Original Report

Parapertussis and Pertussis: Differences and Similarities in Incidence, Clinical Course, and Antibody Responses

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ABSTRACT

Objectives: To compare the incidence, clinical course, and serologic response to *Bordetella* antigens in patients with parapertussis and pertussis.

Design: Two studies were performed in Sweden during the 1990s, when pertussis vaccines were used only in clinical trials. Study I was a retrospective study of patients with positive *Bordetella* cultures obtained in clinical routine, and study II involved an active search for patients with *Bordetella* infections during a placebo-controlled trial of a pertussis toxoid vaccine.

Results: Study I includes 58, and study II 23 patients with parapertussis. In study I, the incidence of parapertussis was 0.016 cases per 100 person years in children 0 to 6 years old and 0 in older children and adults. In study II, the incidence rates of parapertussis and pertussis were 0.2 and 16.2 per 100 person years, respectively, in children followed from 3 months to 3 years of age. The median number of days with cough was 21 in parapertussis and 59 in pertussis. The proportions of children with whooping and vomiting were lower in parapertussis than in pertussis. Geometric mean serum filamentous hemagglutinin IgG increased from 6 to 63, and pertactin IgG from 4 to 12 units/mL in parapertussis patients, which was similar to increases in children with pertussis.

Conclusions: Disease caused by *Bordetella parapertussis* is diagnosed less commonly and is milder and of shorter duration than disease caused by *Bordetella pertussis*. Parapertussis induced serum IgG against filamentous hemagglutinin and pertactin of similar magnitude as does pertussis, and did not induce serum IgG against pertussis toxin.

Key words: *filamentous hemagglutinin (FHA), incidence, parapertussis, pertactin, pertussis, pertussis toxin*

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Bordetella parapertussis is closely related to *Bordetella pertussis*. The diseases caused by the two organisms are, according to several studies, similar, but parapertussis is milder and of shorter duration than pertussis.[1-3] However, this finding has been questioned by others.[4,5] Symptomatic parapertussis infections are much less common than symptomatic pertussis infections.[1-3] A classic Danish study of parapertussis suggests, that infections with the two organisms are equally common, but in parapertussis, the ratio of subclinical to clinical infection is much higher than in pertussis.[1]

Pertussis toxin (PT) is one of the major virulence factors in *B. pertussis*. In contrast, *B. parapertussis* does not express PT, although it possesses the complete genome for the toxin.[6] Both organisms have at least two surface proteins, filamentous hemagglutinin (FHA) and pertactin, which are identical or show a high degree of similarity.[7,8] Virtually all patients with pertussis have a serum antibody response to FHA, and a majority also respond to pertactin.[9,10] To date, little is known about the serologic response to FHA and pertactin in patients with parapertussis.

The aims of the present two studies were to compare parapertussis and pertussis concerning incidence, clinical course, and the serum antibody response to FHA, pertactin, and PT.

MATERIAL AND METHODS

This report is based on two studies performed in Göteborg and surroundings in western Sweden from 1991 through 1997. The incidence of pertussis had been high in Sweden since the beginning of the 1970s, because the

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Swedish whole-cell pertussis vaccine had become ineffective around 1970, owing to several changes in vaccine production.[11] From 1979 through 1995 there was no licensed pertussis vaccine in the country.

Study I: Patients with Positive Parapertussis Cultures Found in Clinical Routine

The Department of Clinical Bacteriology in Göteborg is the only laboratory that cultures *Bordetella* in the city of Göteborg and seven surrounding communities. The area had a mean total population of 642,000 from 1992 through 1997. The mean population aged 0 to 6 years was 59,000. Cultures sent to this laboratory are obtained by physicians in primary health care and hospitals from patients seeking medical attention for suspected whooping cough. Between November 1991 and November 1997 all patients from whom *B. parapertussis* was isolated were identified from the laboratory records. If possible, their parents were interviewed concerning symptoms, with the use of a structured questionnaire. Furthermore, a serum sample from the child was requested. These samples were obtained 7 to 30 weeks after onset of symptoms. For comparison, all patients from whom *B. pertussis* was isolated during the same time period were identified. They were not interviewed, and serum was not obtained from them. Individuals with positive parapertussis and pertussis cultures found in a vaccine efficacy trial (study II) are not included in study I. One individual from whom both *B. pertussis* and *B. parapertussis* were isolated is not included in this report.

