

## A PLACEBO-CONTROLLED TRIAL OF A PERTUSSIS-TOXOID VACCINE

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**Abstract Background.** Although many whole-cell vaccines have been effective in preventing pertussis, these vaccines are difficult to standardize and can produce side effects. In Sweden, pertussis became endemic during the 1970s despite vaccination. Because of its limited efficacy, the Swedish-made whole-cell vaccine was withdrawn in 1979.

**Methods.** To evaluate the efficacy of an acellular vaccine consisting of pertussis toxin inactivated by hydrogen peroxide (pertussis toxoid), we conducted a randomized, double-blind, placebo-controlled trial in Sweden. Infants were vaccinated with either diphtheria and tetanus toxoids alone (DT toxoids, 1726 infants) or diphtheria, tetanus, and pertussis toxoids (DTP toxoids, 1724 infants) at 3, 5, and 12 months of age.

**Results.** There were no serious reactions. With the pertussis vaccine there were slightly more local reactions than with the DT toxoids alone, but the rates of postvaccination fever were the same. The main period of surveil-

lance, which began 30 days after the third vaccination, continued for a median of 17.5 months. There were 312 cases of pertussis (72 in the DTP-toxoids group and 240 in the DT-toxoids group) that met the clinical criterion (paroxysmal cough lasting  $\geq 21$  days) and laboratory criteria for pertussis as defined by the World Health Organization. The efficacy of this acellular vaccine was 71 percent (95 percent confidence interval, 63 to 78 percent). The recipients of DTP toxoids who had pertussis had cough of shorter duration than the recipients of DT toxoids, and fewer had whooping and vomiting. The vaccine efficacy after two doses was 55 percent (95 percent confidence interval, 12 to 78 percent), on the basis of 14 cases in the DTP-toxoids group and 31 in the DT-toxoids group that met the definition of the World Health Organization.

**Conclusions.** A pharmacologically inert, acellular pertussis-toxoid vaccine that is easily standardized is safe and confers substantial protection against pertussis. (N Engl J Med 1995;333:1045-50.)

VACCINES consisting of inactivated *Bordetella pertussis* induce protection against pertussis, but estimates of their efficacy vary from 40 to 90 percent.<sup>1</sup> There is no consensus about what the protective moieties are in whole-cell vaccines or what mechanism induces protection. The potency of whole-cell vaccines is estimated by the mouse protection test, which has considerable variability.<sup>2,3</sup> Local reactions and fever are frequent after the administration of whole-cell vaccines, which are also associated with hypotonic-hyporesponsive events, persistent crying, and convulsions.<sup>4</sup> These limitations of whole-cell vaccines have stimulated the development of vaccines made with purified antigens, which should have fewer side effects and be easier to standardize. All acellular vaccines contain detoxified pertussis toxin; some also contain filamentous hemagglutinin, pertactin, and fimbriae.<sup>5</sup>

Because every vaccine component has the potential for causing side effects, we evaluated the safety and efficacy of a single-component, pharmacologically inert pertussis toxoid. Pertussis toxin is a protein with a central role in the pathogenesis of pertussis.<sup>2,6</sup> Open studies and a double-blind study indicate that pertussis toxoids induce protection.<sup>7-9</sup> The present trial was performed in Sweden, where pertussis has been endemic since the early 1970s, when changes in production made the Swedish-made whole-cell vaccine ineffective.<sup>10</sup>

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When the incidence of pertussis increased despite vaccination rates of about 90 percent, the recommended infant vaccine was withdrawn in 1979. Since then, Sweden has had no licensed pertussis vaccine. Accordingly, it was possible to perform a placebo-controlled study. Inclusion of a whole-cell vaccine was considered undesirable because renewed licensure of a whole-cell vaccine in Sweden was highly unlikely.

## METHODS

### Study Design

The study was double-blind and placebo-controlled. Infants were randomly assigned to be vaccinated with diphtheria and tetanus toxoids (DT toxoids) or to receive the same DT toxoids with pertussis toxoid (DTP toxoids). Vaccines were injected subcutaneously in the anterolateral part of the thigh at 3, 5, and 12 months of age, as is recommended for DT toxoids in Sweden.

The study protocol was reviewed by the institutional review board of the National Institute of Child Health and Human Development; the Food and Drug Administration (FDA); the Medical Products Agency, Uppsala, Sweden; and the Ethics Committee, Göteborg University. All parents gave written consent after receiving oral and written information.

