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## Short communication

## Human parainfluenza virus type 4 infection in Chinese children with lower respiratory tract infections: A comparison study

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## ABSTRACT

**Background:** Human parainfluenza viruses (HPIVs) are a leading cause of acute respiratory tract infections (ARTIs). Although HPIV-4 has been associated with mild ARTIs for years, recent investigations have also associated HPIV-4 infection with severe respiratory syndromes and with outbreaks of ARTIs in children. **Objectives:** To characterize the role of HPIV-4 and its clinical features in children with acute lower respiratory tract infections (ALRTIs) in Beijing, China.

**Study design:** Nasopharyngeal aspirates were collected from 2009 hospitalized children with ALRTIs between March 2007 and April 2010. RT-PCR and PCR analyses were used to identify HPIV types and other known respiratory viruses.

**Results:** HPIVs were detected in 246 (12.2%) patients, of whom 25 (10.2%) were positive for HPIV-4, 11 (4.5%) for HPIV-2, 51 (20.7%) for HPIV-1, 151 (61.4%) for HPIV-3, and 8 (3.3%) were co-detected with different types of HPIVs. Like HPIV-3, HPIV-4 was detected in spring, summer, and late fall over the study period. Seasonal incidence varied for HPIV-1 and -2. The median patient age was 20 months for HPIV-4 infections and 7–11 months for HPIV-1, -2, and -3 infections, but the clinical manifestations did not differ significantly between HPIV-1, -2, -3, and -4 infections. Moreover, co-detection of HPIV-4 (44%) with other respiratory viruses was lower than that of HPIV-1 (62.7%), HPIV-2 (63.6%), and HPIV-3 (72.7%).

**Conclusions:** HPIV-4 plays an important role in Chinese paediatric ALRTIs. The epidemiological and clinical characteristics reported here improve our understanding of the pathogenesis associated with HPIV-4.

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## 1. Background

Human parainfluenza viruses (HPIVs) are a leading cause of acute respiratory tract infections (ARTIs).<sup>1–3</sup> HPIVs account for 6.8% of hospitalizations due to fever and/or acute respiratory illnesses in children younger than 5 years of age.<sup>1–9</sup> Four types of HPIVs, 1

through 4, have been identified. Studies of HPIVs have focused primarily on HPIV-1 to -3, and detection methods include mainly cell culture and immunofluorescence. As the recovery rate of HPIV-4 in cell culture is inherently low and most clinical laboratories do not screen for HPIV-4,<sup>10,11</sup> the role of HPIV-4 in ARTIs is unclear and may be underestimated.

Recent studies indicate that HPIV-4 is associated with respiratory infections including bronchitis and pneumonia.<sup>10–14</sup> However, the prevalence and clinical characteristics of HPIV-4 in Chinese paediatric patients with acute lower respiratory tract infections (ALRTIs) have not been addressed fully.

## 2. Objectives

To characterize the role and clinical features of HPIV-4 in children with ALRTIs in Beijing, China.

**Abbreviations:** HPIVs, human parainfluenza viruses; ARTIs, acute respiratory tract infections; ALRTIs, acute lower respiratory tract infections; NPA, nasopharyngeal aspirates; RSV, Respiratory Syncytial Virus.

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**Table 1**  
Characteristics of paediatric patients with human parainfluenza virus (HPIVs) infections.

