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Retrospective Analysis of Pertussis Infection in Tai'an Area

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Abstract: *Bordetella pertussis belongs to the genus Botrytis, which is highly infectious and has a high case fatality rate. In recent years, the number of people infected with pertussis has been increasing each year, despite the popularization of WPV vaccination in many areas. Therefore, we studied the epidemiological characteristics of pertussis in Tai'an, statistically analyzed the basic clinical data of the patients, and summarized the incidence trend of pertussis disease in Tai'an in recent years, with the aim of guiding clinicians in the diagnosis and treatment of pertussis in children. The aim of this study is to collect data and statistics on pertussis patients in terms of epidemiological characteristics, collect the basic data of pertussis patients in Tai'an Children's Hospital from March 2016 to December 2018, and analyze the epidemiological characteristics of the disease in patients in the region.From March 2016 to December 2018, a total of 1,942 patients with whooping cough were found in Tai'an Children's Hospital. The age of the population with B. pertussis infection was mainly under 6 years old, with a higher number of cases in the age groups of 3 months to 2 years old and 5 to 6 years old. The hospitalization time of the patients was 2-20 d, with an average of 12d. This study concludes that the high incidence season of pertussis infection in Taian is summer and fall. The mode of transmission has changed from the child-child transmission pattern in the past to the current adolescent/adult-neonate/child transmission pattern. The age of the population with B. pertussis infection was mainly under 6 years old, and mixed infections were common.*

Keywords: *pertussis; case analysis; co-infection*

1. Introduction

Pertussis is a class of respiratory infections caused by Bordetella pertussis, which is mainly characterized by paroxysmal spasmodic cough with end-inspiratory chicken-like echoes, and the most severe clinical manifestations are mostly in infants and young children[1]. Pertussis spreads mainly through air droplets, and the population is most susceptible in childhood, especially in infants and young children, with a high mortality rate, seriously jeopardizing the health of infants and young children. Currently in China, B. pertussis mainly infects children within 5 years of age, and infants and young children within the first few months of life tend to have more severe symptoms and even death. Many countries and regions around the world have introduced pertussis vaccination, which has had a remarkable effect and effectively reduced the infection rate of pertussis[2]. However, in recent years, in countries and regions with high vaccine coverage, pertussis epidemics occur every 3-5 years, and the phenomenon of "reemergence of pertussis" has appeared, and there was even a pertussis outbreak in the United States in 2012[3], so researchers have gradually begun to pay attention to the onset of the disease in adolescents and adults.

In this study, in the PCR laboratory of the Disease Screening Center of Tai'an Children's Hospital, the data of 1942 cases of pertussis patients from 2016 to 2018 were counted, including personal basic information, whether vaccination was completed, hospitalization time, discharge, complications, treatment and prognosis, and the recent.

2. Materials and Methods

2.1. Sample collection

The samples were obtained from the laboratory of the Disease Screening Center of Tai'an Children's Hospital, and the pharyngeal swabs were collected from patients by pressing the patient's tongue with a

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tongue depressor, extending the swab to the isthmus of the patient's pharynx, moderately wiping the posterior pharyngeal wall and the tonsils on both sides for several times, and then rotating the swab to increase the contact surface to avoid contacting the tongue and the oral mucous membranes and other parts of the body. Immediately after sampling, the swab was placed into a sampling tube containing 3 ml of sample solution, and the cap was tightened and sealed for examination. During the sample collection process, the time of onset of the disease, the time of sample collection, the patient's age, gender, home address, and the patient's clinical characteristics were recorded at the same time.

2.2. Main instruments

The main instruments are shown in Table 1.

Table 1: Instrument details.

Instrument Name	Manufacturer
Biological Safety Cabinet	Jinan Xinbeisi Company
Ordinary centrifuge	Thermo Fisher Company
Refrigerator	Haier Company
Autoclave Sterilizer	Panasonic Company
PCR Gene Amplifier	Shanghai Zhi Shan Company
Metal Heater	Cimo Company

3. Materials and Methods

3.1. Sample collection

Extraction method: the sample of the throat swab was shaken and poured into the centrifuge tube and labeled, placed in the centrifuge at 2000 rpm for 3 min, and then 200 μl of the liquid sample at the bottom of the throat swab tube was taken for the extraction of nucleic acid, the process was carried out by using the fully automated nucleic acid extraction kit produced by Sun Yat-sen University D'an Genetics Company Limited to extract the nucleic acid, and all the operations were done in the biosafety cabinet.