### Vaccines

The pertussis toxoid inactivated by hydrogen peroxide was developed at the National Institute of Child Health and Human Development and manufactured by Amvax (Beltsville, Md.). The vaccine elicits serum antibodies that neutralize pertussis toxin.<sup>8,11</sup> The DT toxoids were manufactured by Statens Seruminstitut (Copenhagen, Denmark). One 0.5-ml dose (DT-toxoids lot K36, DTP-toxoids lot K36-567) consisted of 25 flocculation units of diphtheria toxoid and 7 flocculation units of tetanus toxoid adsorbed onto 0.5 mg of aluminum hydroxide with 0.01 percent thimerosal, with or without 40  $\mu$ g of pertussis toxoid. The purity of pertussis toxin was demonstrated by sodium dodecyl sulfate-polyacrylamide-gel electrophoresis. The inactivation of pertussis toxin was verified by tests for histamine sensitization, toxicity in Chinese-hamster-ovary cells, and hemagglutinating activity. The DTP-toxoids vaccine passed the rabbit pyrogenicity and general-safety assays of the FDA. The DT toxoids and DTP toxoids

were identical in appearance. Vials were filled and coded in sets of three, one set for each child, according to a computer-based randomization list. No other vaccine was given with the study vaccines.

### Recruitment and Study Cohort

Full-term, healthy infants in the Göteborg area were eligible for the study if the family had a telephone and if a parent spoke Swedish. About 99 percent of Swedish children visit publicly financed child health centers, where the first information about the study was given to parents. Vaccinations and follow-up were performed by project nurses at five study sites.

During the recruitment period, 7040 newborns were registered at 96 child health centers in the area. The parents of 3450 of 5964 eligible children (58 percent) agreed to participate. Of those, 1724 were randomly assigned to receive DTP toxoids, and 1726 to receive DT toxoids. The 1750 boys and 1700 girls were evenly distributed between the two groups. There were 817 recipients of DTP toxoids and 850 recipients of DT toxoids with one or more older siblings. At 13 months, 8 percent of the DTP-toxoids group attended day-care centers, as compared with 7 percent of the DT-toxoids group; at 27 months, the respective figures were 55 and 51 percent. The first vaccination was given to the 3450 participants between September 1991 and September 1992. The second was given to 3425 children and the third to 3379 between May 1992 and July 1993.

Fifty-two children (1.5 percent; 30 in the DTP-toxoids group and 22 in the DT-toxoids group) were withdrawn from the study, 27 before and 25 after the third vaccination. Twenty-three moved, contraindicating diseases developed in 2, 1 had pertussis before the first vaccination, 1 died, and the parents of 25 declined further participation.

Coughing episodes starting between the first vaccination and July 24, 1994, were included in the study analysis. On that day the median age of the children was 30.5 months (range, 26 to 37). The surveillance period was divided into two parts: from the first vaccination until 29 days after the third, and from 30 days after the third vaccination until July 24, 1994 (the main period of follow-up). The median duration of follow-up during the main period was 17.5 months (range, 13 to 24). The randomization code was broken on November 18, 1994.

### Side Effects

Parents measured the children's rectal temperatures 6, 24, and 48 hours after each vaccination and inspected the injection site daily for seven days, after which time they were interviewed about their children's temperatures, local reactions, and such events as convulsions, symptoms of the hypotonic-hyporesponsive syndrome, and persistent crying. During the study, the parents were contacted monthly by telephone. Visits to doctors and hospitalizations were noted. Between August 1 and November 18, 1994, a final interview was conducted with parents of 3413 of the 3450 children originally enrolled (99 percent).

### Coughing Episodes

Parents were instructed to contact the study nurse if anyone in the family coughed for seven or more days. A nasopharyngeal sample for culture and polymerase-chain-reaction (PCR) analysis and a serum sample were obtained. A serum sample was also obtained during convalescence, at least four weeks later. The duration of cough, paroxysms, whooping, vomiting, fever, and rhinitis and the use of antibiotics were recorded. Follow-up continued for at least 60 days or until the cough ended.

We followed the guidelines of the Swedish Board of Health and Welfare, which recommend prophylaxis with erythromycin for infants under 6 months of age and early treatment for infants 6 to 11 months old and for siblings of infants under 12 months old. All prescriptions of macrolides were documented.

