

Antibody persistence against diphtheria, tetanus, pertussis, poliomyelitis and *Haemophilus influenzae* type b (Hib) in 5–6-year-old children after primary vaccination and first booster with a pentavalent combined acellular pertussis vaccine: immunogenicity and tolerance of a tetravalent combined acellular pertussis vaccine given as a second booster

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Abstract

The main objective of this study was to assess in 5–6-year-old French children ($n = 234$) the persistence of antibodies induced by a primary series vaccination (at 2–4 months of age) and a first booster (at 12–16 months of age) with a pentavalent two-component acellular pertussis combined vaccine (DTaP-IPV-Hib; Pentavac[®]). The second objective was to evaluate in these 5–6-year-old French children the safety and the immunogenicity of a tetravalent acellular pertussis combined vaccine (DTaP-IPV; Tetravac[®]) given as second booster. **Results:** Seroprotective antibody levels against diphtheria, tetanus, types 1–3 poliomyelitis and PRP were maintained 4–5 years after primary-vaccination and first booster with Pentavac[®]. As expected, anti-PT antibodies levels were low, suggesting that children were not colonised by *Bordetella pertussis*. The second booster with Tetravac[®] was well tolerated and elicited a strong booster response for all antigens. **Conclusion:** acellular pertussis combined vaccine, used in primary-vaccination, could be considered as having the same priming effect and the same efficacy as whole cell pertussis vaccine.

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Keywords: Acellular pertussis vaccine; Antibody persistence; Booster

1. Background

Since 1965, extensive use of the wcP vaccine, included in pediatric combination vaccines, has led to a dramatic decrease of pertussis. Although wcP vaccines are generally

considered to be efficacious, concern over their reactogenicity has stimulated the development of acP vaccines made up of selected purified antigens from *Bordetella pertussis*. When acP combination vaccines were compared to the currently licensed wcP combination vaccines, they were

Abbreviations: acP, acellular pertussis; D, diphtheria; DTaP-IPV-Hib, diphtheria, tetanus, acellular pertussis, inactivated polio vaccine, *H. influenzae* type b; DTaP-IPV-PRP-T, diphtheria, tetanus, acellular pertussis, inactivated polio vaccine, polyribosyl ribitol phosphate-tetanus protein; DTwP-IPV-Hib, diphtheria, tetanus, whole cell pertussis, inactivated polio vaccine, *H. influenzae* type b; DTwP-IPV-PRP-T, diphtheria, tetanus, whole cell pertussis, poliomyelitis, polyribosyl ribitol phosphate-tetanus protein; 1/dil, reciprocal dilution; ELISA, enzyme immunoassay; FHA, filamentous haemagglutinin; GMTs, geometric mean of titers; GMTRs, geometric mean of titer ratios; Hib, *H. influenzae* type b; IPV, inactivated polio vaccine; EU/ml, ELISA unit per milliliter; IU/ml, international unit per milliliter; PRP-T, polyribosyl ribitol phosphate-tetanus protein; PT, pertussis toxoid; RIA, radio immuno assay; SC, sero-conversion; SN, sero-neutralisation; T, tetanus; wcP, whole cellular pertussis

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consistently found less reactogenic and as much as immunogenic [1–6]. AcP combined vaccines were introduced into the French national vaccination schedule in 1998 as first booster at 16–18 months of age [7]. Then, a new recommendation in 2001 went beyond with still a priority use for the wCP vaccines, but with also a possible alternative use of acP vaccines in primary vaccination at 2–4 months of age [8]. When new vaccines are implemented into national immunisation programs, a long-term follow-up of antibody persistence is important, for epidemiological reasons and to determine the need, the timing, and the safety of a booster dose.

