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Bordetella pertussis infection in northern Taiwan, 1997-2001

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The clinical presentations of laboratory-confirmed *Bordetella pertussis* infection in Chang Gung Children's Hospital during 1997 and 2001 were analyzed. Of the 46 cases, 25 (54.3%) were male. The patients ages ranged from 24 days to 37 years, with a mean and median of 4.3 years and 10.5 years, respectively. Forty four patients had vaccination records, among them 23 patients (52.2%) had received ≥3 doses of pertussis vaccine. Of the patients who were partially vaccinated (received 1 or 2 doses vaccine) or unvaccinated, 16 (69.6%) presented with whooping cough, 5 (22.2%) with post-tussive vomiting, and 13 (59.1%) with cyanosis. Leukocytosis (white blood cells ≥15,000 cells/μL) and lymphocytosis (lymphocytes ≥10,000 cells/μL) were observed in 17 (47.2%) and 16 (44.4%) of the patients, respectively. Fourteen patients (30.4%) developed complications, among which pneumonia was the most common (92.3%). Among infants ≤1 year of age, 95.2% were partially vaccinated (20/21), compared with 5% (1/20) of the patients >1 year of age ($p<0.05$). The overall complication rate was 37.5%, compared with 18.2% for patients >1 year of age ($p<0.05$). One 2-month-old patient required ventilatory support after the development of cardiopulmonary failure. There was no mortality in this study. In summary, pertussis most commonly occurred in infants who were unvaccinated or partially vaccinated. These patients usually presented with atypical symptoms such as cyanosis or apnea. The importance of vaccination still cannot be overemphasized because immunized patients usually present with milder disease than those who are not immunized.

Key words: *Bordetella pertussis*, mass immunization, retrospective studies, signs and symptoms, whooping cough

Pertussis was a major cause of morbidity and mortality among infants and children during the prevaccine era. Following the introduction and widespread use of whole cell pertussis vaccine combined with diphtheria and tetanus toxoids (DPT) in the mid-1950s, the incidence of reported pertussis cases in Taiwan decreased significantly from 691 in 1955 to only a few in 1970 [1]. Moreover, there has been no mortality due to pertussis reported since 1975. The incidence of pertussis remained low throughout the period from 1971 to 1991 until an outbreak of 226 cases occurred in 1992 [1]. Pertussis incidence has, however, shown a cyclical increase with a peak occurring every 3 to 5 years in Taiwan [1]. The incidence of pertussis steadily declined in the United States since 1951, when the pertussis vaccine was first introduced, but then showed a steady rise since the early 1980s [2]. In 1993 pertussis became the most commonly reported vaccine-preventable disease among children younger than 5 years of age in the United States, with cases reported primarily in non-immunized or under-immunized populations [3-8].

The quality of epidemiological study data on pertussis in Taiwan is poor [1]. Many physicians may be unfamiliar with the disease in the vaccine era. This epidemiologic study was undertaken to analyze the clinical characteristics of *Bordetella pertussis* infections in children.

Materials and Methods

Both inpatients and outpatients clinically suspected of having pertussis infection who were treated in Chang Gung Memorial Hospital and Children's Hospital between January 1, 1997 and December 31, 2001 were included. A clinical case of pertussis was defined as an acute cough illness lasting at least 14 days in a person with at least 1 pertussis-associated symptom (i.e., paroxysmal cough, post-tussive vomiting, or inspiratory whoop) according to the case definition for pertussis recommended by the Council of State and Territorial

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Epidemiologists and the Centers for Disease Control of the USA [9]. A confirmed case was defined as a cough illness of any duration in a person with isolation of *B. pertussis*, or a case that met the clinical definition and was confirmed by polymerase chain reaction (PCR), serology or by epidemiologic linkage to a laboratory-confirmed case. A case was considered epidemiologically linked if there was a close contact with a laboratory-confirmed pertussis case and the onset in the secondary case occurred within 7 to 28 days of the onset in the index case. A probable case met the clinical definition but was not laboratory confirmed or epidemiologically linked to a laboratory-confirmed case.