### Laboratory Assays

Secretions obtained with nasopharyngeal swabs were cultured on Regan-Lowe medium<sup>12</sup> and in an enrichment medium (Regan-Lowe with 50 percent charcoal agar), and PCR was used to detect *B. pertussis* and *B. parapertussis*.<sup>13,14</sup> Colonies of *B. pertussis* and

Table 1. Categories of Coughing Episodes in the Göteborg Classification.

1. Confirmed pertussis
<i>B. pertussis</i> isolated from the nasopharynx
Two criteria in Table 2 met, including at least one major criterion
IgG antibodies against both pertussis toxin and filamentous hemagglutinin $\geq 6000$ in the same single convalescence serum sample
2. Probable pertussis
One major criterion in Table 2 met
3. Clinical pertussis not verified because of absent or suboptimal serum sampling
$\geq 21$ days of paroxysmal cough and contact with a clinical case (except as under criterion C, Table 2)
4. Confirmed parapertussis
<i>B. parapertussis</i> isolated from the nasopharynx
Occurrence of two of the following: <i>B. parapertussis</i> isolated from household contact, a positive parapertussis PCR test, and a significant increase in IgG antibodies against filamentous hemagglutinin without an increase in antibodies against pertussis toxin
5. Bordetella infection unlikely or ruled out
All cases not meeting any of the criteria for categories 1, 2, 3, or 4

*B. parapertussis* were verified by Gram's staining, agglutination, and biochemical tests.

Antibodies against pertussis toxin and filamentous hemagglutinin were measured by enzyme-linked immunosorbent assay<sup>15,16</sup> in duplicate in eight threefold dilutions starting from 1:10 for IgG and 1:100 for IgM and IgA. The pertussis toxin was obtained from Amvax, and the filamentous hemagglutinin from the Institut Pasteur Mérieux, (Marcy l'Etoile, France). The reference was pertussis antiserum lot 3 from the FDA. Titers were defined as the reciprocal serum dilution corresponding to an absorbance of 0.2 above the background level. On the basis of intraassay variation and variation in individual subjects, threefold increases were considered statistically significant if the titer in the serum sample obtained during convalescence was  $\geq 200$ . On the basis of a pilot study, titers of  $\geq 6000$  for IgG antibodies against pertussis toxin and filamentous hemagglutinin concurrently in a single convalescence serum sample were considered a sign of pertussis (data not shown).

Serum was obtained from 3361 children at least four weeks after the third vaccination. IgG antibodies against pertussis toxin and toxin-neutralizing antibodies<sup>17</sup> were measured.

### Case Definitions

Coughing episodes were classified according to the criteria of the World Health Organization (WHO)<sup>18</sup> and according to the Göteborg classification (Tables 1 and 2) developed by the authors. The WHO definition of pertussis includes a paroxysmal cough lasting 21 days or more and at least one of the following: a positive culture, a positive culture in a family member with onset of pertussis within 28 days before or after the onset of the episode studied, and a statistically significant increase in IgG antibodies against pertussis toxin or filamentous hemagglutinin.

The Göteborg classification was developed because the WHO definition accepts only household contact confirmed by culture as a criterion and because an acute-phase serum sample, necessary for the detection of an increase in antibodies, is not always obtained. Moreover, increases in filamentous-hemagglutinin antibodies can be seen in both pertussis and parapertussis and these antibodies cross-react with outer-membrane proteins of nonencapsulated *Haemophilus influenzae*.<sup>19</sup>

Children with confirmed, probable, or unverified pertussis (categories 1, 2, and 3 in Table 1) were considered at risk for pertussis until the first day of cough. Children with parapertussis (category 4) were considered to be still at risk for pertussis.<sup>20</sup>

### Statistical Analysis

The efficacy of the pertussis vaccine was estimated as  $1 - R$ , where  $R$  is the ratio of incidence rates (the number of cases divided by the total time at risk) in recipients of DTP toxoids and DT toxoids. Ninety-five percent confidence intervals for efficacy were estimated by exact calculation<sup>21</sup> based on the conditional binomial distribution that

Table 2. Major and Minor Criteria for Pertussis in the Göteborg Classification.