Studies regarding long-term protection against pertussis after natural infection or specific immunisation with multi-component acellular pertussis vaccines had been carried out, but none out of them had been reported with a two-component acellular pertussis combined vaccine [9,10]. Between 1994 and 1996, a total of 592 French infants was enrolled in two randomised phase II studies to receive a new pentavalent DTacP-IPV-Hib vaccine (Pentavac[®], Aventis Pasteur) consisting of diphtheria (D) and tetanus (T) toxoids, a two-component acP vaccine (pertussis toxoid (PT) and filamentous haemagglutinin (FHA)), an inactivated poliomyelitis vaccine (IPV), and a (*Haemophilus influenzae* type b (Hib) capsular polysaccharide (polyribosyl ribitol phosphate) conjugated to tetanus protein (PRP-T)) vaccine. All children (592) who had been vaccinated by Pentavac[®] (either at 2–4 months or at 2–6 months of age, with a first booster at 12–16 months of age) in these two previous studies were asked to participate in the present study.

The main objective of the study was to describe, for the first time in France, persistence of humoral immunity at 5–6 years of age induced by a primary-immunisation and a first booster with a two-component acP combination vaccine (DTacP-IPV-Hib, Pentavac[®]).

The second objective was to assess humoral immunity and tolerance of a second booster with a two-component acP combination vaccine (DTacP-IPV, Tetravac[®]) at 5–6 years of age.

2. Methods

This open, non-comparative, phase III, multi-center study, conducted in 38 paediatric private practices, was a follow-up of children from two previous randomised phase II studies.

2.1. Subjects

This study potentially involved 592 children who had been vaccinated in the two previous studies by Pentavac[®]. Two hundred and thirty-four (234) healthy children of either sex were enrolled after a written informed consent had been obtained from parents. After a clinical examination, each child received a second booster at 5–6 years of age with a

dose of Tetravac[®] by intramuscular injection into the deltoid muscle using a 16 mm-long, 25 gauge-diameter needle.

2.2. Vaccine

The DTacP-IPV vaccine (Tetravac[®], Aventis Pasteur) is an adsorbed diphtheria, tetanus, acP and inactivated poliomyelitis vaccine. Each 0.5 ml dose of vaccine contains 30 IU purified diphtheria toxoid; minimum 40 IU purified tetanus toxoid; 25 µg purified pertussis toxoid (PT); 25 µg filamentous haemagglutinin (FHA); 40 units of type 1 D antigen, 8 units of type 2 D antigen, and 32 units of type 3 D antigen inactivated poliomyelitis vaccine.

2.3. Immunogenicity assessment

All serologic analysis were performed at the Aventis Pasteur Clinical Sero-Immunology Laboratory (Val de Reuil, France) on blood samples collected before (between D7 and D0: day of vaccination) and 2 months after the second booster (D30 ± 5). Antibodies against D, T, IPV, PT and FHA were measured in the first and second samples, whereas anti-PRP antibodies were only measured in the first sample. Diphtheria and tetanus antibodies titers were measured by enzyme-linked immunosorbent assay (ELISA), with lower limits of detection of 6 mIU/ml [11,12]. Antibodies against pertussis components, PT and FHA, were analyzed by ELISA, each with a lower limit of detection of 2 EU/ml [13]. Neutralizing antibodies to poliovirus types 1–3 were determined by sero-neutralisation (SN) on HEP cells [14]; The results were expressed as reciprocal dilution (1/dil), with a lower limit of detection of 5 (1/dil). Antibodies against PRP were assessed with a Farr-type radio-immunoassay (RIA) [15], with a minimum limit of detection of 0.07 µg/ml.

The primary evaluation endpoints between D7 and D0: day of vaccination were considered as followed. (1) GMTs of antibody titers; (2) percentage of subjects with an anti-PT titer ≥ 4 IU/ml and ≥ 8 IU/ml; (3) percentage of subjects with antibody titers above the protective level for diphtheria, tetanus, poliomyelitis viruses types 1–3 and PRP. The percentage of children with diphtheria and tetanus antibody titers ≥ 0.01 and ≥ 0.1 IU/ml were calculated, considering that these two thresholds reflect a partial and a complete protection, respectively [16]. Anti-pertussis antibody response, induced by acP vaccines, does not very well correlate with vaccine efficacy [16]. The percentage of children with neutralizing antibody titers to poliovirus types 1–3 ≥ 5 (1/dil) were calculated, considering that any level of neutralizing antibodies is generally considered as protective [16]. The percentage of children with anti-PRP antibody titers ≥ 0.15 and ≥ 1 µg/ml were calculated, these two thresholds being associated with a short and a long-term protection, respectively [16].

