RESEARCH PAPER

Immunogenicity and Safety of a Pentavalent Diphtheria, Tetanus, Acellular Pertussis, Inactivated Poliovirus, *Haemophilus influenzae* Type b Conjugate Combination Vaccine (Pentaxim™) with Hepatitis B Vaccine

AK Dutta, VP Verghese*, HK Pemde, LG Mathew* and E Ortiz†

From Lady Hardinge Medical College and Associated Hospitals, New Delhi, India; Christian Medical College Hospital, Vellore, Tamil Nadu, India; and, †Sanofi Pasteur, Lyon, France.*

Correspondence to: Esteban Ortiz, Global Scientific and Medical Affairs, Sanofi Pasteur, 2 Avenue Pont Pasteur, 69007, Lyon, France. esteban.ortiz@sanofipasteur.com

Manuscript received: April 13, 2009; Initial review: May 12, 2009; Accepted: September 30, 2009.

Objective: To obtain immunogenicity and safety data for a pentavalent combination vaccine (diphtheria, tetanus, acellular pertussis, inactivated poliovirus, Hib polysaccharide-conjugate).

Design: Multicenter, open, Phase III clinical study. A DTaP-IPV//PRP~T vaccine (Pentaxim™) was given at 6,10,14 weeks of age; and Hepatitis B vaccine at 0,6,14 or at 6,10,14 weeks of age. Immunogenicity assessed 1 month post-3rd dose; safety assessed for 30 minutes by the investigator, then by parents and investigators to 8 days and 30 days post-vaccination.

Setting: Tertiary-care hospitals.

Participants/patients: 226 healthy Indian infants (6 weeks of age).

Main outcome measures: Immunogenicity and safety.

Results: Immunogenicity was high for each vaccine antigen, and similar to a historical control study (France) following a 2,3,4 month of age administration schedule. Post-3rd dose, 98.6% of subjects had anti-PRP ≥0.15 mg/mL and 90.0% had titers ≥1.0 mg/mL; the anti-PRP GMT was 4.1 µg/mL. Seroprotection rates for diphtheria and tetanus (≥0.01 IU/mL) were 99.1% and 100%; and 100%,99.1% and 100%, for polio types 1,2 and 3 (≥8 [1/dil]) respectively. Anti-polio GMTs were 440.5,458.9, and 1510.7 (1/dil) for types 1,2 and 3 respectively. The vaccine response rates to pertussis antigens (4-fold increase in antibody concentration) were 93.7% for PT and 85.7% for FHA; the 2-fold increase was 97.1% and 92.4%. Vaccine reactogenicity was low with adverse reaction incidence not increasing with subsequent doses.

Conclusion: The DTaP-IPV//PRP~T vaccine, given concomitantly with monovalent hepatitis B vaccine, was highly immunogenic at 6, 10 and 14 weeks of age in infants in India. The vaccine was well tolerated.

Keywords: *pentavalent combined vaccine, primary series, EPI schedule, immunogenicity, safety.*

ClinicalTrials.gov identifier: NCT00259337

Vaccines combining whole cell *Bordetella pertussis* (wP) antigens with diphtheria (D) and tetanus (T) toxoids (DTwP) have been central to the WHO expanded program of immunization (EPI). The valences included in modern combination vaccines reflect current EPI recommendations. In India, DTwP has been a part of the Universal Immunization Program (UIP) since 1985, and the Indian Academy of Pediatrics (IAP) recommends Hib vaccination and inactivated polio vaccine (IPV) for all children who can afford it after one-to-one discussion with parents(1). The use of combination vaccines reduces the number of required injections, the number of clinic visits and the discomfort for children. A number of DTwP-based combination vaccines containing Hib have recently been evaluated in India(2).

In the context of the World Health Organisation (WHO) recommendation to cease OPV use in the

post-eradication era to minimise the impact of circulating vaccine-derived polioviruses (cVDPVs) and vaccine-associated paralytic poliomyelitis (VAPP), the shift towards the use of IPV is vital(3,4), and its incorporation in combination vaccines is an important step.