Major criteria	
A.	Significant increase in IgG antibodies against pertussis toxin* (this finding was discounted if vaccination was performed between the two serum samples or if the first serum sample was obtained $\leq$ 60 days before vaccination)
B.	Significant increase in IgG antibodies against filamentous hemagglutinin*
C.	Household contact with pertussis confirmed by culture* or by serologic studies and PCR and onset of cough not differing by $>$ 28 days between the two cases
Minor criteria	
D.	Significant increase or decrease in IgM or IgA antibodies (or both) against pertussis toxin (assay performed only in cases with significant increases in IgG antibodies against filamentous hemagglutinin but not against pertussis toxin)
E.	Significant increase or decrease in IgM or IgA antibodies (or both) against filamentous hemagglutinin (assay performed only in cases with significant increases in IgG antibodies against pertussis toxin but not filamentous hemagglutinin)
F.	Positive pertussis PCR test
Combined confirmatory criterion	
G.	IgG antibodies against both pertussis toxin and filamentous hemagglutinin $\geq$ 6000 in the same single convalescence serum sample (this finding was discounted if a vaccination was performed $\leq$ 130 days before the serum sample was obtained)

\*Criterion fits the WHO definition if there is paroxysmal cough that lasts  $\geq$ 21 days.

follows from the assumption of a Poisson distribution for cases in each group.<sup>22</sup> Proportions were compared with a two-sided Fisher's exact test, and continuous variables with the two-sided permutation t-test. P values of less than 0.05 were considered to indicate statistical significance.

## RESULTS

### Safety

There were no serious reactions to the vaccines. No child was withdrawn because of an adverse reaction. The frequency of fever within 48 hours of vaccination did not differ between the groups. Redness and swelling increased with the number of vaccinations and were somewhat more common among recipients of DTP toxoids (Table 3). Irritability and feeding and sleeping problems were equally frequent in the two groups. No child had the hypotonic-hyporesponsive syndrome, persistent or high-pitched crying, or afebrile convulsions. Two recipients of DTP toxoids had febrile convulsions within 48 hours after the third vaccination; both had respiratory tract infections. The one child who died was a DTP-toxoids recipient who had pneumococcal endocarditis. Four children, all recipients of DT toxoids, had invasive bacterial infections with favorable outcomes. Malignant diseases developed in five children (two in the DTP-toxoids group and three in the DT-toxoids group). The frequency of other serious diseases was similar in the two groups.

### Bordetella Infections

There were 2037 coughing episodes lasting at least seven days with onset before July 25, 1994, in children in the study. Of those, 465

(160 in the DTP-toxoids group and 305 in the DT-toxoids group) met the criteria for confirmed or probable pertussis (categories 1 and 2, Table 1), including 368 (92 in the DTP-toxoids group and 276 in the DT-toxoids group) that met the WHO definition. Another 14 children (4 in the DTP-toxoids group and 10 in the DT-toxoids group) had clinical pertussis without laboratory confirmation (category 3). Twelve children (seven in the DTP-toxoids group and five in the DT-toxoids group) had confirmed parapertussis. None of the 491 children with bordetella infections were hospitalized.

In family members, 336 of 917 coughing episodes met the WHO definition. Another 98 family members had confirmed or probable pertussis according to the Göteborg classification. Twenty-two episodes met the criteria for category 3. Four family members had confirmed parapertussis.

### Efficacy during the Main Period of Follow-up

Thirty days after the third vaccination, 1670 recipients of DTP toxoids and 1665 recipients of DT toxoids remained at risk. The incidence of pertussis, according to the WHO definition, was 2.96 cases per 100 person-years among the recipients of DTP toxoids and 10.32 cases per 100 person-years among the recipients of DT toxoids, respectively. The efficacy of the pertussis vaccine was 71 percent (Table 4).

Nine children who had paroxysmal cough for 21 days or more (three in the DTP-toxoids group and six in the DT-toxoids group) did not meet the WHO definition but had confirmed or probable pertussis according to the Göteborg classification. Seven had elevated titers of IgG antibodies against both pertussis toxin (7156 to 65,610) and filamentous hemagglutinin (8620 to 21,870) in serum samples obtained during convalescence. Two had household contacts with serologically confirmed pertussis.

Sixty-two children (50 in the DTP-toxoids group and 12 in the DT-toxoids group) received diagnoses of confirmed or probable pertussis with paroxysmal cough lasting less than 21 days. The incidence of pertussis including all children with cough lasting at least seven days was 5.13 cases per 100 person-years among the re-

Table 3. Occurrence of Fever within 48 Hours and Local Reactions within 7 Days in Children Vaccinated with DTP Toxoids or with DT Toxoids.

