Journal of Medical Virology 88:2085–2091 (2016)

Human Parainfluenza Virus Types 1–4 in Hospitalized Children With Acute Lower Respiratory Infections in China

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Human parainfluenza viruses (HPIVs) are an important cause of acute lower respiratory tract infections (ALRTIs). HPIV-4, a newly identified virus, has been associated with severe ALRTIs recently. A total of 771 nasopharyngeal aspirate samples were collected from hospitalized children between March 2010 and February 2011. HPIVs were detected by Nest-PCR, and other known respiratory viruses were detected by RT-PCR and PCR. All amplification products were sequenced. HPIVs were detected in 151 (19.58%) patients, of whom 28 (3.63%) were positive for HPIV-4, 12(1.55%) for HPIV-1, 4 (0.51%) for HPIV-2, and 107 (13.87%) for HPIV-3. Only three were found to be co-infected with different types of HPIVs. All HPIV-positive children were under 5 years of age, with the majority being less than 1 year. Only the detection rate of HPIV-3 had a significant statistical difference ($\chi^2 = 29.648$, $P = 0.000$) between ages. HPIV-3 and HPIV-4 were detected during the summer. Sixty (39.74%) were co-infected with other respiratory viruses, and human rhinovirus (HRV) was the most common co-infecting virus. The most frequent clinical diagnosis was bronchopneumonia, and all patients had cough; some patients who were infected with HPIV-3 and HPIV-4 had polypnea and cyanosis. No significant difference was found in clinical manifestations between those who were infected with HPIV-4 and HPIV-3. Two genotypes for HPIV-4 were prevalent, although HPIV-4a dominated. HPIV-4 is an important virus for children hospitalized with ALRTIs in China. HRV was the most common co-infecting virus. Two genotypes for HPIV-4 are prevalent, HPIV-4a dominated. ***J. Med. Virol. 88:2085–2091, 2016***.

KEY WORDS: human parainfluenza virus; clustal analysis; seasonal incidence; respiratory tract

INTRODUCTION

Human parainfluenza viruses (HPIVs) are a leading cause of acute respiratory tract infection (ARTI) [Laurichesse et al., 1999; Karron and Collins, 2007; Ren et al., 2009]. Among pathogens of ARTI, HPIVs are secondary only to respiratory syncytial virus (RSV) [Weinberg, 2006]. Four types of HPIVs have been identified. Studies of HPIVs have focused primarily on HPIV1, 2, and 3; HPIV-4 has not attracted sufficient clinical attention due to the fact that the illness caused by it is minor [Aguilar et al., 2000; Templeton et al., 2005; Vachon et al., 2006]. Recent studies indicate that HPIV-4 is associated with respiratory infections including bronchitis and pneumonia [Rubin et al., 1993; Lindquist et al., 1997; Lau et al., 2005, 2009; Vachon et al., 2006;]. Furthermore, it was found that this virus had an outbreak in a small range and that children under 2 years of age may encounter serious acute lower respiratory tract infections (ALRTIs) [Lau et al., 2005; Weinberg, 2006; Wang et al., 2012]. However, the prevalence and clinical characteristics of HPIV4 in Chinese

Grant sponsor: Project supported by Natural Science Foundation of Hunan Province; Grant number: 07JJ5055

Conflicts of interest: None of the authors declare a conflict of interest.

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Accepted 18 May 2016

DOI 10.1002/jmv.24580
Published online 30 May 2016 in Wiley Online Library (wileyonlinelibrary.com).

pediatric patients with ALRTIs have not been addressed fully. Therefore, this study collected 771 nasopharyngeal aspirate samples (NPAs) from children with ALRTIs for summarization and analysis of HPIVs (especially HPIV-4) in children with ALRTIs in this region, including prevalence, clinical characteristics, and differences from other HPIV types, in order to lay a foundation for further research.