Acellular pertussis (aP) vaccines containing purified *B. pertussis* antigens are better tolerated than wP vaccines(5). Combination vaccines incorporating an aP component have become widely adopted over the last 10 years, and are now included in the national immunization programs in North America, most western European countries, some Asian countries, Mexico, Turkey and South Africa(6).

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''(7). In addition, the WHO position paper on aP vaccines states that ''although most efficacy and effectiveness studies on aP vaccines have been conducted in industrialized countries, the new DTaP vaccines are expected to be efficacious in all regions of the world.''

Various aP combination vaccines have been developed, including a liquid DTaP-IPV combination used to reconstitute a lyophilized Hib conjugate vaccine (PRP~T) at the time of injection, which was used in the present study. Each antigen is well-known as a stand-alone vaccine; the PRP~T and IPV vaccines are licensed worldwide including in India and both are WHO pre-qualified(8). The DTaP-IPV//PRP~T vaccine has been licensed since 1997 as Pentaxim™/Pentavac™ in >85 countries worldwide, including India in 2007. The exclusion of a hepatitis B (Hep B) component from this pentavalent vaccine affords greater flexibility in the administration schedule of the Hep B vaccine.

The present study was carried out to obtain immunogenicity, reactogenicity and safety data in Indian subjects following administration of this DTaP-IPV//PRP~T pentavalent vaccine in the EPI immunization schedule (6,10,14 weeks of age) with a separate Hep B vaccine being administered at either 0,6,14 or 6,10,14 weeks of age, according to the IAP recommendations for immunization in India.

METHODS

Study Design

This prospective, non-comparative, Phase III, open clinical study was performed at two medical centers in India - Lady Hardinge Medical College and Associated Hospitals in New Delhi and Christian Medical College Hospital, Vellore, Tamil Nadu. The study protocol and consent form were approved by the relevant institutional review boards before study initiation and the study conformed to local regulations, GCP and applicable ICH guidelines, and the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from a parent/legal guardian of each subject before enrolment.

Subjects

Healthy fullterm (≥37 weeks) infants weighing ≥2.5 kg at birth were eligible. Each subject received the DTaP-IPV//PRP~T study vaccine (6,10,14 weeks of age) and hepatitis B (Hep B) vaccine (0,6,14 or at 6,10,14 weeks of age) according to the Indian National Immunization schedule and the clinical practice in each study center. A physical examination and medical review were performed before enrolment, and the inclusion and exclusion criteria were verified. Exclusion criteria included congenital or acquired immunodeficiency; immunosuppressive therapy; systemic hypersensitivity to any vaccine component; chronic illness that could interfere with trial conduct/completion; previous administration of blood or blood-derived products; any vaccination preceding the trial vaccination (except Bacille Calmette-Guérin [BCG] and hepatitis B vaccination); history of, or vaccination against, diphtheria, tetanus, pertussis, poliomyelitis, Hep B or Hib; thrombocytopenia/bleeding disorder contraindicating intramuscular vaccination; and, history of seizures.

Vaccines

The pentavalent vaccine (batch Z2044-1, Pentaxim™) was produced and supplied by Sanofi

Pasteur, France, and stored between 2ºC and 8ºC. Each 0.5 mL dose of DTaP-IPV//PRP~T contained ≥30 IU (25Lf) of diphtheria toxoid, ≥40 IU (10Lf) of tetanus toxoid, 25 µg of pertussis toxoid (PT), 25 µg 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), and 10 µg polyribosyl-ribitol-phosphate (PRP) Hib capsule polysaccharide conjugated to tetanus protein. The lyophilized PRP~T component was reconstituted with the liquid DTaP-IPV vaccine immediately before vaccination. The recombinant Hep B vaccine (Euvax B™, LG Life Sciences, Iksan, Korea, batch UVA05005), contained 10 µg of recombinant HBsAg; this vaccine is licensed and commercially available in India. The pentavalent vaccine and the hepatitis B vaccine were administered by intramuscular injection into the right and left anterior thigh, respectively.