CONDITION	FIRST VACCINATION		SECOND VACCINATION		THIRD VACCINATION	
	DTP TOXOIDS (N = 1724)	DT TOXOIDS (N = 1726)	DTP TOXOIDS (N = 1708)	DT TOXOIDS (N = 1717)	DTP TOXOIDS (N = 1692)	DT TOXOIDS (N = 1687)
	<i>percentage of children</i>					
Temperature ( $^{\circ}$ C)						
$\geq$ 38	6	6	11	11	17	17
$\geq$ 40	0.1	0	0.1	0.1	0.6	0.5
Redness (mm)						
$\geq$ 20	2	2	18*	11*	30*	24*
$\geq$ 60	0	0.1	0.1	0.1	4*	3
Swelling (mm)						
$\geq$ 20	2	2	13*	8*	24*	19*
$\geq$ 60	0	0.1	0.4	0.3	5*	3*

\*P $<$ 0.01 for the comparison between the groups.

recipients of DTP toxoids and 11.09 cases per 100 person-years among the recipients of DT toxoids (efficacy of the pertussis vaccine, 54 percent) (Table 4).

Periods of cough, vomiting, and whooping were significantly shorter in children with pertussis in the DTP-toxoids group than in those in the DT-toxoids group (Table 5). There were no significant differences in efficacy between boys and girls or among the five study sites and no decreasing efficacy with time after the third vaccination.

#### Possible Sources of Bias

Nine children (two in the DTP-toxoids group and seven in the DT-toxoids group), who were not included in efficacy estimates, had clinical pertussis without laboratory confirmation (category 3). Eight (four of whom were positive for *B. pertussis* by PCR) had high values for IgG antibodies against pertussis toxin and filamentous hemagglutinin in both the initial and convalescence serum samples but did not have significant increases or titers of  $\geq 6000$  for both antibodies. Their first serum samples were obtained more than 10 days after the onset of cough. No serum samples were available from one child who was positive by PCR.

Three cases of pertussis in recipients of DT toxoids with onset 30 to 60 days after the third vaccination were not included in efficacy estimates because of the timing of serum collection (Table 2). Two patients had increases in IgG antibodies against pertussis toxin, and one had titers of IgG antibodies against pertussis toxin and filamentous hemagglutinin above 6000. Erythromycin was given to 5 of 125 recipients of DTP toxoids with pertussis (4 percent) and to 17 of 258 recipients of DT toxoids with pertussis (7 percent) within 14 days after onset of cough. The parents of 25 of the 52 children

Table 4. Cases of Pertussis and Efficacy of the Pertussis Vaccine during the Main Period of Follow-up (from 30 Days after the Third Vaccination until the End of the Study).\*

	WHO DEFINITION†			GÖTEBORG CLASSIFICATION		
	CONFIRMED		CONFIRMED + PROBABLE			
<b>Paroxysmal cough lasting <math>\geq 21</math> days</b>						
No. of cases						
DTP-toxoids group	72	58	75			
DT-toxoids group	240	236	246			
Vaccine efficacy — % (95% CI)	71 (63–78)	77 (69–83)	71 (62–78)			
<b>Cough lasting <math>\geq 21</math> days</b>						
No. of cases						
DTP-toxoids group	96	77	99			
DT-toxoids group	245	241	252			
Vaccine efficacy — % (95% CI)	63 (52–71)	69 (60–77)	62 (52–71)			
<b>Cough lasting <math>\geq 7</math> days</b>						
No. of cases						
DTP-toxoids group	121	98	125			
DT-toxoids group	251	244	258			
Vaccine efficacy — % (95% CI)	54 (43–63)	62 (51–70)	54 (42–63)			

\*Numbers of cases are cumulative from top to bottom. CI denotes confidence interval.

†All cases with cough lasting  $\geq 7$  days and meeting at least one laboratory criterion of the WHO definition have been included. The true WHO definition only includes cases with paroxysmal cough lasting  $\geq 21$  days.

Table 5. Comparison of Clinical Symptoms among Recipients of DTP Toxoids and of DT Toxoids during the Main Period of Follow-up, According to the Classification of Pertussis.\*

SYMPTOM	WHO DEFINITION†		GÖTEBORG CLASSIFICATION‡	
	DTP-TOXOIDS GROUP (N = 72)	DT-TOXOIDS GROUP (N = 240)	DTP-TOXOIDS GROUP (N = 125)	DT-TOXOIDS GROUP (N = 258)
	<i>median no. of days (percentage of children with symptoms)</i>			
Cough	49	59	37	59
Paroxysmal cough	42	51	28	50
Vomiting	0 (46)	20 (78)	0 (35)	20 (76)
Whooping	0 (39)	24 (78)	0 (28)	22 (74)

\* $P < 0.001$  for all comparisons between the groups.