B. pertussis culture from nasopharyngeal specimens and PCR was performed in the Department of Clinical Pathology of Chang Gung Memorial Hospital. The nasopharyngeal specimens were collected for culture and the PCR-based assay with calcium alginate and Dacron swabs (Copan Diagnostics, Corona, CA, USA), respectively. Specimens for culture were inoculated into freshly prepared Bordet-Gengou agar plates containing 20% sheep blood and 40 μg of cephalexin per mL. The plates were incubated at 35-36°C for 3 to 7 days in a moist chamber. The suspicious colonies were identified by oxidase reaction and specific fluorescent antibodies (Difco Laboratories, Detroit, MI, USA) [10].

The oligonucleotide primers used for PCR amplification were as described previously by Wadowsky et al [11]. DNA was extracted from nasopharyngeal swabs by a proteinase K-sonication procedure. Two separate DNA targets (153 and 203 bp) amplified simultaneously are from the insertion sequence IS481 and a 438-bp target within the β-actin gene of human DNA (PCR amplification control).

Primers BP1 (5'-GATTCAATAGGTTGTATGCATG GTT-3') (bp 12 to bp 36) and BP2 (5'-AATTdCTGGACC ATTTCGAGTCGACG-3') (bp 164 to bp 149) and primers BP13 (5'-CCGCGCTGTGCCATGAGCTGG-3') (bp785 to bp 805) and primers BP14 (5'-GATGCCTTG GTGGGGTCGATG-3') (bp 987 to bp 967) define 153- and 203-bp targets, respectively within the *B. pertussis* IS481 [11]. Primers HAC3 (5'-ATCATGTTTGA GACCTTCAAC-3') (bp 1954 to bp 1874) and primers HAC5 (5'-CAGGAAGGAAGGCTGGAAGAG-3') (bp 2292 to bp 2272) define a 439-bp target within β-actin gene of human DNA [12].

Fifty μL of extracted DNA was added to 50 μL of PCR mixtures containing 2U *Taq* DNA polymerase (Boehringer Mannheim, Mannheim, Germany), 2.5 mM $MgCl_2$, 100 μM (each) deoxynucleoside triphosphates (GeneTeks BioScience, Inc., Taipei, Taiwan), and 0.25 μM of each oligonucleotide primer in 1 × PCR buffer. Ten ng of human DNA was added to each PCR reaction. PCR tubes were placed in a model 9600 thermal cycler (Perkin-Elmer), denatured at 94°C for 2 min, then subjected to 35 cycles of 94°C for 1 min, 64°C for 30 sec, and 72°C for 1 min. A final extension step at 72°C for 10 min was performed and the products were stocked at 4°C until used. After agarose gel electrophoresis, the ethidium bromide-stained PCR products were visualized under ultraviolet light. Specimens yielding 2 bands corresponding to the 153- and 203-bp products were considered positive.

Sera were processed at the Center for Disease Control in Taiwan. Serology consisted of measurement of immunoglobulin A (IgA) and IgM antibodies against whole cells of *B. pertussis* by enzyme-linked immunosorbent assay (ELISA), according to methods previously described [13,14].

Other etiologies which may cause pertussis-like illness, such as influenza virus, adenovirus, chlamydia or mycoplasma were also checked.

The medical charts of patients were reviewed, and the following data were analyzed: demographic characteristics, underlying diseases, history of vaccination, clinical symptoms and signs, laboratory data, management, and outcome. The medication histories of patients, such as dose and duration of therapy were incomplete and this information was not included in the analysis.

Statistical analysis

Calculations and statistical analyses were performed using the SPSS software (SPSS 9.0, Chicago, IL, USA). Continuous variables were analyzed with independent Student's *t* test. Chi-squared and Fisher's exact 2-tailed tests were used to examine the nominal data. A difference was considered significant if the *p* value was less than 0.05.

