Viral Pathogens of Acute Lower Respiratory Tract Infection in China

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ABSTRACT

***Objectives**: To document the viral etiology of acute lower respiratory tract infection (ALRIs) in Chinese children. **Setting**: Children Hospital, Zhejiang University, China. **Study design**: Cross-sectional. **Participants**: 34885 children with ALRI between January 2001 to December 2006. **Methods**: Nasopharyngeal aspirates were collected from all subjects. Respiratory syncytial virus (RSV), adenovirus (ADV), type 1 to 3 parainfluenza viruses (PIV), and type A and B influenza virus (Flu) were detected by direct immunofluroscence. **Results**: Viruses were identified in 32.3% cases, including RSV (23.6%), PIV 3 (4.3%), Flu A (2.0%), ADV (1.7%), PIV I (0.6%), Flu B (0.2%) and PIV 2 (0.1%). RSV and PIV 3 predominated in younger children while Flu A and Flu B predominated in older children (P<0.001, respectively). PIV 1 was more prevalent in children aged 1 to 3 years. The peak frequency of RSV, PIV 3 and Flu A were in early spring, June to August, and August and September, respectively. Flu B had a peak in the winter and spring. Adenovirus infections occurred in all seasons with a relatively constant frequency. **Conclusions:** Viruses are an important cause of ALRIs in Chinese children constituting 1/3 of total cases. RSV is the most common pathogen.*

Key words: *Adenovirus, Children, Influenza virus, Parainfluenza virus, Respiratory syncytial virus.*

INTRODUCTION

Acute lower respiratory tract infections (ALRIs) are the main cause of morbidity and mortality in younger children. It accounts for 33-50% mortality in children below 5 years of age, most of them in underdeveloped countries(1-3). In developed countries, viruses, including respiratory syncytial virus (RSV), adenovirus (ADV), influenza virus (Flu) and para-influenza virus (PIV), are the most common cause of lower respiratory tract infection(3-5), although with lower mortality. The distribution of viral pathogens differs depending on the population, geographic area and the socioeconomic levels(5,6). ALRIs are one of the main reasons for consultation in pediatrics services in primary and secondary health care units in China. However, the etiologic agents of ALRI are rarely investigated or reported. Therefore, the predominant agents and viruses responsible for ALRI in Chinese children remain unknown. Moreover, most ALRIs cases are treated with antibiotics without considering the probable viral etiology.

We conducted this study to document the contribution of viruses to infective etiology of ALRI in Chinese children at Hangzhou district which is located in the east of China.

METHODS

All children admitted with ALRIs to the Children's hospital of Zhejiang University School of Medicine between January 2001 to December 2006 were enrolled (*n*=34885; 21597 males, 13288 females). A diagnosis of bronchitis, bronchiolitis or pneumonia at discharge was considered as ALRI. Age of

enrolled subjects ranged from 1 month to 13 years (median 10.4 months); 21940 cases were younger than 1 year, 7243 cases between 1 to 3 years, and 5702 cases over 3 years.

Consent was obtained from the ethical committee of the Children Hospital of Zhejiang University School of Medicine.

Nasopharyngeal aspirates (NPA) were collected to obtain epithelial cell from each patient. Aspirates were obtained within 24 h in 34711 subjects; in 174 cases, the aspirates could only be collected on day 2. Samples for virological study were diluted with equal volumes of saline, stored at 4ºC and then processed within 24 h of collection. Indirect immunofluorescence assays were performed to identify the viruses, as per the manufacturer's instructions (D^3 DFA Respiratory Virus Screening and ID Kit, The Diagnostic Hybrids, Inc. USA). We aimed to detect the following respiratory viruses: RSV, Flu A and B, ADV and PIV 1, 2, and 3. Specimen processing and cell culture of viruses were done by special personnel. Positive and negative controls (uninfected and infected cells) provided in the kit were also performed.

Statistical analyses were conducted using SPSS 11.5. Differences in frequencies between different age groups of children and seasons were compared and analyzed by Chi-square test. *P* value less than 0.05 was considered to be significant.

TABLE I COINFECTION OF VIRUSES IN 34885 PEDIATRIC PATIENTS WITH LOWER RESPIRATORY TRACT INFECTION

	ADV	FIu A	Flu B	PIV 1	PIV 2	PIV 3
RSV	3	7	0	5	1	7
ADV	–	0	1	0	0	1
Flu A	–		0	0	0	1
Flu B	–			0	1	0

Flu: Influenza virus; PIV: paraInfluenza virus; RSV: respiratory syncytial virus; ADV: adenovirus

RESULTS

Among all 34885 samples, at least one respiratory virus was detected in 11270 samples (32.3%) and a total of 11297 viral isolates identified. Coinfection with two kinds of virus was identified in 27 samples, 23 (85.2%) of them had coinfection with RSV and another virus (***Table* I**). Of the positive samples, RSV was identified most frequently with 8223 cases (23.6%) which accounted for 72.8% (8223/11297) of the total viral agents. Other viruses detected were (in descending order of frequency) PIV 3, Flu A, ADV, PIV 1, Flu B, and PIV 2 (***Table* II**). During these 6 years, the proportion of viral etiology ranged from 27.1% (2001) to 39.5% (2003) ($P<0.001$). The main viral pathogens identified from ALRIs were all RSV and PIV 3 in each year although the prevalence rates were some what different as shown in ***Table* II**.