In addition, sero-conversion, GMTs, geometric mean of the individual post/pre-vaccination titer ratios (GMTRs), and

percentage of children with four-fold increase antibody titers were calculated as secondary evaluation endpoints at D30 $\pm$ 5 after vaccination. In this study, sero-conversion for PT and FHA components was defined as a post-vaccination antibody titer ≥ 4 times the pre-vaccination antibody titer.

2.4. Safety assessment

Children remained under medical supervision for 30 min after each injection to monitor any immediate local and systemic reactions. Parents filled in the diary card following the advice given by the investigator. Local or systemic reactions occurring, from 30 min to 7 days after vaccination, and from 8 days after vaccination to the end of the study (D30 $\pm$ 5) were reported. Solicited local reactions were local pain, redness or induration/oedema of at least 0.5 cm. Solicited systemic adverse reactions were fever (rectal temperature $\geq 37.5^\circ\text{C}$), unusual irritability, drowsiness, sleeping disorders, headache, myalgia, arthralgia, loss of appetite, nausea, vomiting, diarrhoea. Then, the investigator recorded this information in the case report form. Serious adverse events were reported over the whole study period.

2.5. Statistical analysis

Analysis was descriptive. The immunogenicity parameters concerning the first booster given at 12–16 months of age in the previous studies were calculated for the children included in the present study. For the second booster given at 5–6 years of age, the GMTs and the proportions of children with antibodies above the defined threshold of sero-conversion/sero-protection were described before (pre-booster results) and 30 $\pm$ 5 days after the booster dose (post-booster results). The GMTRs and the percentage of children with a four-fold rise in antibody titers were calculated.

3. Results

3.1. Study population

From May 2000 to February 2001, a total of 234 healthy children were enrolled in this follow-up study. One hundred and eleven (47.4%) of included children were girls and one hundred and twenty three (52.6%) were boys. Mean age at inclusion was 5.2 years. All children completed the study, excepted one child due to voluntary withdrawal. This child received no booster. No child was lost to follow-up or was withdrawn from the study for an adverse event.

3.2. Immunogenicity

All children except one ($n = 233$) were considered for immunogenicity analysis as primary criterion (pre-booster

analysis) and 232 vaccinees were considered for immunogenicity analysis as secondary criterion (post-booster analysis).

The GMTs and the proportion of children with antibodies above defined levels are summarised in Table 1.

At the age of 12–16 months, 1 month after the first booster dose of Pentavac[®], the GMTs against each antigen were high: 136.85 EU/ml for PT, 282.33 EU/ml for FHA, 7.47 IU/ml for tetanus, 1.07 IU/ml for diphtheria, between 2359 and 3980 for the three poliovirus serotypes and 31, 32 $\mu\text{g/ml}$ for PRP. All children (100%) had tetanus, poliovirus 1–3 and PRP antibody titers above protective levels. All children (100%) had anti-PT antibody titers ≥ 4 EU/ml and ≥ 8 EU/ml and 97% of children had diphtheria antibody titers ≥ 0.1 IU/ml.

At the age of 5–6 years, at the time of the Tetravac[®] second booster dose, the GMTs against each antigen decreased dramatically: 2.71 EU/ml for PT, 43.32 EU/ml for FHA, 0.09 IU/ml for tetanus, 0.05 IU/ml for diphtheria, between 58 and 123 for the three poliovirus serotypes and 1.55 $\mu\text{g/ml}$ for PRP. Only 28 and 47% of children had antibody levels ≥ 0.1 IU/ml against diphtheria and tetanus, respectively, and 60% of children had anti-PRP antibody levels ≥ 1 $\mu\text{g/ml}$. At least 94% of vaccinees had neutralizing antibody titers ≥ 5 (1/dil) against the three serotypes of poliovirus. Regarding anti-pertussis antibodies, 41% of children had an anti-PT antibody titer ≥ 4 EU/ml and 19% of children had an anti-PT antibody titer ≥ 8 EU/ml.