Results

From January 1997 to December 2001, a total of 139 patients with pertussis-like syndrome had a nasopharyngeal specimen collected for culture and PCR assay. *B. pertussis* was isolated in 10 of these patients (7.2%), and the PCR result was positive in 25 (54.3%). Blood samples for serological studies were available in 122 cases (87.8%). IgM and IgA were positive for *B. pertussis* in 22 (47.8%) and 4 cases (3.3%). In

Table 1. Laboratory diagnosis of *Bordetella pertussis* infection

Case numbers	Culture	PCR	Serology	
			IgA	IgM
17	-	-	+	-
14	-	+	-	-
8	+	+	-	-
2	+	+	+	-
2	-	-	-	+
2	-	-	+	+
1	-	+	+	-
Total 46	10	25	22	4

Abbreviations: PCR = polymerase chain reaction; IgA = immunoglobulin A; IgM = immunoglobulin M; + = positive; – = negative

addition, respiratory syncytial virus infection was found in 28 patients, chlamydia infection in 7, adenovirus infection in 3, and parainfluenzae infection in 1. These patients were classified as having pertussis-like illness and were excluded from the study. Overall, 46 cases (29.5%) fulfilled the diagnostic criteria for pertussis (Table 1).

Age, gender, and season

Of the 46 patients, 25 (54.3%) were male. The mean age was 4.3 years (range, 24 days to 37 years), and the median age was 10.5 months. The age distribution of these patients is shown in Fig. 1. Twenty four patients (52.2%) were younger than 1 year of age (Fig. 1 inset).

Pertussis cases were reported throughout the year, although the peak incidence occurred in the summer and autumn months. The case numbers of pertussis infection in each year were as follows: 19 (41.3%) in 1997, 4 (8.9%) in 1998, 12 (26%) in 1999, 7 (15.2%) in 2000, and 4 (8.9%) in 2001.

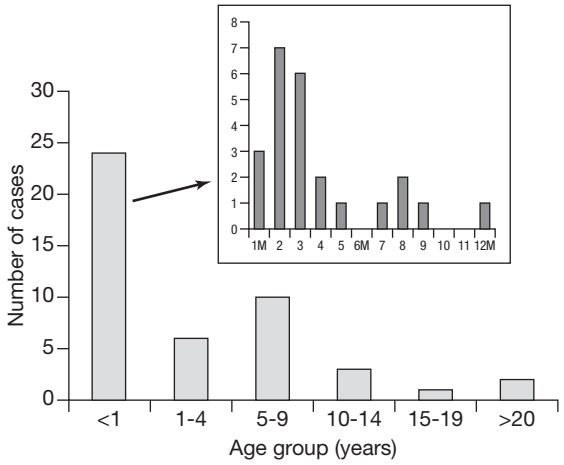

Fig. 1. Cases of pertussis by age group.

Clinical findings

The clinical manifestations of the patients are summarized in Table 2. A high hospitalization rate was noted in patients ≤1 year of age compared with older patients (95.8% vs 54.5%; $p<0.05$). The initial clinical manifestations in patients ≤1 year in age included tachypnea (41.7%), paroxysmal cough (75%), cyanosis (58.3%), feeding difficulties (37.5%), and fever (28.2%). Patients ≥5 years of age tended to present with a prolonged cough lasting longer than 21 days (75% vs 30%; $p<0.05$).

No mortality was reported in this study group [Table 3]. The overall morbidity rate was 30.4% (14/46). Morbidity was more common in young children <1 year of age than in older patients (37.5% vs 18.2%; $p<0.05$). Eight of the 12 patients (66.7%) with pneumonia were younger than 1 year of age. A

Table 2. Clinical presentations of patients with pertussis infection by age group

Characteristics	Age[a]				
	<1 year n = 24	1-4 years n = 6	5-9 years n = 11	≥10 years n = 5	Total n = 46
Admission	23 (95.8)	4 (66.7)	6 (45.5)	2 (40)	33 (71.7%)
Initial cough <7 days	3 (12.5)	0	0	0	3 (6.5)
7-20 days	14 (58.3)	4 (66.7)	3 (27.3)	1 (20)	22 (47.8)
≥21 days	7 (29.2)	2 (33.3)	8 (72.7)	4 (80)	21 (45.7)
Paroxysmal cough	18 (75)	1 (16.7)	7 (63.6)	3 (60)	29 (63)
Whooping cough	4 (16.4)	2 (33.3)	7 (63.6)	1 (20)	14 (30.4)
Tachypnea	10 (41.7)	1 (16.7)	1 (9.0)	0	12 (26)
Post-tussive vomiting	4 (16.4)	2 (33.3)	3 (27.3)	0	9 (19.6)
Fever	7 (29.2)	2 (33)	0	1 (20)	10 (21.7)
Feeding difficulty	9 (37.5)	0	0	0	9 (19.6)
Cyanosis	14 (58.3)	1 (16.7)	0	0	15 (32.6)
Apnea	7 (29.2)	0	0	0	7 (15.2)