3.2. PCR reagent preparation

Duane Gene Pertussis kit to take out the pertussis (Bronchocephalitis, BP) PCR reaction solution A liquid and B liquid, placed at room temperature for a period of time, until the reagents melted, placed in the oscillator mixing, centrifuge on the centrifuge at 8000 rpm speed centrifugation for 10s and then used.BP single reaction amplification system preparation (see Table 2).

Table 2: Amplification system.

BP PCR reaction solution A	BP PCR reaction solution B	Amplification system
17 μl	3 μl	20 μl

3.3. Sample addition (sample preparation area)

Add 5 μl of nucleic acid of the sample to be tested and 5 μl each of the negative and positive quality control products into the above prepared experimental system, centrifuge briefly for a few seconds, and then transfer to the amplification detection zone through the transfer window for amplification.

3.4. PCR amplification (amplification detection zone)

The extracted DNA was amplified using a real-time fluorescent PCR instrument to identify positive samples. All samples were processed after autoclaving at 125℃ 25min in the afternoon of the day the experiment was completed.

3.5. Statistical analysis

Statistical software IBM SPSS Statistics24 was used to statistically analyze the data, and the count data were expressed as t-test of two independent samples for comparison between two groups; the

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independent sample chi-square test was used for comparison between groups of count data, and the difference was statistically significant with P<0.05.

4. Results

4.1. General situation of the survey population

From 2016 to 2018, the number of throat swabs of patients attending the clinic was tested to be 12,097 of which, 1,942 patients were positive for pertussis, the age of the patients was within 60 years, 1,039 (54%) were male patients, and 903 (46%) were female patients, and the age distribution of the study was in 7 age groups, the findings of each group were >0 to =3 months of age for 247 cases (12.7%), and 790 cases (40.7%) at >3 months to =12 months of age, 370 cases (19.1%) at >1 to =2 years of age, 126 cases (6.5%) at >2 to =4 years of age, 256 cases (13.2%) at >4 to =6 years of age, 76 cases (3.9%) at >6 to =13 years of age, and 40 cases (2.1%) at >27 to =60 years of age; the distribution of the seasons of onset of the disease was: spring 352 cases (18%), 754 cases (39%) in summer, 538 cases (28%) in fall, and 298 cases (15%) in winter (see Figures 1-2 for the distribution of patients' age, and season of onset). The pertussis patients were divided into two groups according to age, =1 year old group and =1 year old group, and the two groups were statistically analyzed in terms of gender, season and year of onset (See Table 3).

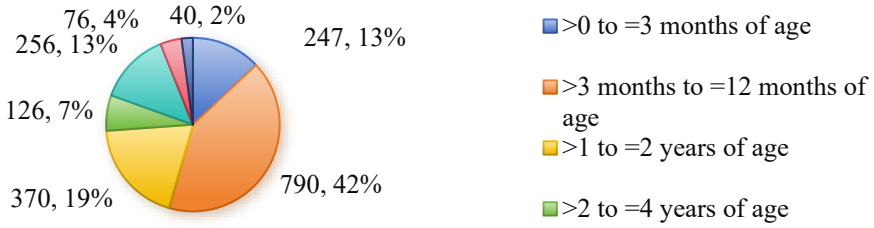

Figure 1: Distribution of patients with pertussis by age

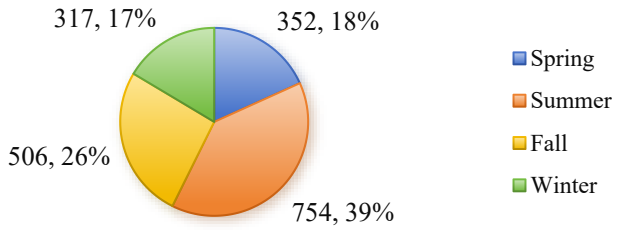

Figure 2: Distribution of patients with pertussis in different seasons

Table 3: Analysis of general condition of patients in different age groups.