MATERIALS, PATIENTS, AND METHODS

Patients and Specimens

NPA samples were collected from 771 children with ALRTIs in the Hunan Province People's Hospital, China, from March 2010 to February 2011. All patients were 14 years of age or younger, and the male: female ratio was 1.75:1. Informed consent was obtained from their parents/guardians. All patients had lower respiratory tract infection symptoms on admission. All NPA samples were collected 1–3 days after the onset of lower respiratory tract infections. Demographic data and details of the clinical findings and severity of disease were recorded in the medical record. These data were obtained via the medical record after their discharge from the hospital. The study protocol was approved by the hospital ethics committee.

Collection and Processing of Nasopharyngeal Aspirate

All NPA specimens were collected and transported immediately to the laboratory at the National Institute for Viral Disease Control and Prevention, China CDC, and stored at −80°C until required for further testing. Viral DNA and RNA were extracted from 140 μl of each nasopharyngeal aspirate specimen using the QIAamp viral DNA and the QIAamp viral RNA Mini Kits (Qiagen, Shanghai, China) according to the manufacturer's instructions. cDNA was synthesized using random hexamer primers with Superscript II RH^- reverse transcriptase (Invitrogen, Carlsbad, CA).

Human Parainfluenza Virus Detection

Nest-PCR was used to amplify the HN gene of HPIV- 1, HPIV- 2, and HPIV- 3 and the P gene of HPIV- 4 [Coiras et al., 2004; Klig and Shah, 2005; Ren et al., 2009; Weinberg et al., 2009].

Detection of Other Respiratory Viruses

Human rhinovirus (HRV), RSV, influenza virus (IFVA, IFVB), Human metapneumovirus (HMPV), and human coronaviruses (NL63, and HKU1) were screened for using a standard reverse transcription-PCR technique [Bastien et al., 2005; Bellau-Pujol et al., 2005]. In addition, AdV and HBoV were screened for using PCR methods [Hierholzer et al., 1993; Allander et al., 2005].

Nucleotide Sequence Analysis

All positive amplification products of HPIV-1, -2, -3, and -4 were sent for determination of their gene nucleotide sequences at Beijing Tianyi Huiyuan Bioscience & Technology Inc. BLAST comparison was conducted between the sequencing results obtained and sequences in the GeneBank database of U.S. National Center for Biotechnology (NCBI). MEGA4 software was used to analyze the sequence of the HN gene of HPIV- 1, -2, and - 3 and the P gene of HPIV-4, and an evolutionary tree was drawn.

Statistical Analysis

The significance of differences in rates among various groups was evaluated using the χ^2 test, Fisher's exact test, or Student's *t*-test. All analyses were performed using SPSS version 16.0 software (SPSS, Inc., Chicago, IL). $P < 0.05$ was considered statistically significant.

RESULTS

Patient Characteristics

The ages of children with acute respiratory infections in this study ranged from 1 day to 14 years (23.65 ± 2.67 months). The majority of patients (91.70%) were 5 years of age and under. The ratio of boys to girls was 1.75:1.

Epidemiology of HPIVs

HPIVs were detected by Nest-PCR in 151 specimens, and the overall frequency of HPIV infection among the 771 children was 19.58%. The difference in the prevalence of HPIVs between males (19.95%) and females (10.3%) was not statistically significant ($\chi^2 = 0.694$, $P = 0.476$). Twenty-eight (3.63%) patients were positive for HPIV-4, 12 (1.55%) for HPIV-1, 4 (0.51%) for HPIV-2, and 107 (13.87%) were positive for HPIV-3 (Table I). All of the children found to have HPIVs were 5 years of age or younger. The detection rate of HPIVs varied significantly between

TABLE I. Epidemiologic Characteristics of Children with HPIV Infections

	$HPIV_S$ (%)			
Parameters	HPIV-1	HPIV-2	HPIV-3	HPIV-4
No. of positive specimens	12	4	107	28
Detection rate (%)	1.55	0.51	13.87	3.63
Age, months				
~0 m	0	0	35 (20.46)	5 (2.92)
~6 m	3 (1.73)	2 (1.15)	35 (20.23)	7 (4.04)
~12 m	7 (2.91)	2 (0.83)	30 (12.50)	9 (3.75)
~36 m	2 (1.62)	0	7 (5.69)	7 (5.69)
≥60 m	0	0	0	0
Gender (M/F)	7/5	3/1	71/36	19/9