Study II: Active Search for Parapertussis Cases during a Vaccine Efficacy Trial

In a placebo-controlled, double-blind efficacy trial of an acellular pertussis vaccine, consisting of pertussis toxoid alone, 3450 infants were randomized to vaccination with diphtheria-tetanus toxoids alone (DT, Statens Seruminstitut, Copenhagen, Denmark) or to the same DT with pertussis toxoid (DTaP, North American Vaccine Inc., Beltsville, Maryland, USA) at 3, 5, and 12 months of age.[12,13] The trial was performed in the Göteborg area of western Sweden during 1991 to 1995.

During the follow-up period, *Bordetella* infections were actively sought and diagnosed in both study children and family members. All families were interviewed by a study nurse once a month. Parents were instructed to contact the nurse if a study child or a family member coughed for 7 days or more. A nasopharyngeal culture and an "acute" serum were obtained. A convalescent serum was taken about 4 to 6 weeks later. A total of 3450 study children, 2940 siblings, and 6466 parents were followed prospectively. The observation periods were 9008, 7104, and 17,610 person years in study children, siblings, and parents, respectively. In this study pertussis was defined as cough for 7 days or longer and one or more of the following criteria: (a) positive pertussis culture, (b) isolation of *B. pertussis* from a family member whose cough began within 28 days of the onset of the episode studied (before or after), (c) significant rise in PT IgG, and (d) significant rise in FHA IgG. These laboratory criteria are included in the definition of pertussis proposed by the World Health Organization (WHO).[14] To these were added (e) significantly elevated IgG antibodies against PT and FHA in a convalescent serum if no acute serum was available.[12] Parapertussis was defined as cough for 7 days or longer and a positive parapertussis culture or fulfilment of at least two of the following criteria: (a) significant rise in FHA IgG without significant rise in PT IgG, PT IgM, and PT IgA, (b) parapertussis DNA detected by the polymerase chain reaction (PCR), and (c) isolation of *B. parapertussis* from a family member whose cough began within 28 days of the onset of the episode studied (before or after). The clinical follow-up of patients with pertussis and parapertussis continued until the cough ended, or for at least 60 days. Three individuals with concurrent laboratory-confirmed pertussis and parapertussis infections are not included in this report.

Laboratory Assays

Nasopharyngeal swabs taken in clinical routine (study I) were cultured on Regan-Lowe medium.[15] In the vaccine efficacy trial (study II), nasopharyngeal swabs were cultured on Regan-Lowe medium and in an enrichment medium (Regan-Lowe with 50% charcoal agar). *Bordetella pertussis* and *B. parapertussis* colonies were verified by Gram-stain, agglutination, and biochemical tests. In study II, the secretions from the nasopharyngeal swabs also were examined by PCR for *B. pertussis* and *B. parapertussis* DNA.[16,17]

Serum IgG antibodies against PT, FHA, and pertactin were measured by enzyme-linked immunosorbent assay (ELISA).[9,12] Sera were tested in eight threefold dilutions, starting with a 1:10 dilution. All sera from the same child were assayed on the same microtiter plate. Petussis toxin was obtained from NAVA Inc., Beltsville, Maryland, USA. Filamentous hemagglutinin (lot 10,000) was obtained from Institut Pasteur-Mérieux, Marcy l'Etoile, France. Pertactin was obtained from Chiron Vaccines, Siena, Italy. The reference sera used were the United States Food and Drug Administration (US FDA) reference pertussis antisera lot 3 (for PT and FHA) and lot 4 (for pertactin). Lot 3 contained 200 units/mL of PT IgG and FHA IgG. Lot 4 contained 90 units/mL of pertactin IgG. Antibody concentrations were calculated with the reference line assay.[18] The mimimal level of detection was 1 unit/mL for all assays. Sera with IgG of less than 1 unit/mL were arbitrarily assigned a value of 0.5 units/mL. For all IgG antibodies, an increase of twofold or more from the acute to the convalescent serum was considered significant if

