

Sustained Efficacy During the First 6 Years of Life of 3-Component Acellular Pertussis Vaccines Administered in Infancy: The Italian Experience

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ABSTRACT. *Background.* In 1992–1993, a randomized, double-blind, placebo-controlled clinical trial of two 3-component acellular pertussis vaccines was started in 4 of Italy's 20 regions. During the trial, the children had been randomized to receive 3 doses of 1 of 2 acellular pertussis vaccines combined with diphtheria and tetanus toxoids (DT) or of a DT vaccine only, at 2, 4, and 6 months of age. Both diphtheria-tetanus-acellular pertussis (DTaP) vaccines, 1 manufactured by SmithKline Beecham (DTaP SB; Infanrix) and 1 manufactured by Chiron Biocine (DTaP CB; Triacelluvax), contain pertussis toxin (PT), filamentous hemagglutinin, and pertactin. The results of the first period of follow-up, which ended in 1994 (stage 1), showed that both vaccines had a protective efficacy of 84% in the first 2 years of life; when the trial's follow-up was extended under partial blinding until the participating children had reached 33 months of age (stage 2 of the follow-up), these high levels of efficacy had persisted. Therefore, the objective of this study was to estimate the persistence of protection from 3 to 6 years of age of the 2 3-component DTaP vaccines administered as primary immunization in infancy.

Methods. An unblinded prospective longitudinal study of vaccinated and unvaccinated children in 4 Italian regions, with active surveillance of cough, was conducted by study nurses, and *Bordetella pertussis* infections were confirmed laboratory. The present study (stage 3) included those children who completed stage 2 of the follow-up and were still under active surveillance as of October 1, 1995, accounting for 4217 children who had received DTaP SB (representing 94% of the vaccine's recipients in the initial phase of the trial), 4215 who had received DTaP CB (95% of the original recipients), and 266 who had received DT only (18% of the original recipients). Because the parents of most of the original DT placebo group accepted pertussis vaccination during stage 2 in 1995, an additional 856 children were recruited in the DT group at the initiation of stage 3. These additional children were identified from the census list of children born in the same period and living in the same areas as the trial participants but who had been vaccinated in infancy with DT only. Eligible children were included in stage 3 if they had no history of either per-

tussis or pertussis vaccination and if a serum sample obtained at the time of enrollment had undetectable immunoglobulin G (IgG) against PT. Parental consent to participate in the study was obtained. Active surveillance for pertussis was conducted in the field by 72 study nurses through monthly contact with each family in the study. A cough episode that lasted ≥ 7 days was considered to be a laboratory-confirmed infection by *Bordetella pertussis* if at least 1 of the following 5 criteria (listed in hierarchic order) was met: 1) *B pertussis* was obtained from nasopharyngeal culture (culture-confirmed infection); 2) the enzyme-linked immunosorbent assay (ELISA) IgG or IgA titer against PT in the convalescent-phase serum sample increased by at least 100% compared with the acute-phase sample; 3) the PT-neutralizing titers in Chinese hamster ovary assay in the convalescent-phase sample increased by at least 4-fold compared with the acute-phase sample; 4) the ELISA IgG or IgA titer against filamentous hemagglutinin in the convalescent-phase sample increased by at least 100% and the culture or the polymerase chain reaction assay on the nasopharyngeal aspirate was negative for *B parapertussis*; and 5) the ELISA IgG PT titer in 1 of the 2 serum samples exceeded the geometric mean titer computed on convalescent sera of the children with a culture-confirmed *B pertussis* infection in each study group. Incidence of laboratory-confirmed *B pertussis* infection, using case definitions that varied in terms of duration and type of cough, was computed and the proportion of cases prevented among DTaP recipients in comparison with DT recipients was calculated.