One month after the second booster dose of Tetravac[®], a significant increase of antibody titers against each vaccine antigens was observed. The antibody titers achieved levels at least similar to those obtained 1 month after the first booster dose of Pentavac[®] (at 12–16 months): 129.31 EU/ml for PT, 467.04 EU/ml for FHA, 10.25 IU/ml for tetanus, 3.68 IU/ml for diphtheria and between 3174 and 3847 for the three poliovirus serotypes. The GMTRs (post/pre-booster) were 107.61 for tetanus, 73.80 for diphtheria, above 30 for the three poliovirus serotypes. All children had antibody titers above the thresholds considered as protective for all these antigens. Regarding anti-pertussis antibody response: the GMTRs were 49.41 for PT and 11.00 for FHA; a four-fold rise of antibody titers was elicited in 98% of children for anti-PT antibodies and in 79% of children for anti-FHA antibodies.

3.3. Tolerance

Safety data were available for 233 children. Two hundred and fourteen children experienced at least one adverse event, regardless of the relationship to the study vaccine. Fifty-five adverse events, considered as non-related to the study vaccine, were reported in 38 infants (16.3%).

At least one local reaction was reported in 83.7% of children after Tetravac[®] injection. The most frequently reported local reactions, occurring from 30 min to 7 days after injection, were: local pain (71.7%), redness (64.8%),

Table 1

Immune response to vaccine antigens after the first booster (at 12–16 months of age) of PENTAVAC[®] and before/after the second booster (at 5–6 years of age) of TETRAVAC[®] in children having received a 3-dose primary series with PENTAVAC[®]

Vaccine antigens	Criteria for evaluation	First booster 12–16 months DTacP-IPV-Hib Pentavac [®]	Second booster 5–6 years DTacP-IPV Tetravac [®]		
			Pre-booster vaccination	Post-booster vaccination	Post/pre vaccination
PT (EIA-EU/ml)	<i>N</i> (all-missing)	69 (234–165)	232 (233–1)	231 (232–1)	230 (232–2)
	GMT ^a	136.85	2.71	129.31	
	[95% CI]	[117.23; 159.75]	[2.28; 3.21]	[117.52; 142.28]	
	% ≥ 4	100	41	100	
	% ≥ 8	100	19	100	
	GMTR ^b				49.41
FHA (EIA-EU/ml)	% ≥ four-fold rise				98
	<i>N</i> (all-missing)	70 (234–164)	232 (233–1)	231 (232–1)	230 (232–2)
	GMT	282.33	43.32	467.04	
	[95% CI]	[236.07; 337.64]	[36.25; 51.78]	[432.94; 503.82]	
	GMTR				11.00
	% ≥ four-fold rise				79
Tetanus (EIA-IU/ml)	<i>N</i> (all-missing)	70 (234–164)	226 (233–7)	231 (232–1)	224 (232–8)
	GMT	7.47	0.09	10.25	
	[95% CI]	[6.16; 9.06]	[0.08; 0.11]	[9.19; 11.42]	
	% ≥ 0.01	100	99	100	
	% ≥ 0.1	100	47	100	
	GMTR				107.61
Diphtheria (EIA-IU/ml)	% ≥ four-fold rise				100
	<i>N</i> (all-missing)	69 (234–165)	228 (233–5)	231 (232–1)	226 (232–6)
	GMT	1.07	0.05	3.68	
	[95% CI]	[0.82; 1.40]	[0.04; 0.06]	[3.20; 4.23]	
	% ≥ 0.01	100	95	100	
	% ≥ 0.1	97	28	99.6	
Polio 1 (SN-1/dil)	GMTR				73.80
	% ≥ four-fold rise				99.6
	<i>N</i> (all-missing)	71 (234–163)	229 (233–4)	231 (232–1)	227 (232–5)
	GMT	2359.45	57.79	3173.66	
	[95% CI]	[1685.43; 3303.00]	[46.98; 71.08]	[2843.28; 3542.42]	
	% ≥ 5	100	94	100	
Polio 2 (SN-1/dil)	GMTR				55.02
	% ≥ four-fold rise				95
	<i>N</i> (all-missing)	71 (234–163)	230 (233–3)	231 (232–1)	228 (232–4)
	GMT	2760.37	77.59	3050.51	
	[95% CI]	[2093.08; 3640.38]	[63.34; 95.05]	[2719.46; 3421.85]	
	% ≥ 5	100	96	100	
Polio 3 (SN-1/dil)	GMTR				39.85
	% ≥ four-fold rise				93
	<i>N</i> (all-missing)	71 (234–163)	230 (233–3)	231 (232–1)	228 (232–4)
	GMT	3980.34	122.98	3846.87	
	[95% CI]	[3115.19; 5085.75]	[100.56; 150.40]	[3396.07; 4357.50]	
	% ≥ 5	100	96	100	
PRP (RIA-μg/ml)	GMTR				31.95
	% ≥ four-fold rise				93
	<i>N</i> (all-missing)	70 (234–164)	229 (233–4)	0 (232–232)	0 (232–232)
	GMT	31.32	1.55		
	[95% CI]	[23.83; 41.16]	[1.30–1.86]		
	% ≥ 0.15	100	97		
	% ≥ 1	100	60		