Serology

Blood samples (4 mL) were collected just before the first vaccine dose at 6 weeks of age, and at 18 weeks of age, approximately 1 month after the third vaccination, and serologic analyses were performed at the Sanofi Pasteur central laboratory in Swiftwater, Pennsylvania, USA. Anti-HBs antibody titer determinations were not performed as it was not possible to prospectively plan the proportion of subjects who would receive the hepatitis B vaccination at 0,6,14 weeks or at 6,10,14 weeks of age, due to different Hep B vaccination schedules between study centers.

Reactogenicity and Safety

Safety data were only collected for the pentavalent vaccine. Following an initial assessment by the Investigator of immediate adverse events occurring in the 30 minutes after each vaccination, parents/ legal guardians recorded solicited injection site reactions (redness, swelling and tenderness) and solicited systemic reactions (fever [axillary temperature ≥37.4ºC], vomiting, abnormal crying, drowsiness, loss of appetite and irritability) on diary cards daily for 8 days after each vaccination. Unsolicited injection site and systemic reactions (with onset date, resolution, and intensity) were recorded for 30 days after each vaccination. Serious adverse events (SAEs) were reported throughout the conduct of the study. Solicited and unsolicited local and systemic adverse events were graded according to the scales described below as mild, moderate, or severe. Mild, moderate or severe tenderness were defined as 'minor reaction when injection site is touched', 'cries and protests when injection site is touched', and 'cries when injected limb is moved, or the movement of the injected limb is reduced'. For erythema and swelling, a diameter of <2.5 cm was graded as mild, 2.5 to 5 cm as moderate and ≥5 cm as severe. Mild, moderate and severe fever were defined as axillary temperature ≥37.4ºC to 37.9ºC, ≥38ºC to 38.9ºC, and ≥39ºC, respectively.

Statistical Analysis

Seroprotection and vaccine response rates were calculated with their corresponding 95% confidence intervals (CI) using the exact binomial method. Pre-defined seroprotection levels were: anti-PRP ≥0.15 and ≥1.0 µg/mL; anti-polio ≥8 (1/dil); anti-diphtheria and anti-tetanus ≥0.01 IU/mL. The pertussis antigens were assessed using ≥4-fold and ≥2-fold increases in antibody concentration from pre- to post-vaccination state.

Geometric mean titers (GMTs) were calculated with 95% CIs using the normal approximation. Reverse Cumulative Distribution Curves (RCDCs) for pre- and post-vaccination antibody titers were also derived. The statistical analysis was descriptive; no hypothesis was tested.

The sample size calculation of 226 subjects allowed an indirect, descriptive comparison with a historical control study conducted in France using the same combined vaccine but given at 2, 3 and 4 months of age(26). A drop-out rate of 20% was assumed in order to ensure at least 180 evaluable subjects.

RESULTS

Study population

A total of 226 infants were enrolled. There were slightly more male (53.1%) than female (46.9%) infants. The mean age (±standard deviation) when

the first vaccine dose was administered was 45.0±2.9 days (about 6.4 weeks) and the mean weight was 4.5±0.5 kg. Ten subjects did not complete the study; six were withdrawn by parents (one due to migration away from the area and five due to personal reasons not related to an adverse event), two were withdrawn because of protocol violations (receipt of oral polio vaccine during the study), and two were lost to follow up. There were no withdrawals because of adverse events.

Immunogenicity

The seroprotection and vaccine response rates for the study vaccine and historical control study are summarized in ***Table* I**. In addition to the criteria presented in ***Table* 1**, 90.0% of subjects had anti-PRP ≥1.0 µg/mL after the third dose, and 97.1% and 92.4% of subjects had ≥2-fold increases in antibody titer for PT and FHA. The tetanus seroprotection rate was very high before the first injection, with 99.5% of subjects having anti-tetanus antibody titers ≥0.01 IU/mL and 97.2% having titers ≥0.1 IU/mL. GMTs increased strongly following the primary vaccination (***Table* II**). ***Fig.*1** shows a strong, linear increase in antibody titers for anti-PRP antibodies (µg/mL); a similar effect was seen for the remaining antigens.