†Only patients who had  $\geq 21$  days of paroxysmal cough are included, since this is one of the criteria in the WHO definition.

‡All confirmed and probable cases with  $\geq 7$  days of cough are included.

withdrawn from the study were interviewed during the autumn of 1994 before the randomization code was broken. Three children (two in the DTP-toxoids group and one in the DT-toxoids group) had culture-confirmed pertussis after leaving the study.

#### Comparison of Diagnostic Assays

Cultures were performed for 357 of 383 children with confirmed or probable pertussis. The proportion of positive cultures was lower in DTP-toxoids recipients than in DT-toxoids recipients (49 percent [56 of 115] vs. 64 percent [154 of 242],  $P < 0.01$ ). Results of the PCR were similar: 55 of 108 DTP-toxoids recipients with pertussis (51 percent) and 155 of 225 DT-toxoids recipients with pertussis (69 percent) were positive ( $P < 0.01$ ).

Paired serum samples were available for 345 of 383 children with confirmed or probable pertussis. Significant increases in IgG antibodies against pertussis toxin were less common in recipients of DTP toxoids than in recipients of DT toxoids (31 percent [33 of 107] vs. 89 percent [201 of 226],  $P < 0.001$ ) because 80 percent of DTP-toxoids recipients had values above 1000 for these antibodies in the acute-phase serum samples, as compared with 20 percent of DT-toxoids recipients. Significant increases in IgG antibodies against filamentous hemagglutinin were seen in 94 percent of the DTP-toxoids recipients (104 of 111) and 92 percent of the DT-toxoids recipients (216 of 234).

A significant increase in IgG antibodies against filamentous hemagglutinin was the only criterion met in 29 cases of pertussis (22 in the DTP-toxoids group and 7 in the DT-toxoids group). In all cases there was a clinical picture of bordetella infection, with known exposure, paroxysmal cough, and little or no rhinitis or fever. Nineteen cases (15 in the DTP-toxoids group and 4 in the DT-toxoids group) met the WHO definition of at least 21 days of paroxysmal cough. Seventeen involved whooping or vomiting. Four of seven DT-toxoids recipients with increases only in IgG antibodies against filamentous hemagglutinin did not have IgG, IgM, or

IgA antibodies against pertussis toxin in their acute-phase and convalescence serum samples, suggesting parapertussis rather than pertussis. The other three DT-toxoids recipients had IgG antibodies against pertussis toxin in both serum samples, indicating pertussis. The 22 recipients of the DTP toxoids had IgG antibodies against pertussis toxin in both serum samples.

#### Efficacy before the Main Period of Follow-up

From the first vaccination to 29 days after the second, 13 children (7 in the DTP-toxoids group and 6 in the DT-toxoids group) had confirmed or probable pertussis with cough lasting at least 7 days. Eleven of these cases met the WHO definition.

From 30 days after the second vaccination to 29 days after the third, there were 69 cases with cough lasting at least 7 days (28 in the DTP-toxoids group and 41 in the DT-toxoids group; efficacy of pertussis vaccine, 31 percent; 95 percent confidence interval, -14 to 59 percent). Of the 69 cases, 45 met the WHO definition (14 in the DTP-toxoids group and 31 in the DT-toxoids group; efficacy of the pertussis vaccine, 55 percent; 95 percent confidence interval, 12 to 78 percent).

#### Serologic Responses

Geometric mean antibody titers were determined at least four weeks after the third vaccination in 71 DTP-toxoids recipients who later had pertussis according to the WHO definition. The values were 5407 for IgG antibodies against pertussis toxin (range, 881 to 77,990) and 200 for neutralizing antibodies (range, 40 to >640). Corresponding titers for 433 randomly selected children in whom pertussis did not develop were 7801 (528 to 65,610) and 244 (40 to >640).

#### DISCUSSION

This study shows the safety of the acellular pertussis-toxoid vaccine. Apart from slight increases in local reactions in recipients of DTP toxoids as compared with recipients of DT toxoids, no adverse reactions were observed.

