systemic reaction	Any	68/123	55.3	(46.1-64.3)
	Grade 3	1/123	0.8	(0.0-4.4)
Fever	Any	13/123	10.6	(5.7-17.4)
	Grade 3	1/123	0.8	(0.0-4.4)
Headache	Any	30/123	24.4	(17.1-33.0)
	Grade 3	0/123	0.0	(0.0-3.0)
Malaise	Any	40/123	32.5	(24.4-41.6)
	Grade 3	0/123	0.0	(0.0-3.0)
Myalgia	Any	54/123	43.9	(35.0-53.1)
	Grade 3	0/123	0.0	(0.0-3.0)

n: number of subjects experiencing the endpoint listed in the first column
M: number of subjects with available data for the relevant endpoint
Any, all cases, irrespective of intensity. Any fever, axillary temperature ≥38.0ºC
Grade 3 Pain, prevents performance of usual activities; Grade 3 Erythema/Swelling, longest d ≥5 cm; Grade 3 Fever, axillary temperature ≥39.0ºC; Grade 3 Headache/Malaise/Myalgia, prevents daily activity

unsolicited AE. Injection site reactions were reported by 101 participants (82.1%); systemic reactions by 68 (55.3%). The incidence of solicited reactions is presented in Table 2. The most frequent injection site reaction was pain, reported by 75.6% of participants, followed by erythema and swelling, which were reported in 48.8% and 36.6% of subjects, respectively. Grade 3 injection site reactions were observed in 4.9% of subjects (1 participant with injection site pain, 5 with erythema, and 3 with injection site swelling). The most frequently reported systemic reaction was myalgia (43.9% of subjects); followed by malaise (32.5% of subjects), headache (24.4%), and fever (10.6%). Most solicited reactions were Grades 1 and 2, occurred within 3 days after vaccination and were transient. Overall, Grade 3 systemic solicited reactions were observed in 0.8% of participants, with only one report of Grade 3 fever.

A total of 40 subjects (32.5%) experienced at least 1 unsolicited AE within 30 days after booster vaccination. The most frequently reported unsolicited AEs were upper respiratory tract infections (22.8% of participants). Four subjects (3.3%) reported injection site induration. No Grade 3 unsolicited AE were reported. No hypotonic, hyporesponsive episodes or seizures with
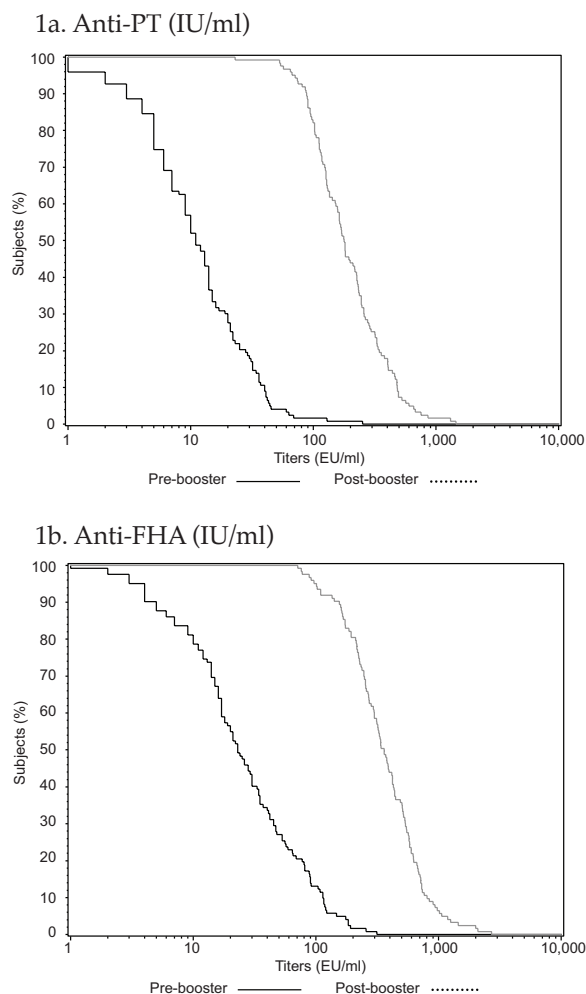
1a. Anti-PT (IU/ml)