In this study, viral frequency was analyzed with

TABLE II THE FREQUENCY OF VIRUS ISOLATION BETWEEN 2001 TO 2006

	2001 *n*=2564	2002 *n*=5068	2003 *n*=6010	2004 *n*=6721	2005 *n*=7585	2006 *n*=6937	Total *n*=34885
RSV	441(17.2)	1312(25.9)	1762(29.3)	1496 (22.3)	1540 (20.3)	1672(24.1)	8223(23.6)
ADV	36(1.4)	57(1.1)	96(1.6)	108(1.6)	153(2.0)	137(2.0)	587(1.7)
Flu A	50(1.9)	82(1.6)	200(3.3)	100(1.5)	108(1.4)	148 (2.1)	688(2.0)
Flu B	9(0.4)	11(0.2)	13(0.2)	4(0.1)	21(0.3)	14(0.2)	72(0.2)
PIV 1	40(1.6)	33(0.7)	22(0.4)	16(0.2)	52(0.7)	37(0.5)	200(0.6)
PIV 2	7(0.3)	7(0.1)	7(0.1)	9(0.1)	1(0.01)	5(0.1)	36(0.1)
PIV 3	112(4.4)	141(2.8)	272(4.5)	375(5.6)	328(4.3)	263(3.8)	1491(4.3)
Total	695(27.1)	1643(32.4)	2372(39.5)	2108(31.4)	2203(29.0)	2276(32.8)	11297

Flu: Influenza virus; PIV: paraInfluenza virus; RSV: respiratory syncytial virus; ADV: adenovirus

TABLE III THE FREQUENCY OF VIRUS ISOLATION IN ALRI IN DIFFERENT AGE GROUPS

	<1 yr *n*=21940	1-3 yr *n*=7243	>3 yr *n*=5702	Total *n*=34885	*P* value
RSV	6594 (30.1)	1317 (18.2)	312 (5.5)	8223 (25.3)	<0.001
ADV	215 (1.0)	209 (2.9)	163 (2.9)	587 (1.7)	<0.001
Flu A	378 (1.7)	199 (2.7)	111 (1.9)	688 (2.0)	<0.001
Flu B	34 (0.2)	17 (0.2)	21 (0.4)	72 (0.2)	0.006
PIV 1	109 (0.5)	61 (0.8)	30 (0.5)	200 (0.6)	0.003
PIV 2	18 (0.1)	9 (0.1)	9 (0.2)	36 (0.1)	0.23
PIV 3	1193 (5.4)	227 (3.1)	71 (1.2)	1491 (4.3)	<0.001
Total	8541 (38.9)	2039 (28.2)	717 (12.6)	11297	<0.001

Flu: Influenza virus; PIV: paraInfluenza virus; RSV: respiratory syncytial virus; ADV: adenovirus.

TABLE IV FREQUENCY OF VIRUS ISOLATES IN ALRI IN DIFFERENT MONTHS

	Number	RSV	ADV	Flu A	Flu B	PIV 1	PIV 2	PIV 3	Total
Jan	3647	1608(44.1)	45(1.2)	65(1.8)	15(0.4)	13(0.4)	4(0.1)	137(3.8)	1887(51.7)
Feb	3690	1991(54.0)	35(0.9)	53(1.4)	17(0.5)	11(0.3)	1(0.03)	96(2.6)	2204(59.7)
Mar	3914	1596(40.8)	63(1.6)	92(2.3)	5(0.1)	7(0.2)	1(0.03)	173(4.4)	1937(49.5)
Apr	3289	765(23.3)	85(2.6)	65(2.0)	6(0.2)	3(0.1)	1(0.03)	186(5.7)	1111(33.8)
May	2678	299(11.2)	68(2.5)	21(0.8)	4(0.1)	9(0.3)	1(0.04)	99(3.7)	501(18.7)
Jun	2298	74(3.22)	58(2.5)	23(1.0)	1(0.04)	12(0.5)	4 (0.2)	131(5.7)	303(13.2)
Jul	2202	26(1.2)	39(1.8)	29(1.3)	2 (0.1)	14(0.6)	0	164(7.4)	274(12.4)
Aug	2395	54(2.2)	50(2.1)	125(5.2)	3(0.1)	15(0.6)	1(0.04)	146(6.1)	394(16.5)
Sept	2189	96(4.4)	35(1.6)	78(3.6)	2(0.1)	18(0.8)	1(0.05)	84(3.8)	314(14.3)
Oct	2416	205(8.5)	25(1.0)	24(1.0)	4(0.2)	49(2.0)	13(0.5)	91(3.8)	411(17.0)
Nov	2744	413 (15.0)	35(1.3)	43(1.6)	5(0.2)	33(1.2)	4(0.1)	99(3.6)	632(23.0)
Dec	3423	1096(32.0)	49(1.4)	70(2.0)	8(0.2)	16(0.5)	5(0.1)	85(2.5)	1329(38.8)
Total	34885	8223	587	688	72	200	36	1491	11297