Safety and Reactogenicity

Solicited symptoms after any dose and after each dose are summarized in ***Table* III**. Injection site tenderness was the most common symptom, but was severe only after two injections (0.3% of doses). The incidence of erythema and swelling was also low. All solicited injection site reactions occurred within 3 days after vaccination except one case of erythema/redness that occurred between Days 4 and 7. The percentage of subjects with a specific adverse reaction did not increase with subsequent doses. Fever was the most frequent systemic reaction, with at least one episode being reported by 33.0% of subjects (15.3% of doses), followed by irritability and drowsiness. Only two subjects (0.9%) had severe fever (≥39.0ºC axillary temperature) and only one case of drowsiness was severe.

Overall, 119 subjects (53.1%) reported at least one unsolicited AE following vaccine administration. Only one unsolicited AE, a mild macular rash occurring one day after the first injection and lasting for 7 days was assessed by the investigator as related to the vaccination. Eleven subjects experienced at least one SAE; none was considered to be related to the vaccination and most were diagnoses commonly observed in infancy such as bronchopneumonia, bronchiolitis, and gastroenteritis.

TABLE I SEROPROTECTION/VACCINE RESPONSE RATES TO DTaP-IPV/PRP~T PENTAVALENT VACCINE

	Historical control* Rate % (95% CI)	Study vaccine† Rate % (95% CI)
Anti-diphtheria ≥0.01 IU/mL	100 (95.9;100)	99.1 (96.6;99.9)
Anti-tetanus ≥0.01 IU/mL	100 (95.9;100)	100 (98.3;100)
Anti-polio 1 ≥8 (1/dil)	97.0 (91.5;99.4)	100 (98.3;100)
Anti-polio 2 ≥8 (1/dil)	100 (96.4;100)	99.1 (96.6;99.9)
Anti-polio 3 ≥8 (1/dil)	99.0 (94.6;100)	100 (98.3;100)
Anti-PRP ≥0.15 µg/mL	98.0 (93.0;99.8)	98.6 (95.9;99.7)
Anti-PT ≥4-fold increase EU/mL	89.6 (81.7;94.9)	93.7 (89.5;96.6)
Anti-FHA ≥4-fold increase EU/mL	89.5 (81.5;94.8)	85.7 (80.2;90.1)

** DTaP-IPV//PRP~T pentavalent vaccine at 2, 3 and 4 months of age (French historical control study - Mallet et al, 1997[8]); † DTaP-IPV// PRP~T pentavalent vaccine at 6, 10 and 14 weeks of age. Anti-PRP measured by Farr-type radioimmunoassay (RIA) in comparison to an American Food and Drug Administration (FDA) human reference serum, lower limit of quantification (LLOQ) 0.06 µg/mL; anti-FHA and anti-PT measured by ELISA in comparison to sanofi pasteur reference standards, LLOQ 2 EU/mL; anti-tetanus measured by ELISA in comparison to a WHO reference standard, LLOQ 0.01 IU/mL; anti-polio were titrated by microneutralization following a modified WHO standardized procedure using Vero cells and wild-type poliovisuses, LLOQ 4 (1/dil); anti-diphtheria were titrated using a micrometabolic inhibition test against a WHO reference standard, LLOQ of 0.005 IU/mL.*

TABLE II GEOMETRIC MEAN TITERS (GMTs) BEFORE THE FIRST DOSE AND ONE MONTH AFTER THE THIRD DOSE OF THE STUDY VACCINE

	Pre-first dose GMT (95% CI)	Post-priming* GMT (95% CI)
Anti-diphtheria (IU/mL)	0.028 (0.022; 0.033)	0.046 (0.040; 0.053)
Anti-tetanus (IU/mL)	1.96 (1.69; 2.28)	0.93 (0.86; 1.0)
Anti-polio 1 (1/dil)	18.1 (15.1; 21.5)	440.5 (363.4; 533.9)
Anti-polio 2 (1/dil)	20.4 (16.6; 25.2)	458.9 (361.4; 582.6)
Anti-polio 3 (1/dil)	9.9 (8.6; 11.5)	1510.7 (1283.9; 1777.6)
Anti-PRP (µg/mL)	0.11 (0.09; 0.14)	4.17 (3.52; 4.93)
Anti-PT (EU/mL)	4.9 (4.0; 5.9)	321.1 (294.0; 350.8)
Anti-FHA (EU/mL)	5.1 (4.3; 5.9)	97.6 (94.6; 99.2)

