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ORIGINAL ARTICLE

Clinical characteristics of 967 children with pertussis: a single-center analysis over an 8-year period in Beijing, China

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Abstract

The purpose of this study is to understand children's clinical characteristics with pertussis and analyze risk factors on critical pertussis patients. Demographic data from patients with pertussis at Children's Hospital affiliated to the Capital Institute of Pediatrics between March 2011 and December 2018 were collected. We retrospectively gathered more information with the positive exposure, vaccination, antibiotic usage before diagnosis, clinical manifestation, laboratory tests, therapy, and complications for hospitalized children. We divided the patients into severe and non-severe groups, comparing related factors and clinical characteristics among each group. In particular, we summarize the clinical features of the severe patients before aggravation. A total of 967 pertussis cases were diagnosed, of which 227 were hospitalized. The onset age younger than 3 months old accounted for the highest proportion, and 126 patients received hospitalization. For those patients, the incidence of post-tussive vomiting, paroxysmal cyanosis, post-tussive heart rate decrease, hypoxemia, severe pneumonia, and mechanical ventilation was significantly higher than that in the ≥ 3-month-old group ($p < 0.05$). Among 227 hospitalized patients, 54 suffered from severe pertussis. Risk factors for severe patients included early age of onset, pathogen exposure, and unvaccinated status. Cough paroxysms, post-tussive vomiting, paroxysmal cyanosis, facial flushing/cyanosis/fever during cough, increased WBC, and chest X-ray revealing pneumonia/consolidation/atelectasis were important indications of severe pertussis. Unvaccinated status was an independent risk factor for severe pertussis. The most vulnerable population was infants < 3 months old to pertussis, and may be on the severe end of the disease. Pediatricians must detect and treat severe cases promptly and recommend timely vaccination for all eligible children.

Keywords *Bordetella pertussis* · Children · Clinical characteristics · Beijing

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Introduction

Pertussis is a highly contagious acute respiratory infection mainly caused by *Bordetella pertussis* (*B. pertussis*). Since the introduction of pertussis vaccine in the 1940s and 1950s, the incidence of pertussis has declined remarkably worldwide, with 266,000 cases per year reported in the USA before the introduction of the vaccine, and only approximately 1000 cases per year reported in the 1980s after the introduction of the vaccine [1]. The incidence of pertussis in China has also declined significantly since the introduction of universal immunization programs in 1978; consequently, the incidence dropped from 100−200 per 100,000 in the 1960s and 1970s to less than 1 per 100,000 in the late 1990s and stabilized at less than 0.2 per 100,000 from 2006 to 2010 [2]. In the past decade, however, epidemiological surveillance in developed countries such as those in Europe and the USA, where vaccine coverage is high, has revealed a gradual increase in the incidence of pertussis, known as the "resurgence of pertussis" [3, 4]. In the USA, the incidence of pertussis has increased over the past decade to 20,000−40,000 cases per year [1]. I n the Netherlands, the incidence of pertussis began to increase from 2010, with rates of 3.3, 4.3, and 12.2 per 100,000 in 2010, 2011, and 2012, respectively [5–7]. In the past 5 years, the incidence of pertussis has increased significantly in some regions of China [8], including Jinan, Shandong Province, where the average incidence of pertussis increased from 0.38/100,000 in 2009–2013 to 4.70/100,000 in 2015.

Since the beginning of the expanded immunization program in 1978, the combined diphtheria, tetanus, and pertussis (i.e., "DTP") vaccine has been offered free of charge to the entire population of China. The immunization protocol involves 3 doses of basic immunization at 3, 4, and 5 months of age and 1 dose of booster immunization at 18−24 months of age [9]. However, booster immunization is not administered to pregnant mothers, and mothers are not tested for pertussis antibodies before pregnancy and during gestation. There is a high incidence of pertussis among infants aged < 3 months, and they are prone to serious complications because of inadequate maternal antibody protection and not reaching the age for pertussis vaccination after birth [10]. Andrade et al. [11] reported that infants aged < 3 months accounted for 75% of the total children with pertussis in the 0–1-year age group.

To understand the clinical characteristics of children with pertussis, especially those with severe pertussis; to describe pertussis in small infants aged < 3 months at an earlier stage; and to explore risk factors for the development of severe pertussis, we analyzed children who had a confirmed pertussis and were treated for pertussis at a Grade A national pediatric medical center in Beijing.

Materials and methods

Study population

Children with a diagnosis of pertussis at the Children's Hospital affiliated to the Capital Institute of Pediatrics (Beijing, China) between March 2011 and December 2018, including those seen in outpatient clinics and those hospitalized, were included. The study was approved by the hospital ethics committee, and all patients had written informed consent sign by their legal guardian.

Diagnostic criteria for pertussis

Diagnostic criteria were based on those published by the World Health Organization (WHO) and the United States Centers for Disease Control and Prevention (USCDC; Atlanta, GA, USA) [12, 13]. Clinically suspected pertussis was defined as persistent cough lasting for ≥ 2 weeks with one or more of the following symptoms: paroxysmal spasmodic cough, cockcrow-like inspiratory roar after coughing, and/or vomiting after coughing. A confirmed diagnosis of pertussis was defined as laboratory isolation of *B. pertussis*, a positive multiplex polymerase chain reaction (PCR) result for *B. pertussis*, or significantly elevated levels of the serum antibody IgG titer during the recovery period. Diagnostic criteria for severe pertussis included children with pertussis experiencing any of the following: recurrent apnea; hypoxemia (arterial partial pressure of oxygen < 80 mmHg [1 mmHg = 0.133 kpa]); pertussis encephalopathy; or cardiovascular dysfunction [14]. Severe pneumonia was defined as having any of the following: altered mental status, hypoxemia, high fever, dehydration, severe chest X-ray findings, and extra-pulmonary complications. A positive contact history of pertussis was defined as a history of prolonged (≥ 2 weeks) cough in a close contact that preceded the child's condition [15].

Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) age < 18 years, (2) presence of symptoms consistent with the clinical diagnosis of pertussis and a positive multiplex PCR test result for *B. pertussis* from respiratory secretions. The exclusion criteria were as follows: (1) presence of cough caused by congenital abnormal development or malformation of the airway, (2) presence of cough due to airway compression from various causes, (3) a history or family history of allergic diseases and non-specific inflammatory reactions of the airway due to allergic factors such as

allergic cough and asthma, (4) postnasal drip syndrome, eosinophilic bronchitis, and gastroesophageal reflux cough, (5) cytomegalovirus pneumonia and pulmonary tuberculosis.

Research methods

Patient information

Demographic information from all children with pertussis, including age, sex, and season of onset, was collected to study the corresponding changes in characteristics. The children were divided into five groups depending on age according to the current immunization protocol in China: < 3 months (unvaccinated), 3−6 months (not fully or just fully vaccinated), 7−12 months (months after vaccination), 1−5 years (booster shot received), and > 5 years (years after full vaccination). They were also divided into four groups depending on the season of onset: winter (December to February), spring (March to May), summer (June to August), and autumn (September to November). For hospitalized children, data of patient contact history, history of antibiotic treatment before confirmed diagnosis, clinical characteristics, laboratory data, treatment course, and complications were retrospectively collected from the inpatient electronic medical records system. The patients were divided into two groups according to the severity of pertussis—severe and non-severe—and relevant factors and clinical characteristics were compared between the groups, especially clinical features before progression to severe disease (Fig. 1).

Pertussis pathogen detection

Specimen collection On the second day of patient admission, a dedicated individual collected respiratory secretions in the morning from hospitalized children who were on overnight fast. After disinfection of the oral cavity, respiratory secretions were collected using a disposable suction tube and a sputum collector connected to a negative-pressure aspirator. After aspiration, the specimen was placed in 2 mL of normal saline and stored at − 20℃ until testing. For children visiting the outpatient clinic, respiratory secretions or nasopharyngeal swabs and specimens for routine blood tests were collected on the same day as that of the outpatient visit and stored at − 20℃ until testing.

B. pertussis test This test was performed by the central laboratory of the Children's Hospital affiliated to the Capital Institute of Pediatrics. Sputum or nasopharyngeal swabs were tested using *B. pertussis* multiplex PCR, with reagents purchased from Tiangen Biotech Co., Ltd (Beijing, China). Bacterial total DNA extraction was performed using a commercially available kit according to the manufacturer's instructions (INC Inc.). Two pairs of primers [16, 17] were designed by selecting the *B. pertussis* insertion sequence IS481 gene fragment and the PT gene fragment of the pertussis toxin promoter region as target genes. The primers were synthesized by Genewiz Biotechnology Co., Ltd. (Beijing, China). The multiplex PCR assay included both screening and confirmatory PCR, both of which were used to assess PCR positivity.

Other tests

All children underwent chest X-ray and laboratory exams for the evaluation of disease severity, including C-reactive

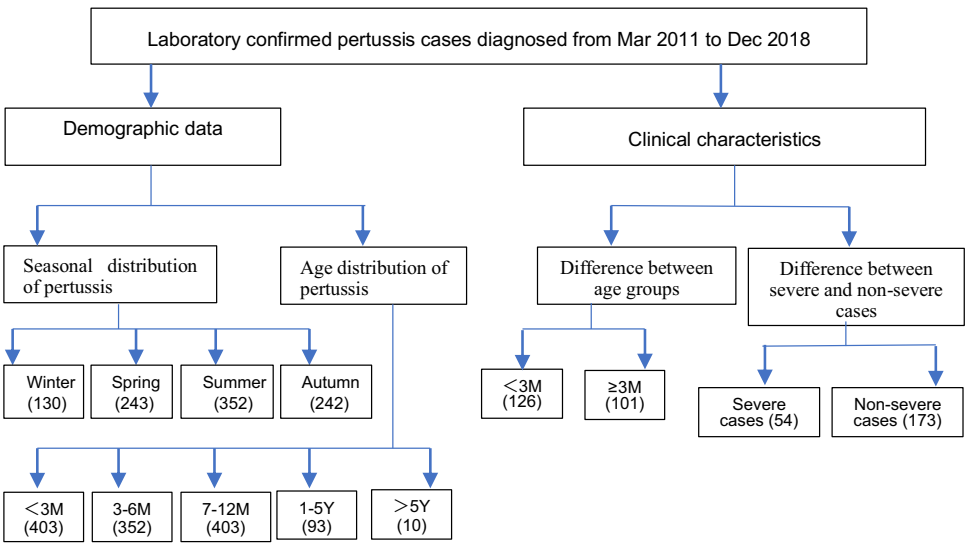

Fig. 1 Study design. The design of this single-center retrospective case–control study

protein (CRP), brain natriuretic peptide (BNP), and echocardiography.