^a GMT: geometric mean of titers.

^b GMTR: geometric mean of titers ratio.

Table 2

Description of safety after a second booster with TETRAVAC® at 5–6 years of age in children ($N = 233$) having received a primary series and a first booster with PENTAVAC® (four doses)

	Adverse events, n	Subjects $N = 233$	Percentage	[95% CI]
Any local reaction	532	195	83.7	
Any immediate local reaction	3	3	1.3	[0.3; 3.8]
Any local reaction with onset (30 min to 7 days)	520	193	82.8	[71.6; 95.3]
Solicited reactions	501	193	82.8	[71.6; 95.3]
Local pain	169	167	71.7	[61.3; 83.4]
Redness	152	151	64.8	[54.9; 76.0]
Induration	120	120	51.5	[42.7; 61.6]
Oedema	60	60	25.8	[19.7; 33.1]
Unsolicited reactions	19	19	8.2	[4.9; 12.7]
Any local reaction occurring from 8 days	9	5	2.2	[0.7; 5.0]
Any systemic reaction	352	163	70	
Any immediate systemic reaction	1	1	0.4	[0.0; 2.4]
Any systemic reaction with onset (30 min to 7 days)	349	162	69.5	[59.3; 81.1]
Solicited reactions	335	161	69.1	[58.9; 80.6]
>1% ^a				
Fever	128	112	48.1	[39.6; 57.8]
Myalgia	48	48	20.6	[15.2; 27.3]
Headache	36	35	15.0	[10.5; 20.9]
Irritability	32	32	13.7	[9.4; 19.4]
Drowsiness	28	28	12.0	[8.0; 17.4]
Sleep disorders	22	22	9.4	[5.9; 14.3]
Loss of appetite	14	14	6.0	[3.3; 10.1]
Arthralgia	12	12	5.2	[2.7; 9.0]
Diarrhoea	6	5	2.2	[0.7; 5.0]
Nausea	5	5	2.2	[0.7; 5.0]
Vomiting	4	4	1.7	[0.5; 4.4]
Unsolicited reactions	14	13	5.6	[3.0; 9.5]
>1% ^a				
Pruritus	5	5	2.2	[0.7; 5.0]
Abdominal pain	3	3	1.3	[0.3; 3.8]
Any systemic reaction occurring from 8 days	2	1	0.4	[0.0; 2.4]

^a Details of reactions noted for more than 1% of subjects.

induration (51.5%) and oedema (25.8%). Local reactions occurred mainly within the first 3 days after vaccination, were considered as mild or moderate, and lasted from 1 to 4 days in most cases. One child presented with a large inflammatory reaction including redness, induration and oedema from shoulder to elbow at the injection site associated with moderate pain and 38.8 °C rectal temperature. The fever lasted 2 days and the local reaction resolved in 5 days.