Parameters	HPIV (%)			
	HPIV-1	HPIV-2	HPIV-3	HPIV-4
No. of patients <sup>a</sup>	51	11	151	25
Age, months				
0 to <6	13 (25.5) <sup>a</sup>	2 (18.2)	63 (41.7)	8 (32.0)
≥6 to <12	15 (29.4)	3 (27.3)	36 (23.8)	3 (12.0)
≥12 to <36	19 (37.3)	3 (27.3)	38 (25.2)	3 (12.0)
≥36 to <72	1 (2.0)	1 (9.1)	9 (6.0)	7 (28.0)
≥72 to 192	3 (5.9)	2 (18.2)	5 (3.3)	4 (16.0)
Year				
2007	20 (39.2)	2 (18.2)	32 (21.2)	11 (44.0)
2008	9 (17.6)	8 (72.7)	66 (43.7)	10 (40.0)
2009	8 (15.7)	1 (9.1)	31 (20.5)	2 (8.0)
2010	14 (27.5)	0	22 (14.6)	2 (8.0)
Gender (M/F)	34/17	6/5	108/43	14/11
Clinical symptoms				
Cough	48 (94.1)	11 (100.0)	148 (98.0)	22 (88.0)
Sputum production	27 (52.9)	10 (90.9)	108 (71.5)	16 (64.0)
Chilly	5 (9.8)	3 (27.3)	11 (7.3)	2 (8.0)
Running nose	20 (39.2)	5 (45.5)	39 (25.8)	6 (24.0)
Sneezing	11 (21.6)	4 (36.4)	23 (15.2)	6 (24.0)
Wheezing	18 (35.3)	3 (27.3)	50 (33.1)	8 (32.0)
Vomiting	3 (5.9)	3 (27.3)	2 (1.3)	2 (8.0)
Diarrhoea	2 (3.9)	1 (9.1)	11 (7.3)	1 (4.0)
Pneumonia	32 (62.7)	7 (63.6)	94 (62.3)	20 (80.0)
Bronchopneumonia	8 (15.7)	1 (9.1)	23 (15.2)	2 (8.0)
Bronchitis	6 (11.8)	2 (18.2)	21 (13.9)	3 (12.0)
Peribronchitis and others	5 (9.8)	1 (9.1)	13 (8.6)	0

<sup>a</sup> Numbers in parentheses indicate the percentages of positive detections reported to the total number of positive patients, while cases co-infected by two types of HPIVs are excluded.

<sup>\*</sup>  $\chi^2 = 18.906$ ,  $P = 0.0008$ .

### 3. Study design

#### 3.1. Clinical specimen and data collection

Nasopharyngeal aspirates (NPA) were collected from children with ALRTIs, upon admission to Beijing Children's Hospital between March 2007 and April 2010. No repeat samples or lower respiratory tract samples were collected. To exclude typical bacterial infections, physicians enrolled patients with body temperature  $\geq 38^\circ\text{C}$ , respiratory symptoms and normal or low leukocyte count. Similar numbers of samples were collected during each season over the study period, except in August 2009 when only eight samples were collected.

#### 3.2. RT-PCR screening

Nucleic acids were extracted from NPA using NucliSens easyMAG<sup>TM</sup> (bioMérieux, France). Nested RT-PCR was used to detect HPIVs, as described previously.<sup>3,5</sup> Invariant  $\beta$ -actin gene was used as an internal control for efficient extraction and amplification of viral nucleic acids. Analytic sensitivity of RT-PCR was 1–10 copies for HPIV-2 and -4 and 10–100 copies for HPIV-1 and -3. To avoid contamination, the PCR process (including master mixture preparation for PCR, nucleic acid extraction, reaction installation and electrophoresis) was performed in different rooms. Strict controls were used during the process of nucleic acid extraction and PCR analysis to monitor contamination. All PCR products were verified by sequencing. Each specimen was also screened for other respiratory viruses, as described elsewhere.<sup>3,15,16</sup>

#### 3.3. Statistical analysis

Distribution frequencies of HPIVs were compared using Pearson's Chi square test or Fisher's exact test. Continuous variables for

population parameters such as age, maximum temperature and duration of fever, laboratory investigations and other parameters were compared using one-way analysis of variance.  $P$  values  $<0.05$  were statistically significant.