Statistical processing

Statistical analysis was performed using SPSS, version 25.0 (IBM Corporation, Armonk, NY, USA). Measurement data conforming to normal distribution are expressed as mean ± standard deviation, and enumeration data are expressed as *n* (%). The independent-samples *t* test was used to compare measurement data between groups, and the chi-square test (Fisher's exact probability method) and chi-square trend test were used for enumeration data. We used univariate logistic regression analysis to find out the related factors of severe pertussis, and then use multivariate logistic regression to analyze the high-risk factors of severe pertussis. Differences with $p < 0.05$ were considered to be statistically significant.

Results

Demographic characteristics of children with pertussis

A total of 967 children were diagnosed with laboratory-confirmed pertussis between March 2011 and December 2018, of whom 23.5% (227/967) were hospitalized. Among the 967 children with pertussis, 56.0% (542/967) were male and 44.0% (425/967) were female. As shown in Table 1, the analysis of seasonal distribution revealed small differences among the seasons, with a slightly higher proportion of children experiencing disease onset in summer (36.4% [352/967]), but there was no statistically significant difference among seasons ($p = 0.956$). The largest proportion of patients were infants < 3 months (41.7% [403/967]). Figure 2 shows the number of pertussis patients diagnosed at the hospital from 2011 to 2018, with an increasing trend. Figure 3 shows the age distribution each year, and Fig. 4 shows seasonal distribution.

Table 1 The characteristics of all the enrolled patients with pertussis from March 2011 to December 2018

Year	*n*	Onset season [*n* (%)]				Onset age [*n* (%)]				
		Winter	Spring	Summer	Autumn	< 3 M	3–6 M	7–12 M	1–5Y	> 5Y
2011	26	2 (7.7)	11 (42.3)	8 (30.8)	5 (19.2)	13(50)	10(38.5)	1 (3.8)	2 (7.7)	0
2012	33	2 (6.0)	12 (36.4)	15 (45.5)	4 (12.1)	27 (81.8)	3 (9.1)	0	3 (9.1)	0
2013	24	2 (8.4)	6 (25.0)	11 (45.8)	5 (20.8)	16 (66.7)	4 (16.7)	1 (4.1)	3 (12.5)	0
2014	116	12 (10.3)	25 (21.6)	42 (36.2)	37 (31.9)	52 (44.8)	34 (29.3)	10 (8.6)	15 (12.9)	5 (4.3)
2015	108	21 (19.4)	30 (27.8)	42 (38.9)	15 (13.9)	41 (38.0)	51 (47.2)	7 (6.5)	9 (8.3)	0
2016	149	25 (16.8)	27 (18.1)	43 (28.9)	54 (36.2)	79 (53.0)	50 (33.5)	11 (7.4)	8 (5.4)	1 (0.7)
2017	230	31 (13.5)	56 (24.3)	80 (34.8)	63 (27.4)	95 (41.3)	82 (35.7)	30 (13.0)	21 (9.1)	2 (0.9)
2018	281	35 (12.5)	76 (27.0)	111 (39.5)	59 (21.0)	80 (28.5)	118 (42.0)	49 (17.4)	32 (11.4)	2 (0.7)
Total	967	130 (13.5)	243 (25.1)	352 (36.4)	242 (25.0)	403 (41.7)	352 (36.4)	109(11.3)	93 (9.6)	10 (1.0)

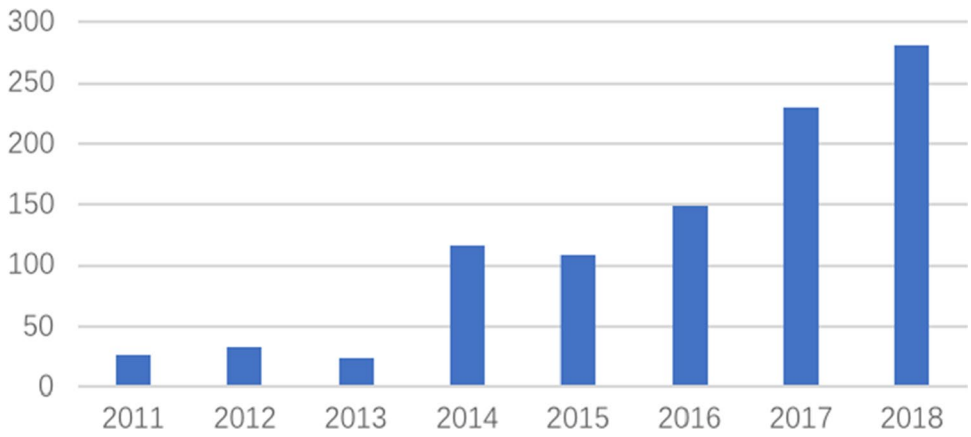

Fig. 2 Number of pertussis diagnosed at the hospital between 2011 and 2018. An increasing trend of number of pertussis patients diagnosed at the hospital was observed