** DTaP-IPV//PRP~T pentavalent vaccine at 6, 10 and 14 weeks of age.*

TABLE III SOLICITED LOCAL ADVERSE REACTIONS AND SYSTEMIC REACTIONS THAT OCCURRED WITHIN 8 DAYS (DAYS 0-7) AFTER EACH DOSE AND AFTER ANY DOSE OF PENTAVALENT VACCINE

		DTaP-IPV//PRP~T vaccine			
		Dose 1 *n* = 224 % of doses	Dose 2 *n* = 217 % of doses	Dose 3 *n* = 217 % of doses	Any dose *n* = 658 % of doses
Local reactions					
Tenderness	Any	21.9	15.7	14.3	17.3
	Severe	0.4	0.5	0.0	0.3
Redness	Any	9.4	5.1	3.7	6.1
	Severe	0.0	0.0	0.0	0.0
Swelling	Any	6.3	3.7	5.1	5.0
	Severe	0.4	0.0	0.0	0.2
Systemic events					
Fever	Any	17.0	12.4	16.6	15.3
	Severe	0.4	0.5	0.0	0.3
Vomiting	Any	17.0	11.1	11.5	13.2
	Severe	0.4	0.0	0.0	0.2
Abnormal crying	Any	14.3	11.1	11.5	12.3
	Severe	0.4	0.0	0.9	0.2
Drowsiness	Any	16.5	6.9	6.9	10.2
	Severe	0.4	0.0	0.0	0.2
Appetite loss	Any	11.6	7.4	7.8	9.0
	Severe	0.0	0.5	0.0	0.2
Irritability	Any	15.6	12.0	13.4	13.7
	Severe	0.0	0.0	0.0	0.0

Four cases of chikungunya or probable chikungunya occurred. One case of seizure/convulsion was reported 22 days after the third injection. No hypotonic hyporesponsive episode (HHE) was reported. All subjects with an SAE recovered, and no subject was withdrawn due to an adverse event.

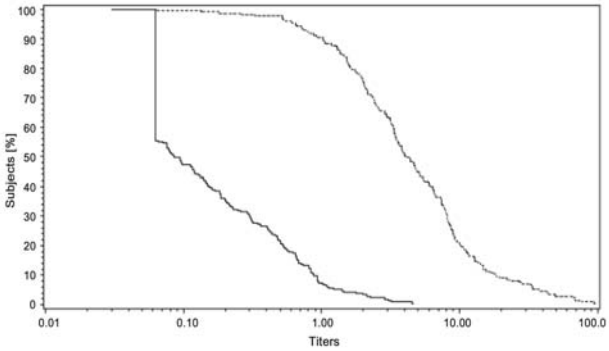

FIG.1 *RCDCs for anti-PRP antibody titers before (6 weeks of age) and 1 month after primary vaccination (18 weeks of age).*

DISCUSSION

This clinical study evaluated the immunogenicity and safety of a DTaP-IPV//PRP~T combination vaccine (Pentaxim™) for primary immunization at 6,10,14 weeks of age, with a monovalent recombinant hepatitis B vaccine (Euvax B™) given at either 6,10,14 weeks of age or 0,6,14 weeks of age. No control group wP was included since wP vaccines are routinely available in India, meaning that participation in such an arm of the study would have offered no benefit to the infant. Instead, the study design included a descriptive comparison to a historical control study group given the same vaccine at 2,3,4 months of age in France, where this vaccine has been routinely used(9). At the time of the study, no historical clinical data on the immunogenicity of this vaccine given at 6,10,14 weeks of age were available.

Seroprotection and vaccine response rates were similar to the control study and also to those subsequently seen in a later study evaluating the same vaccine for primary vaccination in infants in the Philippines following the 6,10,14 weeks of age EPI schedule(10). The statistical comparison was performed using the French data rather than the Philippine data since at the time of the design and set-up of the study reported in this article, the Philippine data were not available.