Variable	Total (N=1942)	=1 year old (n=1272)	>1 year old (n=670)	χ^2	P value
Sex				1.203	0.273
Male, n(%)	103(54%)	692(0.54%)	347(0.52%)		
Female, n(%)	903(0.46%)	580(0.46%)	323(0.48%)		
Year				87.298	<0.001
2016, n(%)	99(0.51%)	55(0.43%)	44(0.66%)		
2017, n(%)	918(0.47%)	699(0.55%)	219(0.33%)		
2018, n(%)	925(0.48%)	518(0.41%)	407(0.61%)		
Season				20.593	<0.001
Spring, n(%)	352(0.18%)	249(0.20%)	103(0.15%)		
Summer, n(%)	754(0.39%)	484(0.38%)	242(0.36%)		
Fall, n(%)	538(0.28%)	312(0.25%)	226(0.34%)		
Winter, n(%)	298(0.15%)	227(0.18%)	99(0.15%)		

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4.2. Vaccination status of pertussis patients

Of the 1942 cases of pertussis patients, 669 cases (34.4%) were unimmunized, 689 cases (35.5%) were incompletely immunized, 545 cases (28%) were fully immunized, and 39 cases (2%) had unknown vaccination histories, of which 247 cases (12.7%, 247/1942) were under the age of immunization; the low rate of complete immunization against combined DPT vaccine (DPT) resulted in children younger than one year of age become the main susceptible population for pertussis (see Table 4).

Table 4: Vaccination status of pertussis patients.

Vaccination status	Not vaccinated	Not fully vaccinated	Vaccination completed	Vaccination history unknown
Number of persons (n=1942)	669(34.4%)	689(35.5%)	545(28%)	39(2%)

4.3. Comorbidities in patients with pertussis

Of the 1942 patients with pertussis, 91% (1767/1942) had complications (mainly in children). Among them were bronchiolitis 5.3% (103/1942), pneumonia/bronchopneumonia 85% (1553/1942), severe pneumonia 15% (291/1942), prolonged pneumonia 5.1% (99/1942), pulmonary solid lesions 7.3% (142/1942), pulmonary atelectasis 2.7% (52/1942), pleural lesions 2.1% (40/1942), malnutrition 0.5% (9/1942), thrombocytosis 61% (1184/1942), pertussis encephalopathy 3.2% (62/1942), hepatic impairment 7.2% (140/1942), and anemia 2.8% (54/1942), and the complications in pertussis patients of different age groups were statistically analyzed. years of age and age >1 year of age patients, the difference was statistically significant in terms of whether complications of severe pneumonia would occur (see Table 5).

Table 5: Comorbidities in patients with pertussis.

Variable	Total (N=1942)	=1 year old (n=1272)	>1 year old (n=670)	χ^2	P value
Bronchitis n (%)	103(5%)	65(5%)	38(6%)	0.276	0.6
Pneumonia n (%)	1653(85%)	1075(85%)	578(86%)	1.068	0.301
Severe pneumonia n(%)	291(15%)	142(11%)	149(22%)	42.255	<0.001
Lung solid changes n(%)	142(7%)	85(7%)	57(9%)	2.157	0.142
Pulmonary atelectasis n(%)	36(2%)	20(2%)	16(2%)	1.605	0.205
Pleural lesions n(%)	30(2%)	18(1%)	12(2%)	0.408	0.523
Thrombocytosis n(%)	1032(53%)	673(53%)	359(54%)	0.08	0.777
Pertussis encephalopathy n(%)	62(3%)	34(3%)	28(4%)	3.221	0.073
Malnutrition n(%)	29(1%)	17(1%)	12(2%)	0.616	0.432
Hepatic impairment n(%)	140(7%)	90(7%)	50(7%)	0.098	0.754