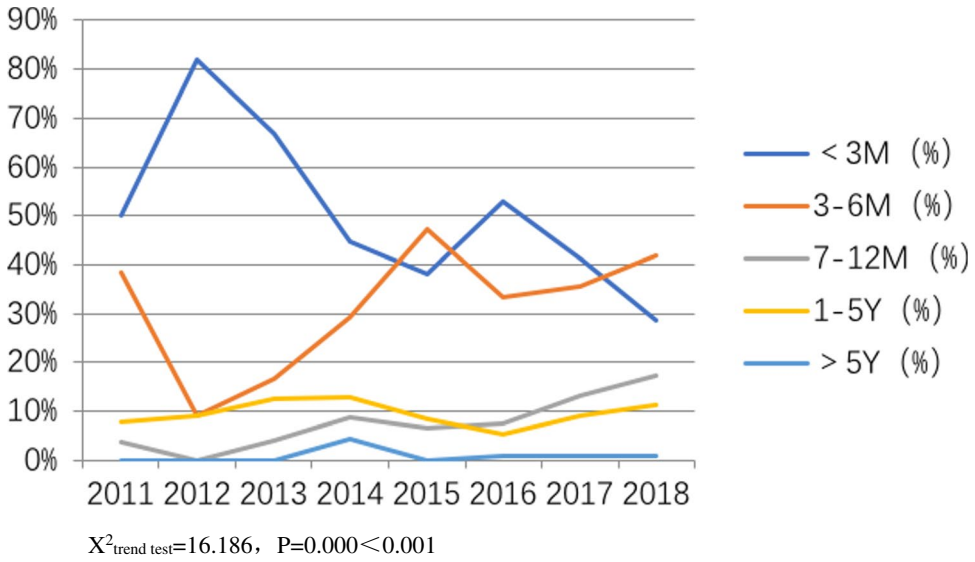

Fig. 3 Age distribution of pertussis patients diagnosed at the hospital between 2011 and 2018. $X^2_{\text{trend test}} = 16.186$, $p = 0.000 < 0.001$. Within each year, the predominant age groups of pertussis patients are < 3 months and 3–6 months. The increasing trend in the number of patients in the 3–6 months age group in recent years is statistically significant

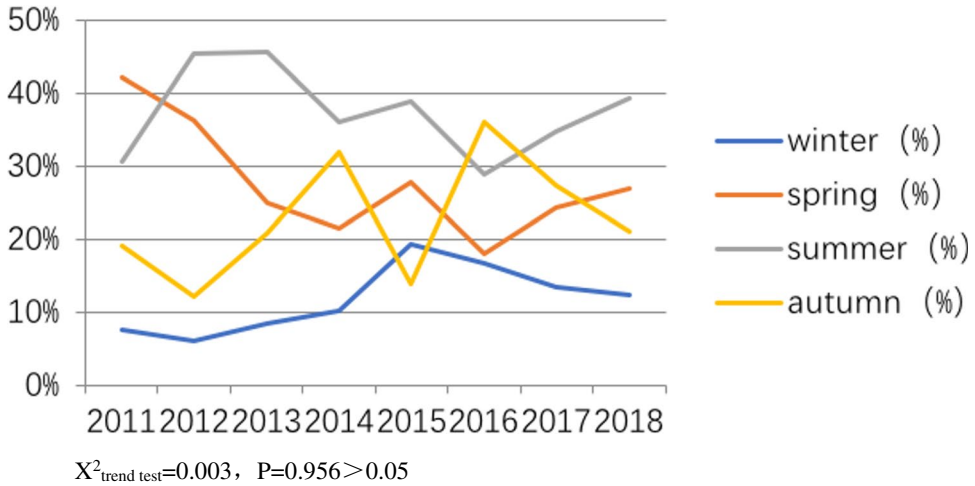

Fig. 4 Seasonal distribution of pertussis diagnosed at the hospital between 2011 and 2018. $X^2_{\text{trend test}} = 0.003$, $p = 0.956 > 0.05$. Within each year, there is a slight increase in pertussis cases in summer, but overall the difference is insignificant

Clinical characteristics of hospitalized children with pertussis

General information

In total, 227 patients with pertussis were hospitalized, of whom 85.95 (195/227) were < 1 year old, and 55.5% (126/227) were < 3 months old. As shown in Table 2, 75.3% (171/227) patients were not vaccinated, including 45 non-vaccinated children ≥ 3 months of age. 26.9% (61/227) of the children had a history of positive household contact. From disease onset to hospitalization, only 42.7% (97/227) of the children experienced cough for ≥ 14 days, and 85.0% (193/227) had paroxysmal spasmodic cough; however, only 18.1% (41/227) experienced cough followed by cockcrow-like roar and post-tussive vomiting. Laboratory investigations revealed that 71.3% (162/227) of children exhibited a white blood cell count of $\geq 15 \times 10^9$/L, and 80.2% (182/227) had an elevated lymphocyte ratio (> 60%). 37.9% (86/227) of patients were complicated by infections with other pathogens, including 28 cases of parainfluenza virus III, 12 cases of respiratory syncytial virus, 10 cases of mycoplasma pneumoniae, 6 cases of *Streptococcus pneumoniae*, 6 cases of *Klebsiella pneumoniae*, 4 cases of *Escherichia coli*, 4 cases of human rhinovirus, 4 cases of *Chlamydia pneumoniae*, 3 cases of haemophilus influenzae, 4 cases of Epstein-Barr virus, 3 cases of influenza B virus, and 2 cases of *Chlamydia trachomatis*. Those who had combined infections had more signs of fever and lung rales and crackles than those who did not. In total, 226 children underwent chest imaging, which suggested pneumonia in 70.9% (161/227), of whom 6.17% (14 /227) exhibited pulmonary consolidation and/or atelectasis, 5.3% (12/227) had interstitial lung lesions, and 4.9% (11/227) had pulmonary ground-glass opacities. All children received erythromycin or azithromycin for pertussis and other symptomatic treatments involving cough-suppressants,