The good seroprotection and GMT response to the IPV antigens are of particular interest because of the vaccination schedule. During the study, no subject that was analysed for immunogenicity received OPV, although the possibility of an effect of herd immunity is acknowledged since OPV is used routinely in India. However, this putative herd immunity is difficult to quantify, and a similarly strong response to the same inactivated polio antigens has been observed in countries where OPV is no longer used (*e.g* France, Sweden) (9,11). As such, we do not consider the polio response to be markedly augmented due to routine local OPV use. Overall, these results are consistent with the IPV immunogenicity seen for this vaccine in studies in various European countries, Chile, and Turkey following vaccination schedules of 2,3,4 months of age, 2,4,6 months of age, and 3,5,12 months of age(11,12,13). The strong IPV response is particularly relevant in the context of OPV cessation in the post-eradication era and the planned switch to routine IPV use.

As there are no recognized serological correlates of protection for pertussis, 4-fold increases from pre- to post-vaccination were used to evaluate the anti-PT and FHA response. These data and the strong increase in GMTs accord 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(14). The long-term effectiveness of the pentavalent vaccine on pertussis incidence has been documented over the past 10 years by the National Surveillance Program in Sweden using a 3, 5, 12 month schedule(15,16). Although the schedule in Sweden differs from that in India, we believe that the Swedish surveillance data are applicable to the EPI administration schedule since high immunogenicity has been demonstrated from a range of primary series schedules(11-13). These data have shown that routine primary vaccination with aP vaccines, including the present pentavalent vaccine (Pentaxim™), has resulted in a marked decrease in the incidence of pertussis cases after the second and third doses and that protection remains high after the third dose for 8 to 9 years(17). In addition, the WHO position paper on aP vaccines(7) states that ''although most efficacy and effectiveness studies on aP vaccines have been conducted in industrialized countries, the new DTaP vaccines are expected to be efficacious in all regions of the world.''

WHAT IS ALREADY KNOWN?

- Pentavalent (DTaP-IPV-Hib) vaccine (Pentaxim™) is safe and immunogenic in over 85 countries.

WHAT THIS STUDY ADDS?

- Additional immunogenicity and safety data of Pentaxim™ following a primary vaccination EPI (6, 10, 14 weeks) schedule in India, in particular a strong immune response to three polio antigens.

The anti-PRP antibody response is consistent with both the Philippine study and the control study. Furthermore, this anti-PRP response is similar to that observed in previous studies in India conducted with ActHib given either alone, concomitantly or combined with DTwP vaccines(18,19), highlighting the strong immune response to the Hib component of the pentavalent vaccine.

The relatively high incidence of minor adverse events and occasional SAEs associated with wP vaccines has prompted the development of aP vaccines, and the safety results of this study reflect the good reactogenicity documented for all aP-based combination vaccines(20,21).

In summary, the DTaP-IPV//PRP~T pentavalent vaccine was highly immunogenic for all antigens and well tolerated when administered in the 6,10,14 weeks of age EPI schedule, consistent with previous data. The inclusion of IPV makes it easier to incorporate this vaccine successfully into the UIP in India, in the context of the future cessation of OPV in the post-eradication era.

ACKNOWLEDGMENTS

The authors would like to thank the participating clinicians at each study site and Clement Weinberger (Le Stylo Communications) for assistance with the draft manuscript preparation. The authors would like to acknowledge Fabrice Guitton and Ranjeet Kaur for study monitoring, Roy Fernando for data management, Valérie Bosch-Castells for statistical analysis, and Andrew Lane for additional assistance in finalising the manuscript. FG, RK, RF, VB and AL are employees of Sanofi Pasteur.

Contributors: AKD, VPV, HKP and LGM were responsible for trial conduct, data acquisition, data interpretation, manuscript review and approval. EO was responsible for study design, data interpretation, manuscript review and approval.

Funding: Sanofi Pasteur, Lyon, France.

Competing interest: EO is an employee of Sanofi Pasteur which manufactures Pentaxim™.