At least one systemic reaction was reported in 70% of children after Tetravac® injection. Systemic adverse reactions occurred mainly within the first 3 days after injection. The most frequently reported systemic reactions were fever (48.1%), myalgia (20.6%), headache (15%), irritability (13.7%) and drowsiness (12%). Fever (rectal temperature = 37.5 °C) was reported in 112 children. Fever episodes started within 3 days of vaccination in ninety seven children (86.6%) and lasted 1 day in 68 children (60.7%). Fever episodes were considered as mild (37.5–38.4 °C) in 42.1% of children, moderate (38.5–39.4 °C) in 6% of children and severe

(≥39.5 °C) in one child (0.4%). In this latter case, fever reached 39.5 °C. The other systemic adverse events included sleeping disorders (9.4%), loss of appetite (6%) arthralgia (5.2%), nausea (2.2%), diarrhoea (2.2%) and vomiting (1.7%).

Only one serious adverse event was reported in a 5-year-old girl who experienced a radio-cubital fracture, assessed as non-vaccine related. Details of reported reactions in more than 1% of subjects are presented in Table 2.

4. Discussion

For the first time in France, we are reporting the antibody persistence against diphtheria, tetanus, pertussis (PT and FHA), poliovirus types 1–3 and PRP the capsular polysaccharide of Hib, in 5–6-year-old French children who received a primary vaccination and a first booster with a two-component acP combination vaccine (Pentavac®). Antibody responses and safety of a second booster with a

two-component acP combination vaccine (Tetravac[®]) were also evaluated.

4.1. Persistence of antibodies 4 years after a first booster with Pentavac[®]

More than 95% of children were still protected against tetanus and diphtheria (≥ 0.01 IU/ml) and the three serotypes of poliovirus 4 years after the first booster. Nevertheless, results indicated a decrease in antibody concentrations reflected by the fact that only 28% of children for diphtheria and 47% for tetanus had antibody levels above the considered long-term protection threshold (≥ 0.1 IU/ml). These antibody titers are in the same range of those reported in children receiving others combination vaccines in infancy, containing a wCP vaccine [17]. These results highlight the need for a booster injection at 5–6 years of age in order to reinforce humoral immunity against tetanus, diphtheria and poliomyelitis.

Despite a strong decrease in anti-PRP antibody concentration (31.32–1.55 μ g/ml), 97% of children still had an antibody titer ≥ 0.15 μ g/ml, showing that vaccinated children were protected until the end of the at risk period for invasive Hib disease. These anti-PRP antibody concentrations at 6 years of age were in the same range of those reported in children receiving other Hib conjugated combination vaccine and similar to those reported in non-vaccinated healthy children of the same age [18].

Antibody concentrations against pertussis (PT and FHA) declined considerably after vaccination: while post-vaccination anti-PT GMT levels observed 1 month after the first booster injection at 12–16 months of age were high (GMT = 136.85 EU/ml), pre-vaccination GMT levels before the second booster injection at 5–6 years of age were very low (GMT = 2.71 EU/ml). Nineteen percent of the population had anti-PT antibody titers ≥ 8 EU/ml but only fourteen children (6%) had an antibody titer ≥ 20 EU/ml, and five (2%) an antibody titer ≥ 100 EU/ml. One child had a titer of 4222 EU/ml.