Table 2 Clinical characteristics of patients with pertussis from March 2011 to December 2018

Characteristic	*n* (%): mean ± SD
Gender (M:F)	131 (57.7):96 (42.3)
Cough duration before admission(days)	16.64 ± 14.69
≤ 7	48 (21.2)
8–14	82 (36.1)
15–30	76 (33.5)
31–45	13 (5.7)
> 45	8 (3.5)
Received antibiotics before admission	
No	74 (32.6)
Yes	153 (67.4)
Household contacts history	
Negative	166 (73.1)
Positive	61 (26.9)
Pertussis vaccination status	
Non-vaccinated (0 dose)	171 (75.3)
Incompletely vaccinated (1 dose or 2 doses)	22 (9.7)
Completely vaccinated (3 doses)	34 (15.0)
Manifestations	
Paroxysmal spasmodic cough	193 (85.0)
Inspiratory cockcrow-like roar	41 (18.1)
Nasal congestion, rhinorrhea	17 (7.5)
Paroxysmal cyanosis	61 (26.9)
Facial flushing and/or cyanosis during coughing	186 (81.9)
Post-tussive vomiting	41 (18.1)
Subconjunctival bleeding	3 (1.3)
Apnea	10 (4.4)
Hypoxemia	91 (40.1)
Decreased heart rate during coughing	19 (8.4)
Pertussis encephalopathy	9 (4.0)
Seizure	7 (3.1)
Fever	75 (33.0)
Respiratory failure	25 (11.0)
Cardiac failure	6 (2.6)
Severe pneumonia	33 (14.5)
Severe pertussis	54 (23.8)
Supportive care procedures	
No	157 (69.2)
Oxygen therapy	32 (14.1)
Nasal continuous positive airway pressure	21 (9.3)
Invasive mechanical ventilation	17 (7.5)
Laboratory examination	
WBC ($\times 10^9$/L)	22.88 ± 14.27
≤ 10	12 (5.3)
10–15	53 (23.4)
15–20	60 (26.4)
20–30	59 (26.0)
30–50	33 (14.5)
> 50	10 (4.4)
Lymphocyte ratio	0.68 ± 0.10
< 60%	45 (19.8)
≥ 60%	182 (80.2)
C-reactive protein	
< 8 mg/L	111 (92.9)
≥ 8 mg/L	16 (7.1)
Pathogen co-infected	
No	141 (62.1)
Yes	86 (37.9)
Chest X-ray	
No inspection	1 (0.4)
Normal	1 (0.4)
Lung texture thickening	64 (28.2)
Pneumonia	161 (70.9)

anti-wheezing, antispasmodics, and sedatives. Of the 227 patients, 99.6% (226/227) had an improved condition or were cured, and 0.4% (1/227) died. The deceased patient's age of disease onset was 13 days. He was hospitalized at the age of 28 days. He was intubated and mechanically ventilated at the time of NICU admission, and received leukoreduction therapy (exchange transfusion). He died from multiple organ dysfunction syndrome (MODS) at the age of 30 days.

Clinical characteristics of children with pertussis onset at different ages

The children were divided into two groups according to age: < 3 months ($n = 126$) and ≥ 3 months ($n = 101$). Indicators that were significantly higher in the < 3 months age group than in the ≥ 3 months group included a history of positive exposure (36.5% [46/126] vs. 14.9% [15/101]), post-tussive vomiting (23.8% [30/126] vs. 10.9% [11/101]), paroxysmal cyanosis (34.9% [44/126] vs. 16.8% [17/101]), decreased heart rate after coughing (14.3% [18/126]vs. 2.0% [2/101]), hypoxemia (32.5% [41/126] vs. 8.9% [9/101]), and severe pneumonia (19.1% [24/126] vs. 8.0% [8/101]), all of which showed statistically significant differences ($p < 0.05$). White blood cell count, proportion of patients who received antibiotics before admission, and the duration of cough (days) were significantly lower in the < 3 months age group ($p < 0.05$) (Table 3).

Analysis of clinical characteristics and factors associated with severe pertussis

Among the 227 children hospitalized for pertussis, 54 had severe disease, and the mean age at onset was significantly

Table 3 Comparison of clinical characteristics, < 3 months group vs ≥ 3 months group