the concentration in the convalescent serum was 7 or more units/mL. In study II, 42 children with pertussis were randomly selected for determination of PT, FHA, and pertactin IgG, for comparison with the 18 parapertussis patients from whom paired sera were available.

Statistics

Proportions were compared with Fisher's exact test. Duration of clinical symptoms was compared with Mann-Whitney test. Geometric mean values were compared with paired t-test. All P-values are two-tailed.

Ethics

Study I was approved by the Ethics Committee, Göteborg University. All parents gave oral consent after receiving oral and written information. Study II was approved by the NICHD, the US FDA, the Medical Products Agency, Uppsala, Sweden, and the Ethics Committee, Göteborg University. All parents gave written consent after receiving oral and written information.

RESULTS

Incidence, Age, and Sex Distribution

Study I

Bordetella parapertussis was isolated from 58 and *B. pertussis* from 3481 individuals from November 1991 through November 1997 at the Department of Clinical Bacteriology in Göteborg after exclusion of all patients with positive cultures who are included in study II. *Bordetella parapertussis* constituted 1.6% of all *Bordetella* isolates. Of the 58 parapertussis patients, 25 were boys and 33 girls. Their ages ranged from 2 to 81 months (median 25 mo). In the age group 0 to 6 years the documented incidence of parapertussis was 0.016 per 100 person years. The documented incidence rates of parapertussis and pertussis in all age groups were 0.0015 and 0.09 per 100 person years, respectively.

Study II

During the vaccine efficacy trial, parapertussis was diagnosed in 23 (18 culture-confirmed) and pertussis in 1213 (618 culture-confirmed) individuals. Table 1 presents numbers of cases, incidence rates, and proportions of parapertussis among all *Bordetella* cases in the different groups. The incidence of parapertussis was 0.20 per 100 person years in all study children (vaccinated plus non-vaccinated). The difference in incidence of parapertussis between children who were (0.24 per 100 person years) and were not (0.16 per 100 person years) vaccinated with pertussis toxoid was not significant. The proportion of parapertussis cases among all *Bordetella* infections was significantly higher in the pertussis toxin-vaccinated group (3.5% vs. 1%, $P < 0.01$). Of the 23 parapertussis cases, 14 were boys and 9 girls. Their ages ranged from 4 to 114 months (median 23 mo). Of 1126 children with pertussis in study II, 51% were girls. The median age of the children with pertussis was 30 months.

Clinical Picture

Table 2 summarizes data on cough, whooping attacks, and vomiting in patients with parapertussis and pertussis in studies I and II. There was no clinical difference between parapertussis in study I, in which cases were found passively from the laboratory, and study II, in which cases were actively sought among patients with cough. The duration of cough and paroxysmal cough and the duration and frequency of whooping attacks and vomiting were significantly lower in parapertussis than in pertussis patients in study II.

In study I none of the 58 parapertussis patients were hospitalized, whereas 125 of the 3481 patients (3.6%) with pertussis were hospitalized. In study II no patient with *Bordetella* infection was hospitalized.