[a]Values are number (%).

Table 3. Complications in patients with pertussis infection by age group

Complications	Age[a]				
	<1 year n = 24	1-4 years n = 6	5-9 years n = 11	≥10 years n = 5	Total n = 46
Pneumonia	8 (33.3)	2 (33.3)	2 (18.2)	1 (20)	13 (28.3)
Seizure	1 (4.2)	0	0	0	1 (2.2)
Cardiopulmonary failure	1 (4.2)	0	0	0	1 (2.2)

[a]Values are number (%).

previously healthy young infant aged 2 months had seizure, with electroencephalogram study showing abnormal diffuse cortical dysfunction in bilateral hemispheres. Cerebrospinal fluid study was negative in this patient. Two newborn infants with pertussis received intensive care; both were cured without any sequelae. A 2-month-old infant received 2 weeks of assisted ventilator support after developing cardiopulmonary failure. He recovered fully.

All patients were prescribed macrolides once pertussis infection was suspected. In 43 patients (93.4%), erythromycin was given for 11.4 ± 4.1 days. One patient was treated with azithromycin for 3 days. Two patients were treated with clarithromycin for 5 and 6 days, respectively. The outcome of illness was comparable between patients with early and late start of macrolide treatment (cough ≤14 days vs cough >14 days).

Patients with early initiation of macrolide treatment were young infants (mean age, 8.8 months vs 5 years; $p<0.05$) and had a higher incidence of cyanosis (63.6% vs 28.6%; $p=0.07$). However, the patients who started treatment with macrolides also tended to have shorter hospitalization (mean, 6.5 ± 4.2 days vs 9.2 ± 4.6 days; $p=0.81$), and were less likely to have paroxysmal cough and whooping cough (45.0% vs 65.7%, $p=0.23$; 54.5% vs 60%, $p=0.75$).

Table 4. Clinical characteristics at presentation by immunization status

Characteristics	DPT ≥3 doses n = 22	DPT ≤2 doses n = 22	*p*
Admission	11 (50)[a]	18 (81.8)[a]	0.050
Cough ≥21days	13 (59.1)	6 (27.3)	0.033
Whooping cough	10 (45.5)	16 (72.7)	0.066
Paroxysmal cough	11 (50)	17 (77.3)	0.116
Post-tussive vomiting	4 (18.2)	5 (22.7)	0.955
Cyanosis	2 (9.1)	13 (59.1)	0.001
Apnea	0	7 (31.8)	<0.05
Pneumonia	5 (22.7)	8 (36.4)	0.51
WBC (/μL)	13,250	18,425	0.131
Lymphocyte (/μL)	6692	12,659	0.015

Abbreviations: DPT = diphtheria-pertussis-tetanus; WBC = white blood cells

[a]Values are number (%).

Immunization history

Information on the status of pertussis vaccination was available for 44 patients. Twenty two (50%) of these patients had received at least 3 doses of pertussis vaccine at the time of the onset of illness. Of the partially immunized patients, 16 (72.7%) had whooping cough, and 5 (22.7%) had post-tussive vomiting, whereas only 10 (45.5%) patients who had received ≥3 doses of vaccine had whooping cough, and 4 (18.2%) had post-tussive vomiting ($p<0.05$). Pneumonia was found in 8 (36.4%) of the patients who were partially vaccinated compared with 5 (22.7%) who were fully immunized ($p=0.743$) [Table 4].