### 4. Results

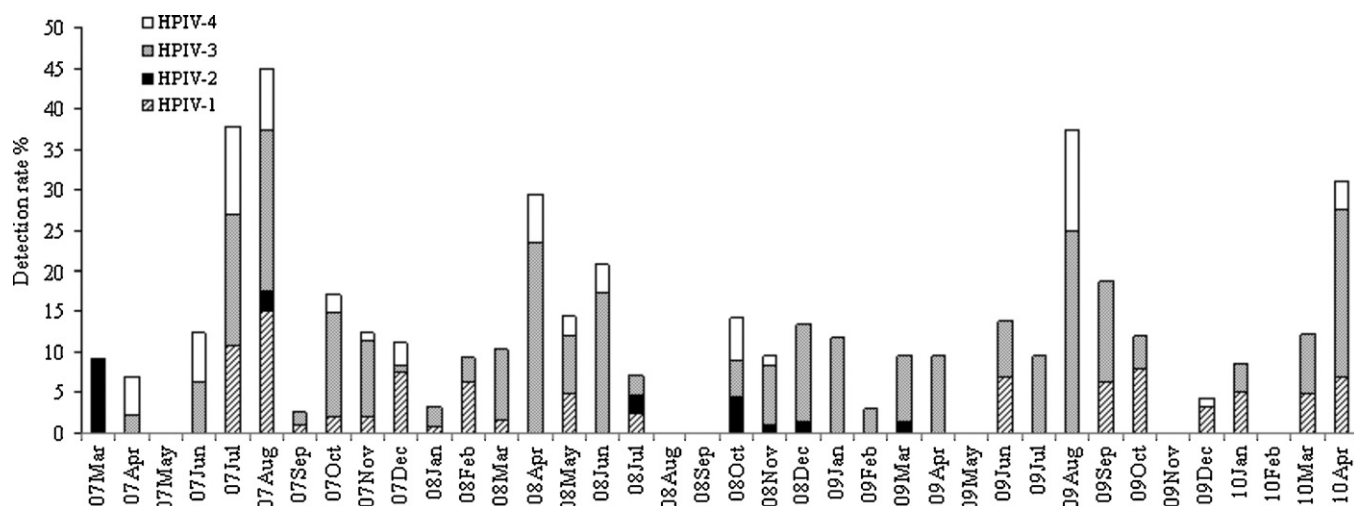
#### 4.1. Incidences of HPIVs

A total of 2009 patients (1278 males and 731 females), 0.5 month to 16 years old (mean 2.5 years, median nine months), were enrolled in this study. HPIV RNA was detected in 246 (12.2%) patients, from 1 month to 13 years old (mean 1.5 years; median nine months). The prevalence of HPIV was not significantly different between males (13.3%) and females (10.3%). Twenty-five (10.2%) patients were positive for HPIV-4, 11 (4.5%) for HPIV-2, 51 (20.7%) for HPIV-1 and 151 (61.4%) for HPIV-3.

The detection rate of HPIVs varied significantly between age groups ( $\chi^2 = 57.680$ ,  $P < 0.001$ ). Compared to HPIV-1 to -3, HPIV-4 was detected at a higher rate in 36–72 months-old patients and at a lower rate in 6–36 months-old patients (Table 1). The median age of HPIV-4 cases (20 months) was higher than that of HPIV-1 (11 months), HPIV-2 (10 months), and HPIV-3 cases (7 months).

#### 4.2. Seasonal distribution

The seasonal distribution of HPIVs fluctuated (Fig. 1). Overall, HPIVs peaked in spring and summer. Every year, HPIV-2 had the lowest and HPIV-3 the highest detection rate. HPIV-4 was detected more often in 2007 and 2008 than in 2009 and 2010. In 2007 and 2008, the highest detection rates of HPIV4 were found in spring, summer and late fall. HPIV-3 peaked in spring and summer. The detection rates of HPIV-1 were higher in 2007 and 2010 than in



**Fig. 1.** Detection rates of human parainfluenza viruses in paediatric samples collected during the study period. The bar graph shows the detection rates of each type of human parainfluenza virus each month.

**Table 2**

Co-detection of HPIVs with respiratory viruses in the paediatric cohort.

	HPIV-1	HPIV-2	HPIV-3	HPIV-4
Positive cases	51	11	151	25
Single detection	19 (37.3) <sup>a</sup>	4 (36.4)	42 (27.8)	14 (56.0)
Co-detection <sup>*</sup>	32 (62.7)	7 (63.6)	109 (72.2)	11 (44.0)
1 virus	25 (49.0)	4 (36.4)	68 (45.0)	7 (28.0)
2 virus	5 (9.8)	1 (9.1)	35 (23.2)	4 (16.0)
3 virus	2 (3.9)	2 (18.2)	4 (2.6)	0
4 virus	0	0	2 (1.3)	0
<b>Frequency of co-detected respiratory viruses</b>				
RSV	14	2	44	5
Rhinovirus	10	4	43	7
Adenovirus	1	1	17	0
Human Metapneumovirus	5	1	11	0
Human Bocavirus	1	2	13	1
Influenza Virus	4	2	9	0
Human Coronavirus	2	0	11	1
Enterovirus	4	0	5	1
WU/KI Polyomavirus	0	0	5	0

RSV, Respiratory Syncytial Virus.