Results. A total of 391 laboratory-confirmed infections were identified in the 3-year follow-up period (138 DTaP SB, 126 DTaP CB, 127 DT recipients, respectively). The mean duration of cough in children with laboratory-confirmed infection was 48, 47, and 70 days for the DTaP SB, DTaP CB, and DT recipients, respectively; the mean duration of spasmodic cough was 15, 13, and 23 days, respectively. When using the primary case definition (ie, laboratory-confirmed *B pertussis* infection and ≥ 14 days of spasmodic cough or ≥ 21 days of any cough), the efficacy was 78% for the DTaP SB vaccine (95% confidence interval [CI]: 71%–83%) and 81% for the DTaP CB vaccine (95% CI: 74%–85%). When using the case definition based on a more severe clinical presentation (≥ 21 days of spasmodic cough), the vaccine efficacy was 86% (95% CI: 79%–91%) for both vaccines. When using the case definition based on milder clinical presentation (any cough for ≥ 7 days), the efficacy was 76% (95% CI: 69%–81%) for the DTaP SB vaccine and 78% (95% CI: 72%–83%) for the DTaP CB vaccine.

Conclusions. The persistence of protection through 6 years of age suggests that the fourth DTaP dose could be

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postponed until preschool age in children who received 3-component acellular pertussis vaccines in infancy, provided that immunity to diphtheria and tetanus is maintained. Additional booster doses could be administered at older ages to reduce reactogenicity induced by multiple administrations and to optimize the control of pertussis in adolescents and young adults. *Pediatrics* 2001; 108(5). URL: <http://www.pediatrics.org/cgi/content/full/108/5/e81>; *pertussis, acellular vaccine, efficacy, follow-up, prospective study, children*.

ABBREVIATIONS. DT, diphtheria-tetanus (vaccine); DTaP, diphtheria-tetanus-acellular pertussis (vaccine); PT, pertussis toxin; FHA, filamentous hemagglutinin; PRN, pertactin; ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin; EU, ELISA units; MLD, minimal level of detection; GMT, geometric mean titer; CI, confidence interval.

Acellular pertussis vaccines are used for primary infant immunization in many Western countries.¹ In 1992–1993, a randomized, double-blind, placebo-controlled clinical trial of two 3-component acellular pertussis vaccines was started in 4 of Italy's 20 regions. The results of the first period of follow-up, which ended in 1994 (stage 1), showed that both vaccines had a protective efficacy of 84% in the first 2 years of life²; when the trial's follow-up was extended under partial blinding until the participating children had reached 33 months of age (stage 2 of the follow-up), these high levels of efficacy had persisted.³ Since 1996, the 2 vaccines have been commercially available in Italy under the names Infanrix (DTaP SB; SmithKline Beecham, Rixensart, Belgium) and Triacelluvax (DTaP CB; Chiron-Biocrine, Siena, Italy).

Although other studies have estimated the protective efficacy of various acellular pertussis vaccines,^{4–11} there are no published reports of observations beyond 2 years of primary immunization for vaccines currently in use. It thus remains to be determined whether the protective efficacy of the acellular vaccines significantly decreases over a prolonged period and whether additional doses are necessary in childhood or adolescence. To this end, the children who had participated in the Italian Pertussis Trial were followed in an unblinded manner for onset of pertussis until the end of 1998 (ie, from 3 to 6 years of age).

METHODS

Study Population

The present study was conducted among children who had been enrolled in the Italian trial in 1992–1993. During the trial, the children had been randomized to receive 3 doses of 1 of 2 acellular pertussis vaccines combined with diphtheria and tetanus toxoids (DTaP) or of a diphtheria tetanus vaccine (DT) only, at 2, 4, and 6 months of age. Both DTaP vaccines contain pertussis toxin (PT), filamentous hemagglutinin (FHA), and pertactin (PRN). The DTaP manufactured by SmithKline Beecham (DTaP SB; Infanrix) contains per dose 25 µg of PT, 25 µg of FHA, and 8 µg of PRN. The DTaP manufactured by Chiron-Biocrine (DTaP CB; Triacelluvax) contains per dose 5 µg of PT, 2.5 µg of FHA, and 2.5 µg of PRN. DTaP CB contains genetically inactivated PT; DTaP SB contains PT inactivated by formalin and glutaraldehyde.