Characteristic	< 3 months ($n = 126$) n (%), mean ± SD	≥ 3 months ($n = 101$) n (%), mean ± SD	Statistic	p value
Male sex	74 (58.7)	57 (56.4)	0.12	0.728
Gestational age < 37 weeks	7 (5.6)	11 (10.9)	2.19	0.139
Positive contact history	46 (36.5)	15 (14.9)	13.38	0.00025
Manifestations				
Cough duration before admission (days)	13.13 ± 9.55	21.02 ± 18.42	− 3.91	< 0.001[b]
Paroxysmal spasmodic cough	111 (88.1)	82 (81.2)	2.1	0.147
Inspiratory cockcrow-like roar	23 (18.3)	18 (17.8)	0.01	0.933
Post-tussive vomiting	30 (23.8)	11 (10.9)	6.32	0.012
Nasal congestion, rhinorrhea	10 (7.9)	7 (6.9)	0.08	0.775
Paroxysmal cyanosis	44 (34.9)	17 (16.8)	9.33	0.002
Facial flushing at the end of paroxysm	108 (85.7)	78 (77.2)	2.73	0.099
Subconjunctival bleeding	2 (1.6)	1 (1.0)	/	1 [a]
Apnea	8 (6.4)	2 (2.0)	/	0.191 [a]
Bradycardia	18 (14.3)	2 (2.0)	10.57	0.001
Fever	38 (30.2)	37 (36.6)	1.06	0.303
Positive pulmonary signs	108 (85.7)	82 (81.2)	0.84	0.359
Laboratory examination				
WBC ($\times 10^9$/L)	21.14 ± 12.50	25.06 ± 16.01	− 2.02	0.045[b]
Lymphocyte ratio	0.68 ± 0.09	0.68 ± 0.12	0.263	0.793[b]
Elevated lymphocyte ratio (≥ 60%)	105 (83.3)	77 (76.2)	1.78	0.183
Elevated C-reactive protein (> 8 mg/L)	7 (5.6)	9 (8.9)	0.96	0.326
Infiltration on chest roentgenogram	91 (72.2)	70 (69.3)	0.23	0.631
Complications				
Hypoxia	41 (32.5)	9 (8.9)	18.22	< 0.001
Respiratory failure	18 (14.3)	7 (6.9)	3.09	0.079
Cardiac failure	4 (3.2)	2 (2.0)	/	0.695 [a]
Pertussis encephalopathy	6 (4.8)	3 (3.0)	/	0.735 [a]
Seizure	5 (4.0)	2 (2.0)	/	0.466 [a]
Pulmonary consolidation and/or atelectasis	8 (6.4)	4 (4.0)	0.64	0.424
Severe pneumonia	24 (19.1)	8 (8.0)	5.73	0.017
Invasive or non-invasive mechanical ventilation	30 (23.8)	7 (6.9)	11.71	< 0.001
Invasive mechanical ventilation	11 (8.7)	6 (5.9)	0.63	0.427
Received antibiotics before admission	73 (57.9)	80 (79.2)	11.54	< 0.001
Delayed diagnosis due to pneumonia	97 (77.0)	58 (57.4)	9.9	0.002
Diagnosed immediately upon first hospital visit	2 (1.6)	1 (1.0)	/	1 [a]

[a]Fisher's exact probability method, [b]t test

lower in the severe group than in the non-severe group (3.16 ± 5.06 vs. 10.63 ± 22.29 months) (Table 4). The incidence of a positive contact history (46.3% [25/54] vs. 20.8% [36/173]) and the non-vaccination ratio (94.4% [51/54] vs. 69.4% [120/173]) were significantly higher in the severe group than in the non-severe group, respectively, and the differences were both statistically significant ($p < 0.001$). The incidences of paroxysmal spasmodic cough, post-tussive vomiting, paroxysmal cyanosis, facial flushing and/or cyanosis during coughing, and fever were higher in the severe group than in the non-severe group, and the differences were statistically significant (all $p < 0.05$). The average white blood cell count in the non-severe and severe group was $19.96 \pm 9.66 \times 10^9$/L and $32.22 \pm 21.19 \times 10^9$/L, respectively, and the proportion of patients with elevated C-reactive protein level were 2.9% and 20.4%, respectively, with statistically significant differences (all $p < 0.001$). The proportions of patients with pulmonary radiography suggestive of pneumonia and combined pulmonary consolidation and/or atelectasis in the severe group were 83.3% [45/54] and 16.7% [9/54], respectively, which were clearly higher than those of 67.1% [116/173] and 0.6% [1/173]in the non-severe group,

Table 4 Comparison of clinical characteristics in severe pertussis group vs non-severe pertussis group

Characteristic	Severe pertussis ($n=54$) n (%), mean ± SD	Non-severe pertussis ($n=173$) n (%), mean ± SD	Statistic	p value
Male sex	29 (53.7)	102 (59.0)	0.47	0.495
Age of onset (month)	3.16 ± 5.06	10.63 ± 22.29	4.085	< 0.001[b]
Gestational age < 37 weeks	6 (11.1)	12 (6.9)	/	0.386[a]
Positive contact history	25 (46.3)	36 (20.8)	13.6	< 0.001
Pertussis vaccination status				
Non-vaccinated (0 dose)	51 (94.4)	120 (69.4)	13.93	< 0.001
Incompletely vaccinated (1 dose or 2 doses)	2 (3.7)	20 (11.6)	2.9	0.088
Completely vaccinated (3 doses)	1 (1.9)	33 (19.1)	9.59	0.002
Manifestations				
Cough duration before admission (days)	14.69 ± 11.18	17.25 ± 15.61	1.12	0.264[b]
Paroxysmal spasmodic cough	52 (96.3)	141 (81.5)	7.07	0.008
Inspiratory cockcrow-like roar	13 (24.1)	28 (16.2)	1.73	0.188
Post-tussive vomiting	17 (31.5)	24 (13.9)	8.62	0.003
Nasal congestion, rhinorrhea	4 (7.4)	13 (7.5)	/	1[a]
Paroxysmal cyanosis	44 (81.5)	17 (9.8)	107.53	< 0.001
Facial flushing at the end of paroxysm	53 (98.2)	133 (76.9)	12.58	< 0.001
Subconjunctival bleeding	1 (1.9)	2 (1.2)	/	0.559[a]
Fever	28 (51.9)	47 (27.2)	11.33	< 0.001
Positive pulmonary signs	50 (92.6)	140 (80.9)	4.11	0.043
Laboratory examination				
WBC ($\times 10^9$/L)	32.22 ± 21.19	19.96 ± 9.66	−4.12	< 0.001[b]
Lymphocyte ratio	0.67 ± 0.09	0.68 ± 0.10	0.76	0.448[b]
Elevated lymphocyte ratio (≥ 60%)	46 (85.2)	136 (78.6)	1.12	0.29
Elevated C-reactive protein (> 8 mg/L)	11 (20.4)	5 (2.9)	/	< 0.001[a]
Infiltration on chest roentgenogram	45 (83.3)	116 (67.1)	5.29	0.021
Complications				
Pulmonary consolidation and/or atelectasis	9 (16.7)	1 (0.6)	/	< 0.001[a]
Other pathogen co-infection	25 (46.3)	61 (35.3)	2.13	0.144
Received antibiotics before admission	36 (66.7)	117 (67.6)	0.02	0.895
Delayed diagnosis due to pneumonia	42 (77.8)	113 (65.3)	2.95	0.086
Diagnosed immediately upon first hospital visit	3 (5.6)	0	/	0.013[a]