<sup>a</sup> Numbers in parentheses indicate the percentages of positive detections reported to the total number of positive samples. Cases co-detected by two types of HPIVs are excluded.

<sup>\*</sup>  $\chi^2 = 8.263$ ,  $P = 0.041$ .

2008–2009, with varying seasonal incidence. HPIV-2 appeared sporadically during the study period (Fig. 1).

#### 4.3. Clinical characteristics

Of the HPIV-positive patients, 63.8% were diagnosed with pneumonia, 15% with bronchopneumonia, 13% with bronchitis and 8.1% with peribronchitis or other conditions. The maximum body temperature was similar for patients with HPIV-4 and patients with other HPIV infections. Congenital heart disease was reported in one (4%) patient with HPIV-4, four (3.9%) with HPIV-1, eight (5.3%) with HPIV-3, and in two (25%) co-infected with HPIV-4 and HPIV-1 or HPIV-4 and HPIV-3. The frequency of symptoms did not differ significantly between patients with different types of HPIV. Chills, diarrhoea and vomiting were observed most frequently in patients with HPIV-2 (Table 1). The percentage of neutrophilic granulocytes, mean lymphocyte count or percentage of lymphocytes was similar between patients infected by different HPIV types. Overall, the clinical manifestation of HPIV-4 infection resembles that of other HPIV types.

#### 4.4. Co-detection of HPIVs and other respiratory viruses

Co-infection was detected in 167 (67.9%) of the HPIV-positive cases, including eight (3.3%) co-detected with different types of HPIVs and 159 (66.8%) co-detected with other respiratory viruses. Co-detection rates of all HPIV types were statistically different (Table 2). HPIV-4 had the lowest co-detection rate, which was significantly lower than that of HPIV-3 ( $\chi^2 = 7.854$ ,  $P = 0.005$ ; Table 2).

Respiratory Syncytial Virus (RSV) (65 cases) and rhinovirus (64 cases) were co-detected with HPIVs most often (Table 2). While co-infections of HPIVs with RSV occurred mainly in spring and winter, those for rhinovirus occurred year round, corresponding to the seasonality of these viruses. Clinical characteristics did not differ significantly between patients with single HPIV infections and those with co-infections.

#### 5. Discussion

We present the first detailed study of HPIV-4 infections in Chinese children with ALRTIs. In our study, we found that HPIV-3 cases are most prevalent, as previously reported,<sup>2</sup> followed by HPIV-1,

then HPIV-4, and HPIV-2 cases. Clinical manifestations did not differ significantly between HPIV-1, -2, -3, and -4 infections, but the median age of patients with HPIV-4 was higher than that for patients with any of the other HPIV types.

In temperate climates, HPIV-1 and -2 infections occur annually during late fall and early winter,<sup>1,2</sup> whereas HPIV-4 infections occur biennially during late fall and winter,<sup>2,11,14,17</sup> and HPIV-3 infections occur mainly during late spring and summer.<sup>2</sup> The seasonal distributions of HPIV-1, -3, and -4 reported here differ from those of previous reports. These discrepancies may be attributed to different geographical regions and study years. However, as the number of positive cases is limited in our study, large-scale investigation of HPIV incidence over a broader geographical range and longer time period is needed to better understand the seasonal patterns of HPIVs.

It is particularly interesting that HPIV-4 was more prevalent during spring and summer in 2007 and 2008 than in 2009 and 2010. We speculate that this finding is associated with the duration of protective immunities. If the protective immunity lasts long, the interval of epidemics could be longer. However, this hypothesis requires testing by immunological studies.

To investigate the rate and role of co-infection, we screened each specimen for HPIVs and other common respiratory viruses. Most HPIV-positive patients (159, 66.8%) were co-infected with other viruses, but the co-detection rate was lowest in patients infected with HPIV-4. Future investigations may want to consider screening for additional pathogens, such as bacteria, and using samples from different geographic locations to provide insight into the clinical significance of co-infections.

Overall, our results confirm that HPIV-4 is an important cause of severe symptoms associated with paediatric ALRTIs, which should be screened for routinely.<sup>13–16</sup>

#### Conflict of interest statement

The authors report no conflicts of interest.

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