The present study (stage 3) included those children who completed stage 2 of the follow-up and were still under active surveillance as of October 1, 1995, accounting for 4217 children who had

received DTaP SB (representing 94% of the vaccine's recipients in the initial phase of the trial), 4215 who had received DTaP CB (95% of the original recipients), and 266 who had received DT only (18% of the original recipients). Because the parents of most of the original DT placebo group accepted pertussis vaccination during stage 2 in 1995,¹² an additional 856 children were recruited in the DT group at the initiation of stage 3. These additional children were identified from the census list of children born in the same period and living in the same areas as the trial participants but who had been vaccinated in infancy with DT only. The families of these children were contacted by local nurses who were specifically hired and trained for the study. Eligible children were included in stage 3 if they had no history of either pertussis or pertussis vaccination and if a serum sample obtained at the time of enrollment had undetectable IgG against PT. Parental consent to participate in the study was obtained.

Surveillance of Pertussis

The active surveillance of pertussis was conducted from October 1, 1995, to October 31, 1998, in an unblinded manner but adopting the same follow-up procedures used in the previous stages.^{2,3} Surveillance was conducted in the field by 72 study nurses. At enrollment, parents were instructed to call the study nurse if the child developed a cough that lasted ≥ 7 days and to record the clinical characteristics of the cough episodes in a daily diary, which was reviewed and transcribed weekly by the study nurses. During the follow-up, the study nurses contacted parents on a monthly basis to ensure that cough episodes had been reported and to encourage reporting. At each monthly contact, the nurses also recorded the frequency of nursery school/kindergarten attendance in the previous month (never, rarely, often, or always) as reported by the parents as a marker of potential exposure to pertussis. The study nurses investigated each reported cough episode that was still ongoing, irrespective of its clinical characteristics, with a nasopharyngeal aspirate and an acute-phase capillary blood sample, both taken at cough detection, and with a convalescent capillary blood sample taken 6 to 8 weeks later.

Laboratory Methods

As in the previous stages of the trial, cultures for *Bordetella pertussis* and *Bordetella parapertussis* were performed on the nasopharyngeal aspirates, and the acute-phase and convalescent-phase serum samples were tested for antibodies immunoglobulin G (IgG) and IgA against PT and FHA.² The serologic testing was performed on blinded paired sera, using the enzyme-linked immunosorbent assay (ELISA) method described by Manclark et al¹³ with reference serum calibrated against reference serum samples provided by the US Food and Drug Administration (serum lot 3 or 4, Bethesda, MD). The reference-line method¹⁴ was used to calculate ELISA units per milliliter (EU/mL). The minimal level of detection (MLD) was set at 2 EU/mL for IgG PT and FHA and at 3 EU/mL and 10 EU/mL for IgA FHA and PT, respectively. Intra-assay variability was monitored by computing the daily coefficient of variability measured on the positive control serum, which was always below 30%. When the quantity of serum was sufficient, PT-neutralizing antibodies also were measured on Chinese hamster ovary cells.² Nasopharyngeal aspirates of children who had cough and were culture negative but who showed an increase only in antibodies against FHA were tested by polymerase chain reaction for *B parapertussis*.^{15,16}

Infections and Case Definitions

As in stages 1 and 2 of the trial, a cough episode that lasted ≥ 7 days was considered to be a laboratory-confirmed infection if at least 1 of the following 4 criteria (listed in hierarchic order) were met: 1) *B pertussis* was obtained from nasopharyngeal culture (culture-confirmed infection); 2) the IgG or IgA titer against PT in the convalescent-phase serum sample increased by at least 100% compared with the acute-phase sample; 3) the PT-neutralizing titers in the convalescent-phase sample increased by at least 4-fold compared with the acute-phase sample; and 4) the IgG or IgA titer against FHA in the convalescent-phase sample increased by at least 100%, and the culture or the polymerase chain reaction assay on the nasopharyngeal aspirate was negative for *B parapertussis*. For stage 3, a fifth criterion was added in the analysis of the results, and cough was classified as laboratory-confirmed infec-

tion by *B pertussis* if the IgG PT titer in 1 of the 2 serum samples exceeded the geometric mean titer (GMT) computed on convalescent sera of the children with a culture-confirmed *B pertussis* infection in each study group.