1b. Anti-FHA (IU/ml)

Fig 1–Reverse Cumulative Distribution Curves before and 1 month after administration of DTaP-IPV booster at 4-6 years of age for anti-PT and anti-FHA.

or without fever were reported. One subject (0.8%) experienced nasopharyngitis, which was reported as an SAE that was not related to the booster vaccination, and recovered without sequelae. There were no study withdrawals or deaths.

DISCUSSION

This study evaluated the antibody persistence against diphtheria, tetanus, PT, FHA and poliovirus types 1-3 in 4-6-year-old Thai children who had received a primary series vaccination at 2, 4 and 6 months and a booster at 18-19 months of age with a DTaP-IPV//PRP-T combination vaccine. The immunogenicity and safety of a second booster with a DTaP-IPV vaccine, containing the same diphtheria, tetanus, pertussis and poliovirus antigens used for the primary vaccination and first booster, were also assessed. Overall, the antibody persistence was high, and the majority of participants were still protected against the vaccine antigens when the second booster was administered.

As expected, and consistent with published data from other vaccines, antibody concentrations and titers had decreased in the interval between first and second booster administration. However, SP rates against polioviruses (≥ 8 1/dil), tetanus and diphtheria (≥ 0.01 IU/ml) remained very high. For tetanus the SP rate at a ≥ 0.1 IU/ml level also remained high, but only 60.2% of children had anti-diphtheria titers ≥ 0.1 IU/ml and anti-PT and -FHA concentrations had fallen to levels comparable to those observed prior to administration of the first booster (Chotpitayasunondh *et al*, 2012). The antibody persistence seen here prior to a second booster was comparable to that reported in other studies in children given either a wP vaccine (Langue *et al*, 2004), or the DTaP-IPV//PRP-T vaccine at 2, 4 and 12-16 months (Mallet *et al*, 2004) or 2, 4, 6 and 12 or 3, 5 and 12 months of age (Carlsson *et al*, 2002). The low anti-PT and -FHA concentrations suggest children immunized with the DTaP-IPV//PRP-T vaccine were probably not naturally boosted by *B. pertussis* colonization in the years following vaccination. Overall, the study results suggest most of the children who participated in this study were still

protected against pertussis when the second booster was given.

One month after the second booster with the combined DTaP-IPV vaccine, all children had high circulating antibody levels, far above the thresholds thought to be associated with long-term protection against diphtheria, tetanus and poliomyelitis. High antibody levels were also induced against PT and FHA, with GMCs similar or greater than those achieved 1 month after the first booster given at 18-19 months of age (Chotpitayasunondh *et al*, 2012). The strong anamnestic responses against each antigen indicated a good immune memory response induced by the primary series vaccination and appropriate timing of booster administration in the study population, and are indicative of long-term protection. These data reflect those of previous studies using similar aP or wP-based combined vaccines (Pichichero *et al*, 2000; Meyer *et al*, 2008; Poovorawan *et al*, 2008).

These results and those of others highlight the need for a booster injection at 4-6 years of age to reinforce humoral immunity, particularly against pertussis and diphtheria. A sero-epidemiologic study in France showed children became susceptible to pertussis infection at about 6 years after their last vaccination (Grimprel *et al*, 1996). Without a second booster vaccination, anti-PT, and other pertussis antibody concentrations decreased rapidly after the fourth injection at 18 months of age but increased approximately 80 months after vaccination, suggesting persistent circulation of *B. pertussis* and exposure to infected persons.