Pertussis is endemic in Sweden, with epidemic peaks about every third year. A peak occurred during the winter of 1993–1994. Seventeen percent of DT-toxoids recipients had pertussis during the trial. After three injections, the efficacy of the acellular vaccine against pertussis with at least 21 days of paroxysmal cough was 71 to 77 percent, depending on the case definition, and 54 percent when all cases with cough lasting at least 7 days were included. The disease had a shorter duration and lower frequency of whooping and vomiting in vaccinated children than in unvaccinated ones.

The evaluation of coughing episodes that resemble pertussis but do not meet laboratory criteria because of suboptimal diagnostic sampling indicated no overestimation of efficacy due to missed cases. The nine cases in category 3 had the same distribution among vaccine groups as cases that met the WHO definition (two in the DTP-toxoids group and seven in the DT-toxoids group).

Two other potential causes of bias — erythromycin use and pertussis among children who were withdrawn from the study — were ruled out. The rate of erythromycin use was low (6 percent) and similar in the two groups, and the withdrawal rate was only 1.5 percent.

It is difficult to make detailed comparisons of the efficacy of this pertussis vaccine and that of others. Randomized, placebo-controlled trials of whole-cell vaccines have not been performed since the 1950s, when diagnostic methods were different.<sup>23</sup> A review of 39 open studies of whole-cell vaccines found efficacy estimates of 40 to 90 percent.<sup>1</sup> Several factors influence the estimates, including case definitions and rates of pertussis in the population studied. Efficacy estimates were higher when only cases of long duration were included, when laboratory confirmation was required, or when the attack rate in the unvaccinated population was relatively low. Furthermore, the amount of pertussis toxoid varies among whole-cell vaccines.<sup>24,25</sup> During the 1980s, a double-blind, placebo-controlled efficacy trial was performed in Sweden with a one-component pertussis toxoid and a two-component vaccine composed of pertussis toxoid and filamentous hemagglutinin.<sup>9</sup> Both vaccines reduced the incidence and severity of pertussis. Since only two doses were given and the children were 6 to 11 months of age when admitted to the trial, valid comparisons with the pertussis toxoid used in our study are not possible.

Because of cross-reactivity between outer-membrane proteins of nonencapsulated *H. influenzae* and filamentous hemagglutinin,<sup>19</sup> there is a risk of overdiagnosing bordetella infections with serologic tests for filamentous-hemagglutinin antibodies. A clinical analysis of all patients with an increase in IgG antibodies against filamentous hemagglutinin as the only positive laboratory finding indicated, however, that all of them had an infection with bordetella rather than *H. influenzae*. Thus, cases with an increase in filamentous-hemagglutinin antibodies as the only laboratory evidence of bordetella infection should be included in calculations of vaccine efficacy, even though a few parapertussis cases could be falsely diagnosed as pertussis. If an increase in IgG antibodies against filamentous hemagglutinin alone had not been accepted as diagnostic in our study, the efficacy of the pertussis toxoid against pertussis with at least 21 days of paroxysmal cough would have been 78 percent instead of 71 percent.

This study reveals an important diagnostic difficulty that occurs when antibodies against a vaccine component are assayed for serologic diagnosis. Pertussis-toxin serologic testing had much lower sensitivity in recipients of DTP toxoids than in recipients of DT toxoids, because the DTP-toxoids recipients already had high values for IgG antibodies against pertussis toxin in the acute-phase serum samples. It was therefore of great value to measure antibodies against an antigen (filamentous hemagglutinin) that was not included in the vaccine. In trials of vaccines containing both pertussis toxin and filamentous hemagglutinin, the serologic di-

agnosis will be even more difficult. Furthermore, cultures and the PCR were less sensitive in vaccinated children than in unvaccinated ones.

In conclusion, a pertussis-toxoid vaccine was safe and immunogenic and reduced the incidence and severity of pertussis. Furthermore, recovery of the organism was reduced. We propose that pertussis toxoid is both essential and sufficient for the vaccination of children and adults. It is the simplest and thus theoretically the safest vaccine for pertussis. We are now investigating whether mass vaccination of children with pertussis toxoid will eliminate pertussis, as happened in the case of diphtheria, another noninvasive, toxin-mediated respiratory disease. Mass vaccination with diphtheria toxoid eliminated diphtheria even though the toxoid confers incomplete individual protection under endemic or epidemic conditions.<sup>26,27</sup>

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