When estimating vaccine efficacy, the primary case definition of pertussis was a laboratory-confirmed *B pertussis* infection, as defined above, plus ≥ 14 days of spasmodic cough or ≥ 21 days of any cough. Additional analyses were performed using definitions based on both milder and more severe clinical presentation (ie, children with laboratory-confirmed infection plus ≥ 7 days of any cough or ≥ 21 days of spasmodic cough, respectively).

Seroprevalence at the End of Follow-Up

In 1998, children who were entering primary school and had received 3 doses of a DTaP during the trial and remained pertussis-free throughout the entire follow-up were offered a booster dose of DTaP.¹⁷ In March 1998, before the booster dose administration, capillary blood was taken from a voluntary sample of 6% of these children to determine the prevalence of circulating IgG PT antibody levels 5 years after primary pertussis immunization.

Sample Size and Statistical Analyses

As calculated for stage 1 of the trial, for the present analysis, the necessary sample size had to consist of 3300 children in each of the 2 DTaP groups and 1100 children in the DT group, based on the following: an 85% probability that the lower limit of a 2-sided 95% confidence interval (CI) for vaccine efficacy would be $>60\%$ if the true efficacy were 80% and the incidence in the DT children were 5%.¹⁸ For stage 3, under the same assumptions, the size of the DTaP groups remained sufficient, whereas the DT control group was increased to its former size to maintain the same study power.

In the statistical analyses, serologic values below the MLD were assigned a value of one half the MLD. Serologic results were analyzed on logarithmically transformed data. GMTs for acute-phase and convalescent-phase sera from culture-confirmed infections were calculated for each study group. Kruskal-Wallis analysis of variance was used to compare the distribution of days of cough and of antibody titers across the 3 study groups for the acute-phase and the convalescent-phase sera.

The individual length of follow-up for the children already under surveillance from the previous stages was calculated as the number of days that had elapsed from the beginning of stage 3 (October 1, 1995); for the children added to the DT group, it was calculated from the date of enrollment. The end of follow-up was the earliest of the following dates: 1) the date of onset of a cough with laboratory-confirmed *B pertussis* infection; 2) the date a child received pertussis vaccine after stage 2 of the trial, including a booster dose; 3) the last date of contact for children who withdrew from the study or for those with a gap in active surveillance longer than 3 months; or 4) October 31, 1998 (last day for onset of cough to be microbiologically investigated).

Vaccine efficacy was estimated in stage 3 using a person-time incidence density approach¹⁹ over the total follow-up period and grouping the follow-up period into 3 separate periods: the first with a duration of 13 months (October 1, 1995, to October 31, 1996) and the other 2 with a duration of 12 months each (from Novem-

ber 1, 1996, to October 31, 1997, and from November 1, 1997, to October 31, 1998). Efficacy was computed as $VE = 1 - R$, where VE is vaccine efficacy and R is the ratio between the incidence density among DTaP recipients and the incidence density among DT recipients. Exact CIs around vaccine-efficacy point estimates were calculated on the basis of conditional binomial distribution of cases in each vaccine group and the total number of cases.²⁰ The statistical package SAS System for Personal Computers (SAS Institute, Cary, NC) and the Statistical Package for Social Sciences for Personal Computers (SPSS Inc, Chicago, IL) were used in the analyses.

RESULTS

Study Population

The type of vaccination, gender, mean months of follow-up per child, total person-months of follow-up, and the frequency of school attendance for the 9554 children included in this analysis are shown in Table 1. The mean age of children was 2.8 years at the beginning of stage 3 and 5.8 years at the end of follow-up. The DT recipients had a lower mean length of follow-up (25 months compared with 31 months for the DTaP recipients) because not all of them had been enrolled at the beginning of stage 3. The DT recipients attended school for a slightly higher proportion of months of follow-up (ie, school attendance was defined as "always" for 57% of months of follow-up for the DT recipients, compared with 54% for the DTaP recipients). This difference was statistically significant.