[a]Fisher's exact probability method, [b]t test

with statistically significant differences (all with $p < 0.05$). According to multivariate analysis, non-vaccination was an independent risk factor for the development of severe pertussis (odds ratio 0.229 [95% confidence interval 0.071−0.736]; $p = 0.013$).

Discussion

The introduction and widespread implementation of pertussis vaccination have led to a significant decrease in the incidence and mortality of the disease. However, in recent years, the incidence of pertussis has gradually increased in China and some developed countries with high vaccine coverage [18–20], a phenomenon known as the "resurgence of pertussis." We determined the incidence of pertussis in children diagnosed at the Children's Hospital of the Capital Institute of Pediatrics between March 2011 and December 2018 and found a significant increase in the number of laboratory-confirmed pertussis cases in the hospital in the past 8 years. All children were divided into five groups according to age at diagnosis (< 3 months, 3−6 months, 7−12 months, 1−5 years, and > 5 years), and pertussis was most prominent in the < 3 months age group. It was also shown that pertussis occurred throughout the year, with some seasonal pattern, with summer associated with the most frequent onset, followed in order by spring

and autumn, and winter, which was generally consistent with the findings of previous investigations [21, 22].

The typical clinical presentation of pertussis is characterized by paroxysmal spasmodic cough, inspiratory cockcrow-like roar at the end of cough, and peripheral blood lymphocytosis[1]; catarrh symptoms such as nasal congestion and rhinorrhea may occur early in the disease course. Catarrh symptoms early in the course of pertussis are non-specific and not easily recognized early, with low rates of suspected and initial diagnosis, as well as a high rate of missed diagnosis before admission. In this study, only 7.49% (17/227) of children experienced catarrh symptoms such as nasal congestion and rhinorrhea, while only 1.3% (3/227) were suspected of pertussis before admission, which also supports this view. This study also found that the percentage of children experiencing paroxysmal spasmodic cough was 85.0% (193/227), and only 18.1% (41/227) exhibited cockcrow-like roar, both suggesting that the clinical manifestations of pertussis are atypical, and for those with suspected pertussis, laboratory tests such as PCR testing of respiratory secretions need to be completed as soon as possible for early diagnosis and treatment.

The duration of cough before diagnosis among hospitalized children in the present study ranged from a minimum of 1 day to a maximum of 90 days (median 13 days), and 57.3% (130/227) experienced cough for ≤ 14 days, which is inconsistent with the current diagnostic criteria for pertussis (requiring cough ≥ 14 days). Andrade et al. [11] reported that 71.5% of children with pertussis experienced cough for < 14 days before diagnosis, and 40.5% experienced cough for < 7 days. Therefore, cough duration ≥ 2 weeks should not be used exclusively as a clinical criterion for the diagnosis of pertussis in clinical practice.

The present study demonstrated that most children experienced elevated leukocyte levels (94.7% (215/227)), with a mean count of 22.88×10^9/L. 44.9% (102/227) of patients had a leukocyte count of $\geq 20 \times 10^9$/L, and 80.2% (182/227) had a lymphocyte ratio of ≥ 60%, which is generally consistent with the findings reported by Berger et al. [23]. Previous research has shown that an elevated peripheral blood leukocyte count in pertussis is positively correlated with disease severity, and a predominance of neutrophils suggests more severe disease. Research has also shown that increased leukocyte levels are an independent risk factor for severe pertussis [24]. A leukocyte count of $> 60 \times 10^9$/L predisposes patients to fatal pertussis, with 90−100% of such deaths being due to severe pneumonia [25]. The present study also found that the leukocyte count among children in the severe pertussis group was significantly higher than that of the non-severe group.

There are currently three methods for the laboratory diagnosis of pertussis[26]: *B. pertussis* culture; pertussis PCR assay; and pertussis serum antibody test. *B. pertussis* culture is the gold standard for diagnosis, with high specificity but low sensitivity [27]. Moreover, the required culture period is long, and the results are influenced by various factors including specimen collection, transport, culture, time of onset, and whether antibiotics were applied. The PCR test for pertussis yields results rapidly and conveniently and has high sensitivity (93.5%) and specificity (97.1%); the sensitivity of the PCR test is higher than that of *B. pertussis* cell culture [28], which can aid in early clinical diagnosis and rapid diagnosis during epidemic outbreaks. The diagnostic criteria for pertussis according to the World Health Organization, the USCDC, Europe, and other countries use the PCR or culture method. Pertussis serum antibody detection plays an important role in diagnosis during the recovery period of the disease but is used mostly for epidemiological surveys; moreover, it does not aid in early clinical diagnosis. A single ELISA test for pertussis toxin (PT)-immunoglobulin G is not recommended in children < 3 months of age, and interpretation of pertussis serological test results is unreliable in children who have been vaccinated against pertussis within 1 year of testing. The Global Pertussis Initiative [29] suggests that PCR assay has the highest sensitivity and can be used as a diagnostic tool in all children. In the present study, multiplex PCR tests were used for laboratory diagnosis in all children.