Antibody Response

Filamentous Hemagglutinin IgG

Geometric mean (GM) values increased significantly both in parapertussis and in pertussis patients in study II. The range and GM in convalescent sera were similar after both diseases. The proportions of patients with significant increases did not differ significantly (parapertussis: 14/18, 77%; pertussis: 40/42, 95%). Filamentous hemagglutinin

Table 1. Numbers of Cases and Incidence Rates of Parapertussis and Pertussis and Proportions of Parapertussis Cases among All *Bordetella* Infections in a Randomized, Prospective Vaccine Efficacy Trial (Study II)

Subjects	*Parapertussis n (Incidence*)*	*Pertussis n (Incidence*)*	*Proportion Parapertussis among All* Bordetella *Infections (%)*
DTaP-vaccinated study children	11 (0.24)	302 (6.60)	3.5
DT-vaccinated study children	7 (0.16)	713 (16.20)	1.0
Siblings	5 (0.07)	111 (1.60)	4.3
Parents	0 (0)	87 (0.49)	0

*Incidence rates = number of cases per 100 person years.

Table 2. Duration and Numbers of Patients with Certain Symptoms in Parapertussis and Pertussis

	Study I*	Study II†		
Symptom	*Parapertussis (n = 46)*	*Parapertussis (n = 23)*	*Pertussis (n = 950)*	*P-Value*
Cough				
Duration (d)	21 (4–80)	21 (7–>60)	59 (7–>60)	< 0.001
Paroxysmal cough				
Duration (d)	14 (0–72)	15 (0–>60)	51 (0–>60)	< 0.001
Number (%)	43 (93%)	22 (96%)	946 (>99%)	NS
Whooping attacks				
Duration (d)	0 (0–25)	0 (0–8)	20 (0–>60)	< 0.001
Number (%)	14 (30%)	2 (9%)	675 (71%)	< 0.001
Vomiting				
Duration (d)	0 (0–30)	0 (0–7)	18 (0–>60)	< 0.001
Number (%)	21 (46%)	9 (39%)	730 (77%)	< 0.001

Duration = median number of days (range).
*In study I, clinical data were obtained from 46 of 58 children with parapertussis. †In study II, clinical data were obtained from all 23 patients with parapertussis. Of the 1213 patients with pertussis in study II, only the 950 children who had not received any pertussis vaccine are included. NS = not significant.

(FHA) IgG levels in the convalescent sera of children in study I were similar to those in study II (Table 3).

Pertactin IgG

Geometric mean values increased significantly both in parapertussis and in pertussis patients in study II. The range and GM in convalescent sera were similar after both diseases. The proportions of patients with significant increases did not differ significantly (parapertussis: 9/16, 56%; pertussis 29/42, 69%). Pertactin IgG levels in the convalescent sera of children in study I were similar to those in study II.

Pertactin IgG in paired sera was a less sensitive diagnostic tool than FHA IgG. Among pertussis patients 40 of 42 (95%) had significant FHA IgG increases, whereas 29 of 42 (69%) had pertactin IgG increases ($P < 0.003$). Corresponding figures for the parapertussis patients were 14 of 18 (78%) for FHA versus 9 of 16 (56%) for pertactin (nonsignificant).

Pertussis Toxin IgG

Of the 42 pertussis patients in study II, 40 had significant PT IgG increases, whereas none of the 18 parapertussis patients had a significant PT IgG increase ($P < 0.001$). Among all 55 parapertussis patients in studies I and II from whom convalescent sera were available, 26 had detectable PT IgG. Of those, 19 were vaccinated with pertussis toxoid, and 7 were never vaccinated with any pertussis vaccine. In three of these seven patients PT IgG was low and possibly nonspecific (1–4 units/mL). One patient with a positive parapertussis culture had high PT IgG in a single convalescent serum (118 units/mL), suggesting mixed pertussis and parapertussis infection. The remaining three patients had PT IgG between 13 and

Table 3. Geometric Mean and Range of Serum Concentrations of IgG Antibodies against Filamentous Hemagglutinin, Pertactin, and Pertussis Toxin, in Patients with Parapertussis and Pertussis