Surveillance of Pertussis

A total of 1953 cough episodes were identified. Of these, 1816 (93%) were investigated with a nasopharyngeal aspirate and an acute-phase serum sample, collected at a median of 10 days from the onset of cough for all 3 study groups (Kruskal-Wallis test for the day when the sample was collected from onset of cough among the 3 study groups = 3.6; $P = .5$). The cough episodes that were not investigated were those reported after the episode had concluded. Of the episodes investigated, 122 were culture-confirmed for *B pertussis*, and an additional 163 episodes were accompanied by a significant increase in serum IgG against PT and/or FHA titers.

Infections Detected

Among the 122 children with culture-confirmed infection, an increase of at least 100% in IgG PT was

TABLE 1. Stage 3 Study Population (Aged 3–6 Years) by Vaccine Received, Gender, Length of Follow-Up, and Frequency of Nursery School/Kindergarten Attendance

	Vaccine Received		
	DTaP SB	DTaP CB	DT
Gender			
Boys	2089	2093	592
Girls	2128	2122	530
Total	4217	4215	1122
Person-months of follow-up	129 324	130 083	27 611
Average per child (mo)	30.7	30.9	24.6
Proportion months in nursery school/kindergarten			
Never	29.9%	29.6%	28.1%
Rarely	2.5%	2.6%	2.6%
Often	13.3%	13.4%	12.0%
Always	54.3%	54.4%	57.3%
Total	100%	100%	100%

observed in 38% of the DTaP SB recipients, in 48% of the DTaP CB recipients, and in 83% of the DT recipients. An increase of at least 100% in IgG FHA among children with culture-confirmed infection was observed in 72%, 74%, and 82% of the DTaP SB, DTaP CB, and DT recipients, respectively. The GMTs of IgG PT in the acute-phase sera were statistically different among the 3 study groups (31 and 32 EU/mL for DTaP SB and DTaP CB recipients, respectively, compared with 3 EU/mL for DT recipients; Kruskal-Wallis test = 27.2, $P < .01$). The GMTs in the convalescent-phase sera were 91 EU/mL for DTaP SB recipients, 97 EU/mL for DTaP CB recipients, and 104 EU/mL for DT recipients; the differences were not statistically significant and were independent of the antibody titer in the corresponding acute-phase specimen (Kruskal-Wallis test = 3.5, $P = .2$). The fifth criterion added for stage 3 (IgG PT titer in 1 of the 2 serum samples greater than the above-indicated GMT for each group, computed on convalescent sera of the children with a culture-confirmed *B pertussis* infection) allowed 106 additional infections to be identified; these were unevenly distributed among the 3 study groups. In Table 2, the 391 laboratory-confirmed infections identified in the 3-year follow-up of stage 3 are shown by study group and by laboratory criterion met.

The mean duration of cough in children with laboratory-confirmed infection was 48, 47, and 70 days for the DTaP SB, DTaP CB, and DT recipients, respectively (Kruskal-Wallis test = 29.2; $P < 10^{-3}$); the mean duration of spasmodic cough was 15, 13, and 23 days, respectively (Kruskal-Wallis test = 21.6; $P < 10^{-3}$). For both any cough and spasmodic cough, the frequency distribution of the number of days of cough was skewed to the right, with median values lower than the average, and only 31% of the children with laboratory-confirmed infections had ≥ 21 days of spasmodic cough, whereas $>80\%$ had ≥ 14 days of spasmodic cough or ≥ 21 days of any cough (Table 3). In all 3 study groups, a greater duration of cough was significantly associated with culture-confirmed infection (Kruskal-Wallis test = 35.4, $P < 10^{-6}$), with a mean duration of 65 days for the DTaP SB recipients, 63 days for the DTaP CB recipients, and 78 days for the DT recipients.