According to the current DTP immunization program in China, infants < 3 months of age are not vaccinated against DTP. Studies [13, 30] have shown that the half-life of maternal-borne antibody decay is only 6 weeks, and with the decay of antibodies, the 0−3 months age group is no longer fully protected by maternal-borne antibodies. In this study, 41.7% (403/967) of all children diagnosed with pertussis and 55.5% (126/227) of the hospitalized children with pertussis were < 3 months of age, suggesting a high susceptibility to pertussis in infants < 3 months of age. In this study, the proportion of non-vaccinated children was 75.3% (171/227), of which 55.5% (126/227) were younger than the vaccination age. It is, therefore, worth discussing whether pertussis vaccination should be administered earlier in China.

Researchers have found that family members are the main source of infection for pediatric pertussis [31–33]. In this study, 26.9% (61/227) of hospitalized patients had a history of positive exposure in the family before disease onset, and the proportion with a history of positive exposure was significantly higher in the < 3 months age group than in the ≥ 3 months age group. Considering that the younger the infant, the more care by the family or nursery staff is needed, many countries have implemented a "cocoon" strategy for pertussis vaccination, recommending acellular pertussis vaccine (APV) for every pregnant woman at 27−32 weeks of gestation [31]. Vaccination of family members and caregivers with whom the infant is

likely to come into contact is effective in reducing the prevalence and mortality of pertussis in children under the vaccination age [34, 35].

In this study, the proportion of children with post-tussive vomiting and paroxysmal cyanosis and the proportion of those with hypoxemia, severe pneumonia, cardiac dysfunction such as decreased heart rate during coughing, and other serious complications that require mechanical ventilation (including both invasive and non-invasive) were significantly higher in the < 3 months age group than in the ≥ 3 months age group. Infants < 3 months of age are prone to severe pneumonia as a comorbidity; the younger the infant, the more severe the disease [36, 37], which is due to the immature development of the immune system and thorax, narrow airways, and weak cough in small infants, as well as delayed ciliary motility caused by pertussis toxin, and mucous airway secretions, causing airway obstruction [23]. A previous study reported that the 20 pertussis-related deaths in the USA nationally in 2012 occurred primarily in infants < 3 months of age [31], also suggesting that infants < 3 months of age are susceptible to severe pertussis.

In this study, pulmonary hypertension was not found in any cases. Some researchers had found that pulmonary hypertension was associated with mortality in pertussis critical illness. Pulmonary hypertension was reported in 75% of patients who died compared to 6% of survivors. Pulmonary hypertension was associated with elevated WBC levels, the need for mechanical ventilation, and death. The pathophysiologic descriptions most commonly used to explain development of pulmonary hypertension are (1) acute vasoconstriction from endothelial dysfunction and/or toxin-related mechanisms, and (2) reduction of pulmonary vascular cross-sectional area [23].

By comparing the severe and non-severe disease groups, pertussis in children with the following characteristics resulted in progression to severe pertussis: younger age at onset; non-vaccination; history of positive exposure; paroxysmal spasmodic cough; post-tussive vomiting; paroxysmal cyanosis; markedly elevated white blood cell count; and pulmonary consolidation and/or atelectasis. In this study, we found that the proportion of non-vaccinated patients was significantly higher in the severe disease group than in the non-severe group, that vaccination status (i.e., non-vaccinated, incompletely vaccinated, and completely vaccinated) was an independent risk factor for severe pertussis, and that pertussis vaccination plays an important role in the prevention of pertussis, especially in reducing the incidence of severe disease. Studies [30, 38] have shown that the effectiveness rate of the 3 doses of pertussis vaccine in infants 6−23 months of age can reach 91.7%, while the effectiveness rate of only 1 dose is only 46%, and that non-vaccinated or incompletely vaccinated infants account for 88.7% of all children with pertussis, thus supporting these findings. The WHO [39] considers pertussis vaccination to be the most important measure to reduce severe pertussis incidence among infants.

The present study had some limitations, the first of which was its single-center design. Although the children were chosen from the entire region, they were not necessarily representative of morbidity in all children. Second, the study did not follow up all cases in detail, and there is insufficient detail regarding the entire disease course. Third, in this study, only confirmed cases of pertussis were analyzed, and there was no control group.

Conclusion

The results of the present study suggest that the number of children diagnosed with pertussis has been increasing annually in the past 8 years and that younger children, especially infants < 3 months of age, are more susceptible to pertussis and prone to severe disease. Furthermore, vaccination status was an independent risk factor for severe pertussis. As such, pertussis vaccination should be administered in a timely manner to all children who have reached the vaccination age. Finally, discussing whether pertussis vaccination should be administered at an early stage in China is warranted.

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Data availability All data generated or analyzed during this study are included in this published article.

Code availability Not applicable

Declarations

Ethics approval The study was approved by the Ethics Committee of Affiliated Children's Hospital, Capital Institute of Pediatrics (SHERLL2021031).

Consent to participate Not applicable

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Conflict of interest The authors declare no competing interests.

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