Laboratory data

Eight of the 10 culture-confirmed patients were younger than 7 months old and 5 had not received any pertussis vaccine. Seven of the 10 culture-positive nasopharyngeal specimens were collected within 2 to 3 weeks after the onset of illness.

Thirty six of the 46 patients had blood samples collected for white blood cell (WBC) analysis. Leukocytosis (WBC ≥15,000/μL) and lymphocytosis (lymphocytes >10,000/μL) were observed in 17 (47.2%) and 16 (44.4%) of the patients, respectively. Only 2 patients had leukopenia, defined as a WBC count <5,000/μL. Lymphocytosis was more common in infants younger than 6 months of age (13,004 vs 6913/μL; $p=0.008$). In contrast, the mean WBC count in the infants ≤6 months old was not different from that in the older patients (18,694 vs 14,322/μL; $p=0.18$). Leukocytosis or lymphocytosis was not correlated with the duration of cough (r=0.182, $p=0.288$; r=0.228, $p=0.181$, respectively), or with any subsequent complications. C-reactive protein levels in patients with pertussis were mildly elevated, from 1 to 14 mg/L.

Discussion

This study analyzed the clinical characteristics of 46 patients with laboratory-confirmed pertussis in Chang-Gung Children's Hospital from 1997 through 2001. In Taiwan, DPT vaccination has been available since 1954, but despite immunization coverage, cases of pertussis have still been reported each year. In this study, 52.2% of the patients with pertussis had received at least 3 doses of vaccine, indicating that the vaccine was not fully protective against the infection. Immunized patients who had received ≥3 doses of whole-cell vaccine or acellular vaccine tended to have milder clinical presentations. In addition, our data revealed a small peak of pertussis in patients aged 5 to 9 years. Immunity to pertussis has been shown to wane after about 3 years following vaccination, and is virtually absent 10 to 12 years post-vaccination [15,16]. This waning of immunity may lead to a growing population of pertussis-susceptible adults and adolescents, and an increasing proportion of cases of infection in older age groups. New formulations of acellular pertussis vaccine are now available. The Steering Committee for Prevention and Control of Infectious Diseases in Asia recommends considering the use of dTap for boosting immunity in preschool, school, adolescent and adult age groups. This strategy may help control transmission from adults to infants and have a significant public health impact including the potential for elimination of pertussis infection.

In the pre-pertussis vaccine era, pertussis in young infants was rarely reported. Although placental transfer of maternal antibodies to many infectious agents affords transient passive protection to the infant, this does not appear to occur with pertussis [17]. Many neonates with pertussis do not develop paroxysmal cough or the characteristic whooping, which makes clinical diagnosis difficult. In such patients, the clinical picture is dominated by gagging, apnea, cyanosis and bradycardia [18-20]. In this study, 34.8% of patients (16/46) were ≤3 months of age, 11 (68.8%) had a short course of cough (<7 days) with cyanosis, 6 (37.5%) developed the complication of pneumonia, 1 required endotracheal intubation and ventilator support, and one 2-month-old infant had seizure attack. These findings indicate that neonatal pertussis is more common than is recognized, and often runs an atypical and complicated course.

Previous study showed that with the exception of infants, pertussis more frequently affects females than males [21]. In England and Wales between 1975 and 1979, the percentage of females among notified cases of pertussis was 49%, 52% to 53%, and 64% for children <1 year, 1 to 14 years, and ≥15 years of age, respectively. However, our results revealed a higher rate of pertussis in males (54.3%). By contrast, Ulrich et al found an even distribution of pertussis between both genders in children (male:female, 49.3%:50.7%) [22]. Data from the Center for Disease Control revealed that up to the age of 15 years, a similar number of boys and girls (50% to 51%) developed pertussis, whereas a female predonderance (55% to 69%) was noted in older age groups.