Flu: Influenza virus; PIV: paraInfluenza virus; RSV: respiratory syncytial virus; ADV: adenovirus

relation to age and 3 groups were studied (*i*) Group 1:<1 yr (*n*=21940); (*ii*) Group 2: 1-3 yr (*n*=7243) and (*iii*) Group 3: >3 years (*n*=5702). The total frequency of viral etiology in the 3 different age groups was 38.8%, 28.1% and 12.6%, respectively. RSV, ADV, Flu A, Flu B, PIV 3 and PIV 1 among 3 different age groups were significantly different ($P<0.05$), while PIV 2 did not show any significant association with the age ($P=0.23$). RSV and PIV 3 associated ALRIs predominated in children young than 1 year while ADV, Flu A and Flu B predominated in older children. The frequency of RSV in children younger than 1 year (30.7%) was 7.4 times (95%CI: 6.6-8.4) of that in children older than 3 years (5.5%). PIV 1 was isolated more frequently in children between 1 to 3 years group (***Table* III**).

To analyze the seasonality of viral ALRIs in children, records were maintained throughout the year (***Table* IV**). Almost all viruses were detected

WHAT IS ALREADY KNOWN?

- Viruses are important causes of acute lower respiratory tract infection in children.

WHAT THIS STUDY ADDS?

- In Chinese children, viruses are responsible for one third of total episodes of acute lower respiratory tract infections; RSV is the most common pathogen.

throughout the year except the PIV 2. RSV was detected in each month and peaked in the early sprint with small differences in different years. In February 2004, the frequency of RSV was up to 67.4%. A higher frequency of PIV 3 was detected during summer, especially from June to August, with small difference during the other 3 seasons. Flu A was detected in higher frequency during August and September while a small peak was also noted in late winter and early spring. Moreover, an epidemic peak was noted in August 2003 with a frequency of 15.7%. Flu B was less frequent with a peak in the winter and spring; the lowest number was attained in autumn. PIV 1 and PIV 2 were less frequent with an increase in the winter and spring which was not statistically significant. ADV was detected with a relatively constant frequency throughout the 4 seasons.

DISCUSSION

RSV, ADV, Flu and PIV are the important causes of ALRIs in China. Primary viral pneumonia or pneumonia secondary to bacterial infections is the primary cause of morbidity with viral infection. In this study, viruses were identified in 32.3% of the children included in the study as sole infectious agents. These results agree with reports by other authors who mention that 30% to 90% of ALRIs are caused by viruses(7-10). Other studies have shown that viruses, such as enteroviruses, rhinoviruses, coronavirus can also cause ALRIs(11-13). Thus, viral infection is a very important cause of ALRIs in Hangzhou area as well and differential diagnosis by a pediatrician must include this category of infections.

In this study, RSV is the major viral pathogens accounting for about 23.6% of the ALRIs and 72.8% of total viral pathogens. This is similar to some previous reports(11,13-15) and confirmed the view that RSV is the primary viral cause of ALRIs in infants and young children.

Previous studies have demonstrated the association of viral infection with climate(16-18). In this study, we noted that the frequency of viral isolation was significantly higher in 2003 and lower in 2001. This might be associated with "cold" winter in 2003 and "warm" winter in 2001, which influenced the rate of RSV infection dramatically. We also found that RSV had a dramatic seasonal variation. Epidemic seasonal peak of RSV is variable, mostly occurring in winter and early spring(5,13,17). PIV was another important viral pathogen in this study. Earlier studies have shown that outbreaks caused by PIV occur during alternate years in the fall (PIV 1 and PIV 2) or throughout the year, with increased activity in the spring (PIV 3)(17). In this series, however, PIV 1 and PIV 2 occurred with low frequency with a non-significant peak in winter and spring. PIV 3 had a significant higher frequency with one significant peak during June to August, but not spring. Flu was found throughout the year. Pandemics of Flu A occur about every 10 to 30 years and epidemics of either Flu A and B occur annually. Infections of Flu are seasonal, typically extending from November to April in the northern hemisphere(11-13). In this study, however, we noted that Flu A was also frequent with a significant peak in the August and September, and a small peak in late winter and early spring. Flu B was less frequent with a peak in the winter and spring, and the lowest number of isolates was attained in autumn. These differences of seasonal variation from some previous studies might be associated with area, climate, economy and race. Further study is required about these factors.

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