Incidence of Pertussis

As shown in Table 4, the incidence of laboratory-confirmed pertussis infections during stage 3 changed over time and reflects the annual incidence

among the general population of the same age in Italy. The incidence was highest in the last 12 months of observation (from November 1, 1997, to October 31, 1998) in all 3 study groups, regardless of the case definition used. When using the definition based on milder clinical presentation (laboratory-confirmed infection with ≥ 7 days of cough), the incidence density was 5.12 per 100 person-years among the DT recipients and 1.24 to 1.12 per 100 person-years among the DTaP recipients. When using the definition with ≥ 21 days of spasmodic cough, the incidence density per 100 person-years was 2.18 among the DT recipients, 0.30 among the DTaP SB recipients, and 0.29 among the DTaP CB recipients.

Efficacy Estimates

The vaccine efficacy calculated using the primary case definition (laboratory-confirmed infections with ≥ 14 days of spasmodic cough or ≥ 21 days of any cough) was 78% (95% CI: 71%–83%) for the DTaP SB vaccine and 81% (95% CI: 74%–85%) for the DTaP CB vaccine (Table 5). When using the case definition based on a more severe clinical presentation (≥ 21 days of spasmodic cough), the vaccine efficacy was 86% (95% CI: 79%–91%) for both vaccines. When using the case definition based on milder clinical presentation (any cough for ≥ 7 days), the efficacy was 76% (95% CI: 69%–81%) for the DTaP SB vaccine and 78% (95% CI: 72%–83%) for the DTaP CB vaccine. The CIs were wider in the first 13 months of follow-up because of the limited number of observations in the DT group and the lower incidence of pertussis.

Seroprevalence at the End of Follow-Up

At the end of stage 3, 68% of the 274 DTaP SB recipients and 57% of the 276 DTaP CB recipients who did not have laboratory-confirmed *B pertussis* infection were found to have IgG PT titers below or equal to the MLD, and the 90th percentile was 8 EU/mL (4 times the MLD). Only 2% of the children tested in both study groups (6 children in each group) had an IgG PT >100 EU/mL, and the cutoff values for confirming pertussis infection (criterion 5 in laboratory confirmation) corresponded to the 98th percentile for both the DTaP SB and the DTaP CB recipients.

DISCUSSION

The estimates of vaccine efficacy in children who were followed from 3 through 6 years of age are

TABLE 2. Number of Cough Episodes and Laboratory-Confirmed Infections, by Hierarchic Diagnostic Laboratory Criteria and by Vaccine Received

	Vaccine Received			Total
	DTaP SB	DTaP CB	DT	
Cough episodes	830	781	342	1953
Laboratory-investigated (%)	94%	93%	95%	93%
Laboratory-confirmed <i>B pertussis</i> infections	138 (100%)	126 (100%)	127 (100%)	391 (100%)
Culture-confirmed	35 (25.3%)	30 (23.8%)	57 (44.9%)	122 (31.2%)
Increase in antibody titers	50 (36.2%)	57 (45.4%)	56 (44.1%)	163 (41.7%)
Single IgG PT titer	53 (38.4%)	39 (30.9%)	14 (11.0%)	106 (27.1%)

TABLE 3. Number of Laboratory-Confirmed *B pertussis* Infections in Children Aged 3 to 6 Years by Duration and Type of Cough and by Vaccine Received

Cough and Duration	Vaccine Received			Total
	DTaP SB	DTaP CB	DT	
Any cough ≥ 7 d	138 (100%)	126 (100%)	127 (100%)	391 (100%)
Spasmodic cough ≥ 14 d or any cough ≥ 21 d	115 (83%)	103 (82%)	117 (92%)	335 (86%)
Spasmodic cough ≥ 21 d	33 (24%)	33 (26%)	54 (43%)	120 (31%)

TABLE 4. Stage 3 Incidence of Laboratory-Confirmed Pertussis by Vaccine Received, by Duration and Type of Cough, and by Interval of Follow-Up*