	Study I	Study II	
	Parapertussis (n = 37) GM (Range)	*Parapertussis (n = 18*) GM (Range)*	*Pertussis (n = 42) GM (Range)*
FHA IgG (U/mL)			
Acute	Not obtained	6.0 (<1–209)	1.5 (<1–16)
Convalescent	46.0 (11–345)	63.0 (12–198)	84.0 (<1–>400)
P-value†		<0.001	<0.001
Pertactin IgG (U/mL)			
Acute	Not obtained	4.3 (<1–21)	2.8 (<1–33)
Convalescent	16.0 (2.7–69)	12.0 (1.8–48)	16.0 (2.2–356)
P-value†		<0.001	<0.001
PT IgG (U/mL)			
Acute	Not obtained	6.4 (<1–116)	1.5 (<1–>400)
Convalescent	2.4 (<1–>400)	5.1 (<1–69)	125.0 (4–>400)
P-value†		NS	<0.001

*For pertactin IgG 16 paired sera were available; †acute versus convalescent.
GM = geometric mean; FHA = filamentous hemagglutinin; PT = pertussis toxin.

42 units/mL in the convalescent sera, which is lower than usually seen in convalescent sera of patients with recent pertussis, suggesting that a clinical or subclinical *B. pertussis* infection may have preceded their parapertussis.

DISCUSSION

A comparison between studies I and II shows, as expected, that registration of cases of parapertussis from laboratory reports underestimates the true incidence of the disease. An active search for cases among patients who cough is necessary to approach the true incidence of symptomatic infections. Study II yielded an incidence of parapertussis in children aged 0 to 3 years that was 12.5 times higher than the incidence in 0- to 6-year-old children in study I. The incidence of pertussis is also considerably underestimated when based on laboratory reports.[11] There are several obvious reasons for this. Many patients with *Bordetella* infections never seek medical attention. If they do, cultures might not be obtained if the pertussis diagnosis is obvious from the clincial picture or if the disease is never suspected by the physician. Furthermore, cultures have a sensitivity of about 50 to 70% when taken under optimal conditions and even lower in clinical practice.[12,19,20]

Two prospective vaccine efficacy trials with active search for *B. parapertussis* infections among infants and young children who coughed were reported previously, one in Italy and one in Germany. These studies yielded incidence rates of 0.21 and 0.6 to 0.9 cases per 100 person years, respectively,[3,10] which can be compared with 0.2 cases per 100 person years in the study children in the vaccine efficacy trial reported here. Even though the incidence rates were similar between the studies, the proportions of parapertussis cases among all *Bordetella* infections in vaccinated and nonvaccinated study children were much higher in the Italian and German studies (12% and 40%, respectively) than in the present study II (1.7%). The incidence of pertussis in Italy and Germany was much lower than in Sweden when the trials were performed. Incidence rates of pertussis fulfilling slightly different variations of the pertussis definition proposed by the World Health Organization in the nonvaccinated control children (DT recipients) were 3.5, 3.0, and 10.3 cases per 100 person years in Italy, Germany, and Sweden, respectively.[10,12,14,21] These pronounced differences in pertussis incidence and the higher ratio of parapertussis to pertussis infections may be attributable to differences in pertussis vaccination rates. In Sweden, virtually no pertussis vaccine was given outside of clinical trials, whereas the vaccination rates with whole-cell pertussis vaccines of infants in Italy and Germany were 15% and 40%, respectively.[10,21]