A comparison of pertussis-specific humoral and cellular immunity in children 5 years after vaccination at 3, 5, 11 and 21 months of age to immunity following natural infection showed the immune responses induced by pertussis vaccination were similar to those in children who recovered from natural infection, and highlighted the need for booster immunization in order to maintain a specific immune response against *B. pertussis*. At 5 years after either vaccination or infection, only a minority of children had significant anti-pertussis serum antibodies or T-cell responses (Esposito *et al*, 2001). Another evaluation of immune responses 6 years after pertussis primary and booster vaccination found low anti-PT antibody levels, consistent with ongoing protection and the absence of pertussis disease. Cell-mediated immune responses continued to be present, also indicative of long-term protection (Guiso *et al*, 2007). Although antibody titers induced by pertussis vaccines do not correlate with vaccine efficacy or protection, published data suggest a concentration of 5 EU/ml to pertussis toxin – achieved by 84.6% of study participants before the booster – would be protective (Taranger *et al*, 2000; Plotkin, 2010).

Vaccine effectiveness data from national surveillance supports immunologic findings of clinical trials, indicating that all currently licensed aP-containing vaccines, including the study vaccine, have been effective in preventing and controlling pertussis (Bonmarin *et al*, 2007; Rendi-Wagner *et al*, 2007; Carlsson and Trollfors, 2009; Hellenbrand *et al*, 2009). The long-term impact of aP vaccines (including Pentaxim) on pertussis incidence has been documented over 11 years by the National Surveillance Program in Sweden, with a 3, 5, 12 month schedule (Swedish Institute for Infectious Disease Control, 2009). Routine vaccination with aP vaccines resulted in a marked decrease in pertussis incidence compared to no vaccination, with continuing protection for 5 to 7 years after the third (booster) dose

(Gustafsson *et al*, 2006). Epidemiologic data have led to adoption of a booster at this age in Germany (Hellenbrand *et al*, 2009), Austria (Rendi-Wagner *et al*, 2007), and other countries in Central and Eastern Europe and the Middle East (Guiso *et al*, 2011), and a recent recommendation by the WHO (2010).

Immunization schedules for IPV vary widely among countries but generally include 2 or 3 doses during the first year of life and at least one booster dose 6-12 months after the last dose of the primary series. The anti-poliovirus antibody persistence and strong IPV booster response observed in our study provide additional immunogenicity data to support IPV administration in a non-western country. The compatibility of IPV and OPV seen in this study is consistent with the use of an OPV/IPV mixed or sequential schedule in case a switch to IPV is contemplated (Bonnet and Dutta, 2008). Booster doses of IPV should be continued either until long-term antibody persistence has been demonstrated without additional doses after the second year of life, or until better worldwide control is achieved. Inclusion of IPV in a DTaP combination vaccine, as in this study, assures vaccination coverage as high as that for the pertussis booster.

As expected for childhood combination vaccines, the incidence of common, solicited adverse reactions tended to be slightly higher with the second booster dose as compared to the first booster or primary vaccination (Plotkin *et al*, 2011). The overall reactogenicity of the study vaccine booster was satisfactory. Grade 3 injection site pain, erythema and swelling were observed in 0.8%, 4.1% and 2.4% of children. The incidence of solicited reactions was comparable to that reported in previous trials with this DTaP-IPV vaccine as a second booster in 4-6-year-old children (Langue *et al*, 2004; Mallet *et al*, 2004). The reactogenicity was in accord with previous clinical trials, which have consistently shown the investigational aP vaccine, as well as other aP vaccines, are less reactogenic than wP vaccines (Plotkin *et al*, 2011) and the frequency of solicited reactions, particularly injection site reactions, increases with age and with the number of doses (Edwards and Decker, 2008). In our study there was no extensive limb swelling or other types of extensive injection site reaction.

In conclusion, the primary vaccination and a booster dose of DTaP-IPV-PRP-T vaccine induced satisfactory persistence of antibodies at 4-6 years of age, and the second booster with DTaP-IPV vaccine was generally well tolerated. As with other AcXim vaccines, a strong immune and anamnestic response was observed for each antigen 1-month post-2nd booster dose, thus long-term protection is expected after this booster dose.

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