Cough and Duration	Vaccine Received†	Interval						Total Stage 3	
		1/10/95–31/10/96		1/11/96–31/10/97		1/11/97–31/10/98		Cases	Rate*
		Cases	Rate*	Cases	Rate*	Cases	Rate*		
Any cough ≥ 7 d	SB	38	0.89	31	0.84	69	2.13	138	1.24
	CB	30	0.70	29	0.78	67	2.06	126	1.12
	DT	11	1.87	35	3.57	81	8.92	127	5.12
≥ 14 d spasmodic or ≥ 21 d any cough	SB	30	0.70	26	0.70	59	1.83	115	1.03
	CB	24	0.56	22	0.59	57	1.75	103	0.92
	DT	11	1.87	27	2.75	79	8.70	117	4.72
≥ 21 d spasmodic cough	SB	10	0.24	7	0.19	16	0.50	33	0.30
	CB	11	0.26	6	0.16	16	0.49	33	0.29
	DT	8	1.36	14	1.43	14	1.43	54	2.18

* Rates per 100 person-years of observation. Notification rates of pertussis for the corresponding birth cohorts and geographical areas in the general population per 1000 inhabitants were 8.9 in 1996, 6.5 in 1997, and 14.8 in 1998.

† SB indicates DTaP SB; CB, DTaP CB.

TABLE 5. Estimates of Vaccine Efficacy and 95% CI by Vaccine Received and by Interval of Follow-up

Cough and Duration	Vaccine Received	Interval			Total Stage 3 (VE % [95% CI])
		1/10/95–31/10/96 (VE % [95% CI])	1/11/96–31/10/97 (VE % [95% CI])	1/11/97–31/10/98 (VE % [95% CI])	
Any cough ≥ 7 d	SB	52 (0–76)	76 (61–86)	76 (66–83)	76 (69–81)
	CB	62 (16–82)	78 (63–87)	77 (68–83)	78 (72–83)
≥ 14 d spasmodic or ≥ 21 d any cough	SB	62 (16–82)	74 (54–86)	79 (70–85)	78 (71–83)
	CB	70 (32–86)	78 (61–88)	80 (71–86)	81 (74–85)
≥ 21 d spasmodic cough	SB	83 (49–93)	87 (65–95)	86 (74–93)	86 (79–91)
	CB	81 (46–93)	89 (69–96)	86 (74–93)	86 (79–91)

VE indicates vaccine efficacy; SB, DTaP SB; CB, DTaP CB.

highly consistent with the results from previous stages, thus demonstrating continued high levels of protection.^{2,3} Furthermore, the degree of protection was similar for the 2 DTaP vaccines, indicating an equivalence of performance of vaccines containing the same antigens.

The ongoing circulation of *B pertussis* in Italy provided an excellent opportunity to assess vaccine efficacy during years of both lower (1996 and 1997) and higher (1998) background incidence. Although vaccination coverage among infants in Italy increased from an estimated 40% for the 1991 birth cohort²¹ to 88% for the 1996 birth cohort,²² pertussis remains a common childhood disease and continues to show a 4-year cyclic pattern, with synchronous epidemic waves across the country, which appear to be sustained by the large number of nonimmunized school-aged children.

Unlike previous retrospective community-based studies and studies that used passive surveillance for estimating long-term effectiveness,^{23,24} we used a prospective approach and active surveillance, thus reducing the likelihood of systematic differences in

diagnostic sensitivity and in case confirmation between vaccinated and unvaccinated groups. For episodes of suspected pertussis, the time elapsed between the onset of cough and the collection of biological specimens and the proportion of coughs that were microbiologically investigated were similar when comparing vaccinated and unvaccinated children. That active surveillance enhanced the sensitivity of case detection in our study is indicated by the high incidence of pertussis, which was at least 10 times higher than that based on statutory notifications.

In the stage 3 follow-up, although we adopted the basic methods used in stages 1 and 2 for pertussis surveillance and for laboratory confirmation, some important modifications were introduced to address the prolonged follow-up period and the need to convert the trial into an observational study, and these modifications may have introduced biases. First, there may have been problems of comparability among the study groups. For any prospective study with long follow-up periods, initially randomized populations may not be fully comparable even after

relatively short follow-up intervals, and potential biases are difficult to identify. In our study, we enrolled new same-aged children in the DT group. To maintain comparability for these children, they were chosen from the same general population used for stage 1 and were required to have had a documented negative history of vaccination and negative serology for *B pertussis* infection. Although these children had a greater risk of exposure to pertussis, as suggested by nursery school/kindergarten attendance and mean family size, it is unlikely that this greatly affected our estimates of vaccine efficacy.