A recent study from Finland concluded that parapertussis is a common disease in a country with a high vaccination rate against pertussis with a whole-cell vaccine. In that study, 32% of the *Bordetella* infections were caused by *B. parapertussis*, and 7% were mixed infections.[22] This could lead to speculations that parapertussis increases if pertussis decreases. However, the incidence of parapertussis was only 0.0034 cases per 100 person years in the Finnish study, which is similar to the present study I (0.0015 per 100 person years) that was performed in a population with little use of pertussis vaccines. In the authors' opinion, both studies show that parapertussis is an uncommon disease, and the twofold difference in incidence between Finland and Sweden can be explained partly by the addition of PCR to cultures in the Finnish study, whereas the present Swedish study used only cultures. Furthermore, the present study II shows that the absolute incidence of parapertussis does not increase in pertussis toxoid-vaccinated children even though its relative incidence compared to pertussis increases. It is even possible, in a longer perspective, that the absolute incidence of parapertussis will decrease in Sweden when the incidence of pertussis decreases owing to vaccination. A study in animals showed that parapertussis infection is facilitated by concurrent infection with pertussis.[23] If that is the case also in humans, a decrease in the incidence of pertussis may decrease spread of and infection with *B. parapertussis*. It is also theoretically possible that pertussis vaccines including antigens common to both organisms also may protect against parapertussis. One study testing two three-component acellular pertussis vaccines (PT, FHA, and pertactin) showed no significant efficacy against parapertussis; another study testing a four-component vaccine (PT, FHA, pertactin and fimbriae-2) showed a tendency to protection that did not reach significance.[3,10]

The present and a previous study show that the age and sex distribution of symptomatic parapertussis infections do not differ from those of symptomatic pertussis infections,[1] with the exception that parapertussis cases in adults were not found in the present studies I and II.

Two recent studies have emphasized the similarities of the clinical picture of pertussis and parapertussis.[4,22] In both studies the prevalence of symptoms were compared at the time the patients sought medical attention, but the patients were not followed until the symptoms ended. The present and several previous studies that have followed the patients until symptoms ended show that parapertussis is a considerably milder disease than pertussis.[1-3] All variables compared (cough, paroxysmal cough, vomiting, and whooping attacks) were of shorter duration or seen in lower frequency in parapertussis than in nonvaccinated pertussis patients. It should be noted that pertussis in vaccinated children was significantly milder ($P < 0.001$ for all variables compared) than in nonvaccinated children in the efficacy trial, on which the current study II is based.[12] Thus, pertussis in vaccinated children may resemble parapertussis.

The possibility that some patients with verified parapertussis were infected with both organisms should be considered, because this may be an underreported occurrence.[22,24] However, in the present two studies with a total of 81 parapertussis cases the authors found four additional patients with confirmed mixed infections (not described in the article). The only way of excluding a coinfection with pertussis in parapertussis patients is to compare pertussis toxin antibodies in acute and convalescent sera.

This study showed, in agreement with other studies, that the antibody response to FHA is an almost universal occurrence in both pertussis and parapertussis patients.[9,10] Notably, parapertussis patients responded to FHA purified from a strain of *B. pertussis*, and the magnitude of the antibody response was similar in the two groups. Likewise, the antibody response to pertactin, derived from a strain of *B. pertussis*, was similar in parapertussis and pertussis patients. Some patients with parapertussis and pertussis had little or no response to pertactin. This is in agreement with two considerably larger studies that showed that an antibody response to pertactin is a less consistent finding than an antibody response to PT and FHA in patients with pertussis.[9,10]

It has been shown previously that pertussis with a typical clinical picture can occur after parapertussis.[25] This study suggests that the reverse also may be true: parapertussis may occur despite previous *B. pertussis* infection. In both study I and study II, a few individuals with parapertussis had pertussis toxin antibodies without having been vaccinated. Since pertussis toxin is unique to *B. pertussis* with no known cross-reacting antigens, the finding of pertussis toxin antibodies in nonvaccinated individuals is strong evidence of previous infection with *B. pertussis*. The lack of cross-protection between the two species and the inability of multicomponent acellular pertussis vaccines containing both FHA and pertactin to protect against *B. parapertussis* cast doubt on their importance as protective antigens in *Bordetella* infections.[3,10]

CONCLUSIONS

The two studies show that *B. parapertussis* infections are underdiagnosed in clinical routine but still are uncommon, even when active search is performed. The clinical presentation of parapertussis is similar to that of pertussis, but the former disease is milder and of shorter duration. Parapertussis induces serum antibodies against FHA and pertactin from *B. pertussis* of similar magnitude to that of pertussis, indicating homology or close similarity of these two surface proteins from the two species.

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