4.4. Combination of pertussis infection with other pathogens at different ages

Patients with pertussis infection were categorized into simple and mixed groups according to whether they were combined with other pathogenic infections. The number of patients with simple pertussis infection was 796 (41.0%) and 1146 (59.0%) were combined with other pathogenic infections.859 (859/1146, 77.3%) children with pertussis were combined with one pathogenic infection, 287 (213/1146, 18.6%) were combined with two pathogenic infections, and 246 (46/1146, 4.1%) combined three pathogen infections. Among them, 366 cases (366/1146, 32.0%) were combined with respiratory syncytial virus infection, 280 cases (280/1146, 24.5%) were combined with parainfluenza virus infection, 151 cases (151/1146, 13.2%) were present with combined Mycoplasma pneumoniae infection, 18 cases (18/1146, 1.6%) were combined with Chlamydia pneumoniae infection, 42 cases (42/1146, 3.7%) combined influenza A virus infection, 265 (265/1146, 23.3%) combined adenovirus infection, and 20 (20/1146, 1.8%) combined rotavirus. The patients with co-infections of other pathogens were divided into two groups according to their age and statistically analyzed, and the results showed that there was no statistically significant difference between patients with age=1 year and age>1 year in terms of co-infections of pathogens. (See Figure 3 and Table 6)

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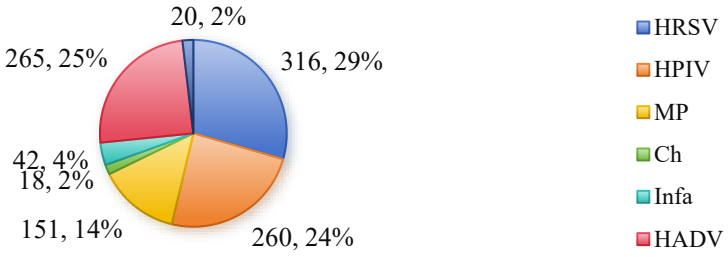

Figure 3: Combined infection with other pathogens in patients with pertussis

Table 6: Combination of other pathogenic infections in patients with pertussis by age.

pathogen	Total (N=1942)	=1 year old (n=1272)	>1 year old (n=670)	χ^2	P value
HRSV	316(16%)	195(15%)	121(18%)	2.4	0.121
HPIV	260(13%)	157(12%)	103(15%)	3.475	0.062
MP	151(8%)	89(7%)	62(9%)	3.117	0.077
Ch	18(0.9%)	10(0.8%)	8(1%)	0.795	0.373
Infa	42(2%)	25(2%)	17(3%)	0.678	0.41
HADV	265(14%)	175(14%)	90(13%)	0.045	0.833
RV	20(1%)	11(0.9%)	9(1%)	0.986	0.321

5. Discussion

In recent years, the phenomenon of "whooping cough reappearance" has appeared in many countries. From 2016 to 2018, the number of whooping cough patients diagnosed and treated in Tai'an Children's Hospital increased year by year, with year-round onset, the onset of the disease season dominated by the summer and the fall, and the number of onset of the disease was highest in July and August, and the onset of disease was dominated by infants and children within 2 years of age, but the number of onset of the disease above 6 years of age was increasing year by year. The incidence of the disease is predominantly in infants and children under 2 years of age, but the number of patients over 6 years of age is increasing year by year.

In this study, more than 50% of the children were younger than 12 months of age, which was significantly higher than other age groups, and the incidence rate was as high as 78% at the age of 3-12 months, which was similar to that reported in the literature [4]. More than half of the infants and young children in this study and in several national publications reported onset of disease before they had completed their scheduled vaccinations. More than 20% of the patients in this study had contact with a coughing patient prior to diagnosis, which is lower than reported in the literature and may be due to lack of careful history taking by the attending physician as well as family members not having obvious symptoms of cough in the early stages.

The pertussis cases in this study were mainly of urban origin, which is different from the previous report in the literature that rural areas have a higher incidence than urban areas[5], probably due to the increase in the urbanization level of the city as well as the geographic location of the hospital, which is located in an urban area.

The present study showed that bronchopneumonia is the most common complication in patients with pertussis. Almost all pertussis deaths occur in infants and children <4 months of age. The mechanism of death is primarily leukocyte accumulation in the small pulmonary arteriovenous and lymphatic tracts leading to irreversible pulmonary hypertension[6].

The mean level of time to diagnosis of pertussis in all infected patients was 13.15 ± 6.19 d. The shortest time to diagnosis in patients was 7 d, and the longest time to diagnosis was 30 d. The duration of hospitalization in patients with pertussis ranged from 2 to 20 d, and the average duration of hospitalization in patients was 12 d.

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