Another source of potential bias is that the cough surveillance in stage 3 was conducted in unblinded conditions (ie, the parents and study nurses were aware of the child's vaccination status). Indeed, that the parents of the DTaP children were aware that their children had received an efficacious vaccine, in addition to their having already participated in the study for several years, may have made them less likely than the parents of the newly enrolled DT children to report cough episodes, despite that active surveillance methods helped to ensure a similar risk of detection of cases. However, the serologic evidence suggests that this was not the case. The seroprevalence of IgG PT at the end of stage 3, which was measured to determine humoral immunity 4 years after primary immunization, also can be used as a marker of missed infections in vaccinated children, whose circulating antibodies against PT, FHA, and PRN from primary immunization have been shown to disappear over time.²⁵ Although the significance of serology in the absence of clinical manifestations is unclear, it is reassuring that only 10% of the DTaP children tested had IgG PT above 8 EU/mL and only 2% above 100 EU/mL (this latter case is probably attributable to the recent exposure to pertussis in the period of peak incidence). Although seroprevalence for IgG PT was not measured at the same time for DT children in stage 3, the percentage of children with high IgG PT is remarkably consistent with that obtained at the end of stage 1 (with surveillance under blinded conditions) in the randomized DT group of children who subsequently received pertussis vaccination. In this group, 3.9% were found to have IgG PT titers higher than the MLD and 2% had titers above 100 EU/mL.¹²

A third source of potential bias is the laboratory criteria of the case definition. In the unvaccinated group, the proportion of culture-confirmed infections seems to be age-dependent: it decreased from 82% in stage 1, when the mean age of children was 24 months, to 72% in stage 2 (mean age: 33 months), and to 45% in stage 3, when these children were between 33 and 69 months of age. The duration of both any cough and spasmodic cough in children with culture-confirmed infection also decreased over the 3 study stages. Furthermore, we found that the serologic diagnosis based on antibody conversion was impaired at older ages by previous vaccination, because more than half of the vaccinated children with culture-confirmed *B pertussis* demonstrated high titers against IgG PT within 10 days of the onset of cough, when most of the acute-phase specimens

were taken. The effects of the lower sensitivity of culture as well as of the rapid increase in antibody titers among the vaccinated children could have resulted in the overestimation of the true efficacy. To compensate for this, we used an additional criterion, the IgG PT titer, in 1 of the serum specimens greater than the GMT for children with culture-confirmed infection in the same study group. The use of IgG PT titer in a single serum specimen has already been reported in the literature,^{26,27} and both humoral²⁸ and cell-mediated immune response to PT²⁹ were found to be the main markers of pertussis infection. If misclassification of cases did occur because of the new criterion, then the bias would have been in the direction of underestimating the vaccine efficacy.

The finding that the duration of both any cough and spasmodic cough was shorter in stage 3, compared with the previous stages, confirms the limitation of using ≥ 21 days of spasmodic cough as the clinical criterion in the case definition in older children. Our primary case definition thus included ≥ 14 days of spasmodic cough or ≥ 21 days of any cough. The duration of any cough and spasmodic cough was longer for infected unvaccinated children compared with infected same-age DTaP recipients. These findings suggest that even 5 years after primary immunization, most of the "vaccine failures" show a milder clinical presentation of pertussis.

CONCLUSION

Our results on the persistence of protection through 6 years of age suggest that the fourth dose of DTaP could be postponed until preschool age in children who received 3-component acellular pertussis vaccines in infancy, provided that immunity to diphtheria and tetanus is maintained. Additional booster doses could be administered at older ages to reduce reactogenicity induced by multiple administrations¹⁷ and to optimize the control of pertussis in adolescents and young adults.

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