These results correspond to the expected rapid decline in antibody levels already observed after primary series vaccination as well as with wCP or acP vaccines [19]. The long-term specific humoral and cellular immunity induced by acellular or whole cell pertussis vaccination seems to be very low and not very different from that observed after natural infection [9,10]. As a matter of fact, antibody titers induced by pertussis vaccines do not correlate with vaccine efficacy or protection: although it had been demonstrated that high levels of antibodies, arbitrarily defined against numerous pertussis antigens acquired by wCP or acP vaccination were associated with lower risk of acquiring the disease. Grimprel et al. showed in 1993 [19] that French children vaccinated with wCP combination vaccine in infancy were protected against the disease despite very low circulating antibody levels, and that vaccine induced a protection which lasted around 8 years. At that time, pertussis started to make

a breakthrough in vaccines, and accordingly, circulating anti-PT antibody levels rose, attesting the contact with *B. pertussis*. The presence of high levels of circulating anti-PT antibodies in few children 4 years after the vaccination in the present study could be also compatible with a recent exposure or sub clinical infection by *B. pertussis*. Therefore, an “a posteriori” search for infectious respiratory diseases or cough was performed on the 14 children with an antibody titer above 20 EU/ml. Among the 13 children for whom information was available, only 2 children presented a coughing episode that could be suggestive of a possible pertussis infection. The first child, who had an anti-PT antibody titer of 22 EU/ml before the second booster, presented a 1 week persistent cough episode 2.5 years earlier. The second child who had an anti-PT antibody titer of 487 EU/ml before the second booster presented a persistent cough in the previous 6 months more suggestive of *Mycoplasma pneumoniae* infection or post viral allergy than a pertussis infection.

In an environment where *B. pertussis* is still circulating, the low proportion of children with high antibody titers 4 years after the pertussis vaccination indicates that most children were well protected and were probably not colonised [19–23]. Table 3 summarises anti-PT antibody persistence from 2.5 to 5 years in children vaccinated in infancy according to different vaccination schedules with different pentavalent pertussis combination vaccines. Regarding the different vaccination schedules, there could be a primovaccination with two doses (at 3 and 5 months) or three doses (at 2–4 months or 2, 4, 6) followed by a first booster between 12 and 18 months. For the different pentavalent pertussis combination vaccines, either children received a whole cell or an acellular vaccine both for primovaccination and first booster, or they received a whole cell vaccine for primovaccination and an acellular vaccine for first booster [17,22,24]. Overall, these findings suggest that, in the French specific pertussis epidemiology context, most of children who received a primary vaccination with an acP two-component pertussis combination vaccine in infancy are protected against pertussis until at least 5–6 years of age as well as children vaccinated in primary vaccination with a wCP combination vaccine.

4.2. Anamnestic response to a second booster with Tetravac[®]

One month after a second booster with a combined DTacP-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 pertussis (PT and FHA), with GMTs similar or greater than those measured 1 month after the first booster. Overall, these results were in the same range of those reported after a booster with an acP vaccine in children primed in infancy with a wCP combination vaccine or with a three component acP combination vaccine. This strong anamnestic response against each antigen indicates that a good immune memory

Table 3

Anti-PT antibody persistence from 2.5 to 5 years in children vaccinated in infancy according to different vaccination schedules with different pentavalent pertussis combination vaccines

Reference	Vaccine used for primary-immunisation (age in months)	Vaccine used for first booster dose (age in months)	Children included in the study (age in years)	Anti-PT antibodies	
				SC ^a (% $\geq$ 8 EU/ml) [95% CI]	GMT [95% CI]
Present study	DT2acP-IPV-PRP-T ^b (2–4 or 2, 4, 6)	DT2acP-IPV-PRP-T (12–16)	232 (5–6)	19.4 [14.5; 25.1]	2.7 [2.3; 3.2]
Langue et al. [17]	DTwacP-IPV-PRP-T ^c (2–4)	DT2acP-IPV-PRP-T (14–16)	156 (5–6)	20.5 [14.5; 27.7]	2.9 [2.4; 3.5]
Carlsson et al. [24]	DT2acP-IPV-PRP-T ^b (3, 5/2, 4, 6)	DT2acP-IPV-PRP-T (12/13)	175 (5–6)	18.3 [12.9; 4.8]	2.7 [2.2; 3.4]
Guiso et al. [22]	DTwacP-IPV-PRP-T ^c (2–4)	DTwacP-IPV-PRP-T (16–18)	100 (2.5–5)	36 [26.6; 46.2]	6.6 [5.7; 7.6]
Guiso et al. [22]	DT3acP-IPV-PRP-T ^d (2–4)	DT3acP-IPV-PRP-T (16–18)	127 (2.5–5)	31.5 [23.5; 40.3]	5.9 [5.3; 6.5]

^a SC: sero-conversion.