Isolation of *B. pertussis* from nasopharyngeal specimens is the gold standard for diagnosis of pertussis due to its high degree of specificity. This method is still widely used although its sensitivity has been shown to be variable. Pertussis is a small Gram-negative rod that cannot be isolated in the laboratory using routine media. *B. pertussis* can be recovered from respiratory tract secretions with the highest rate of isolation within the first 3 weeks of cough [22,23]. In Germany, the culture rate in adults was only 3% [24]. In this study, patients younger than 7 months old and unvaccinated patients had the highest rate of isolation. This indicates that the diagnosis of pertussis by culture is difficult, especially in older patients. In such a setting, serological testing could be helpful. The detection of antibodies to *B. pertussis* by enzyme-linked immunosorbent assay has been the diagnostic method of choice for the last decade. In unvaccinated children, increases in the levels of either IgG or IgA antibodies to a single or various antigens are required to meet the World Health Organization definition of pertussis. The immune response is directed against various antigens of *B. pertussis*, with reactions to pertussis toxin and filamentous hemagglutinin being the most invariable (>90%). Responses to pertactin, lipooligosaccharide, and fimbrae tend to be found somewhat less regularly (30 to 60%) [25]. According to results from a Swedish trial, the increase in the measurable amounts of antibodies necessary for diagnosis was mostly between 50% and 100% [26]. It is still not completely clear whether a high concentration in a single serum sample or in paired serum samples can be used for diagnosis and whether a decrease in antibody levels over a given period of time should also be used as a diagnostic criterion [27]. The diagnosis of pertussis in vaccinated children is more difficult, because these children will have an anamnestic response resulting in a rapid increase in antibody concentrations. IgA antibodies have been used in vaccinated children as an

indicator of infection. However, no single isotype antibody can absolutely distinguish between infection and vaccination [28].

PCR is revolutionizing the diagnosis of many infectious diseases. The best method for the detection of *B. pertussis* is still under investigation; however, various PCR procedures possess a diagnostic sensitivity which is at least comparable to and in many cases superior to that of culture. Loeffelholz et al reported that the sensitivity and specificity of PCR were 93.5% and 97.1%, compared with 15.2% and 100% for culture, respectively [29].

Leukocytosis with lymphocytosis has been recognized as a hallmark of pertussis infection. Pertussis produces a lymphocytosis-promoting factor, pertussis toxin, which causes proliferation of lymphocytes. In a series of 199 children hospitalized for pertussis, Kaufman and Bruyn reported that on admission, 57% of the patients had a WBC count of >20,000/μL [30]. The WBC count, however, was unrelated to age and did not correlate with the subsequent clinical course. On the other hand, Lagergren found that among young infants with pertussis, 33% and 36% had a WBC count ≥15,000/μL and lymphocyte counts ≥10,000/μL, compared with 71% and 63% in older children [31]. In a series of 13 critically ill infants with pertussis, hyperleukocytosis (>100,000/μL) was an independent predictor of death [32]. Leukocytosis and lymphocytosis were observed in about half of the patients in this study and were not correlated with the severity of the disease. However, our finding that lymphocyte counts were higher in infants ≤6 months of age, in conflict with the results of other studies. The reason for this difference is not clear.

Because some of our patients had been treated in clinics before visiting our hospital, we could not reliably assess the role of antibiotics on severity and frequency of symptoms in our patients. It is generally believed and supported by clinical observations that erythromycin is more effective against pertussis when given prophylactically or started early (<7 days) after symptom onset [33,34]. If given to patients in the paroxysmal stage of pertussis, erythromycin does not seem to reduce symptoms. Our data showed that patients with early initiation of macrolides tended to have shorter hospitalization and less severe illness, even though this difference was not significant.

In summary, the results of this study have characterized the clinical features of pertussis infections in northern Taiwan. Most of the infections occurred in unvaccinated children ≤1 year of age. These young infants were also more likely to develop complications associated with the infection. Classical symptoms of pertussis such as prolonged cough, paroxysmal cough, post-tussive vomiting, and whooping cough were observed in most of the patients, while some young infants presented with cough of short duration and cyanosis. A small peak of pertussis infection was noted in the group of children aged 5 to 9 years, suggesting that a booster vaccination with dTap in preschool (4 to 6 years), school, adolescent and adult age groups might reduce the incidence of disease. About half of the patients had leukocytosis and lymphocytosis, but these features were not associated with disease severity.

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