REFERENCES

1. Indian Academy of Pediatrics Committee on Immunization (IAPCOI). Consensus recommendations on immunization, 2008. Indian Pediatr 2008; 45: 635-648.
2. Shah R, Raghu MB, Shivananda A, Mangayarkarashi S, Rao I, Rao R, *et al*. Immunogenicity and safety of an indigenously developd DTPw-hepatitis B combination vaccine in Indian infants. Indian Pediatr 2008; 45: 819-823.
3. Polio Eradication Committee, Indian Academy of Pediatrics, Vashishtha VM, John TJ, Agarwal RK, Kalra A. Universal immunization program and polio eradication in India. Indian Pediatr 2008; 45: 807-813.
4. Mittal SK, Mathew JL. IPV revisited...yet again. Indian Pediatr 2008; 45: 390-395.
5. Edwards KM, Decker M. Pertussis vaccines. In: Plotkin SA, Orenstein WA, editors. Vaccines. 5th ed. Philadelphia PA: Saunders Co.; 2008. p. 467-518.
6. WHO. Vaccine preventable diseases monitoring system: Country profiles. Available from: URL: http://www.who.int/vaccines/globalsummary/immunization/countryprofileselect.cfm. Accessed January 15, 2009.
7. WHO Position Paper. Pertussis vaccines. Weekly Epidemiol Rec 2005; 80: 29-40.
8. WHO. United Nations prequalified vaccines (WHO list of vaccines for purchase by UN agencies as of August 2008). Available from: URL: http://www.who.int/immunization_

standardsn/vaccine_quality/pq_suppliers/en/index.html. Accessed October 29, 2008.

9. 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. The 15th Annual Meeting of the European Society for Paediatric Infectious Diseases (ESPID), Paris, France 1997.
10. 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 Bulletin 2008; 86: 443-451.
11. Carlsson RM, Claesson BA, Selstam U, Fagerlund E, Granstrom M, Blondeau C, *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: 1026-1033.
12. Lagos R, Kotloff, KL Hoffenbach A, San Martin O, Abrego P, Ureta AM, *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.
13. Kanra G, Selier T, Yurdakök K, Yavuz T, Baskan S, Ulukol B, *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 simul-taneously with a hepatitis B vaccine at two, three and four months of life. Vaccine 2000; 18: 947-954.
14. Vidor E, Plotkin SA. Immunogenicity of a two-component (PT & FHA) acellular pertussis vaccine in various combinations. Human Vaccines 2008; 4: 328-340.
15. Swedish Institute for Infectious Disease Control. Pertussis surveillance in Sweden with enhanced follow-up of cohorts immunized with acellular pertussis vaccines 2007 Appendix 2 SP-MSD. Available from: URL: http://www.smittskyddsinstitutet.se/upload/10-y%20report-app%202-%20SP%20MSD.pdf. Accessed December 4, 2008.
16. Olin P, Hallander HO. Marked decline in pertussis followed reintroduction of pertussis vaccination in Sweden. Euro Surveill 1999; 4:128-129.
17. 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. Pediatr 2006; 118: 978-984.
18. Cherian T, Thomas N, Raghupathy P, Durot I, Dutta A. Safety and immunogenicity of *Haemophilus influenzae* type B vaccine given in combination with DTwP at 6, 10 and 14 weeks of age. Indian Pediatr 2002; 39: 427-436.
19. Acharya D, Bhave S, Joshi V, Bavdekar A, Pandit A. *Haemophilus influenzae* type b vaccine in India: need and timing, immunogenecity and tolerance. Indian Pediatr 1997; 34: 9-15.
20. Greco D, Salmaso S, Mastrantonio P, Giuliano M, Tozzi AE, Anemona A, *et al.* A controlled trial of two acellular vaccines and one whole-cell vaccine against pertussis. N Engl J Med 1996; 334: 341-348.
21. Olin P, Rasmussen F, Gustafsson L, Hallander H, Heijbel H. Randomised controlled trial of two-component, three component, and five component acellular pertussis vaccines compared with whole-cell pertussis vaccine. Lancet 1997; 350: 1569-1577.