^b DT2acP-IPV-PRP-T: two-component acellular pertussis pentavalent combination vaccine (Pentavac[®], Aventis Pasteur).

^c DTwacP-IPV-PRP-T: whole cell pertussis pentavalent combination vaccine (Pentacoq[®], Aventis Pasteur).

^d DT3acP-IPV-PRP-T: three-component acellular pertussis pentavalent combination vaccine (Infanrix Quinta[®], Glaxo SmithKline).

was induced by the primary-immunisation with Pentavac[®] during the infancy.

4.3. Safety of a second booster with Tetravac[®]

The second DTacP-IPV booster (i.e. the fifth consecutive dose of acellular pertussis combined vaccine) induced at least one local reaction and one systemic reaction in 83.7 and 70% of children, respectively. The total number of reactions may seem high but it is important to highlight that the reported reactions (local and systemic) were solicited reactions in 5–6-year-old children. Even if they were more frequent than after the primary series (45.7 and 67.5%, respectively) and after the first booster (39.7 and 50.0%, respectively), they remained mild and transient. The incidence of reactions due to the booster is rather comparable to those obtained in previous trials with Tetravac[®] as second booster in 4–7-year-old children and in 8–12-year-old children, primed during the infancy with wcP combined vaccine (Tetravac[®] registration data). In these studies, 76.9% of the 4–7-year-old children and 91.1% of the 8–12-year-old children experienced at least one local reaction, and 47.7% of the 4–7-year-old children and 56.4% of the 8–12-year-old children experienced at least one systemic reaction. These reaction rates and their relative increase with the number of doses are in accordance with the results from previous clinical trials which have consistently shown that acP vaccines are far less reactogenic than wcP vaccines and for both vaccines the frequency of adverse reactions, particularly local reactions, increase with age and with the number of doses administered [2,25,26].

Although the second booster increased antibody levels and was reasonable well tolerated, the implementation of such an intervention should not be systematic. The introduction of a new booster dose into a national immunisation schedule and the recommended age for its administration should take into account local and/or national epidemiological conditions,

vaccination program history, vaccine coverage and current recommendations for infants and children immunisation.

5. Conclusion

The present article reports, for the first time, the persistence of humoral immunity induced by a primary-immunisation and a booster with a two-component acellular DTacP-IPV-Hib vaccine and the humoral immunity and safety of a Tetravac[®] injection as second booster at 5–6 years of age in French children.

The study results demonstrate a satisfactory persistence of antibodies at 5–6 years of age against diphtheria, tetanus, poliomyelitis. For pertussis, as expected, antibodies were very low in almost all children 4 years after the first booster. As already shown with a wcP combined vaccine, these low titers suggest that children immunised with an acP vaccine were probably not naturally boosted by *B. pertussis* colonisation during the several years following vaccination. A two-component (PT and FHA) acellular DTacP-IPV-Hib vaccine used in primary vaccination may therefore be considered as having the same efficacy as a wcP vaccine in French children.

Furthermore, a strong anamnestic response with high antibody levels was observed for each antigen 1 month after a Tetravac[®] injection as second booster, providing evidence for a long-term protection and a good immune memory induced by Pentavac[®] in infancy. Booster injection of Tetravac[®] at the age of 5–6 years, i.e. the fifth acellular combined vaccine injection, was generally well tolerated despite a slight increase of the reaction rates comparing to those reported at a younger age.

These results indicate that a primary vaccination and a booster dose of a two-component acellular DTacP-IPV-Hib vaccine during infancy induce a satisfactory persistence of antibodies at 5–6 years of age, and that a second booster

with Tetravac[®] induce a strong response and is generally well tolerated.

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