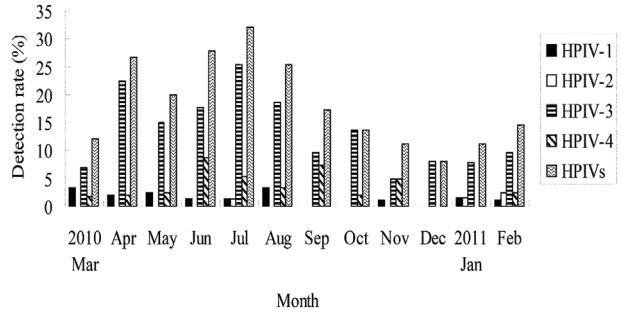

Fig. 1. Seasonal distribution of HPIVs in children with acute respiratory tract infections, March 2010–February 2011.

age groups ($\chi^2 = 25.046$, $P = 0.000$). No child 6 months of age or less was found to have HPIV-1, and only four patients 6–12 months of age were found to have HPIV-2. The detection rate of HPIV-3 was highest in children less than 1 year of age. Compared to HPIV types 1–3, HPIV-4 was detected at a higher rate in patients 36 months of age. Further statistical analysis suggested that only for HPIV-3 was the difference in the detection rate between ages statistically significant ($\chi^2 = 29.648$, $P = 0.000$) (Table I). The seasonal distribution of HPIVs fluctuated, and a phylogenetic tree is shown in Figure 1. Overall, HPIVs peaked during the summer, which is also when the highest detection rates of HPIV-1 and HPIV-4 were found. The detection rate of HPIVs varied significantly depending on the seasonal distribution ($\chi^2 = 22.236$, $P = 0.000$).

HPIV Coinfections

When tested for other respiratory viruses, 39.74% (60/151) of all HPIV-positive children were found to be co-infected with other respiratory viruses, including 24 patients with HRV, 21 with HBoV, 10 with ADV, 9 with RSV, 3 with HMPV, and 1 each with IFVB, HCoV-HKU1, and HCoV- NL63. HRV was the most common additional respiratory virus detected, followed by HRV. Differences in the frequency of age, gender, fever, average days of hospitalization, presence or absence of underlying diseases, average duration of fever, wheezing, vomiting, and diarrhea between the HPIV-3 or HPIV-4 mono-infection and co-infection groups were not significant (Table II).

Phylogenetic Analysis of HPIVs

In genetic sequence comparisons between HPIV-1 and -3 and the reference strains of GeneBank, the nucleotide homology was 97–99%, and HPIV4-type nucleotide homology was 90% to 93%. Based on the HN gene sequence, a phylogenetic analysis was made between HPIV strain types 1–3 of Changsha and the reference strains HPIV-1 (AF016280.1), HPIV-2 (AF2e13352.1), HPIV-3 (AB189961.1), HPIV-3 (EU326526.1), and HPIV-3 (FJ455842.2). The phylogenetic tree is shown in Figure 2. A phylogenetic analysis based on the P gene sequence between the Changsha HPIV-1 strain type and HPIV-4 reference strains HPIV-4a (E03304.1) and HPIV-4b (E03305.1) showed that the 28 cases of Changsha HPIV-4 strains could be divided into two sub-genotypes, with 20 cases (71.42%) of HPIV-4a genotype and 8 cases (28.57%) of HPIV-4b genotype. The phylogenetic tree is shown in Figure 3.

Clinical Characteristics of HPIVs in Children

Information on the clinical characteristics was available for the HPIV-positive patients. The main clinical diagnoses included bronchopneumonia (87.42%), bronchiolitis (9.27%), bronchitis (1.32%), bronchial asthma and pulmonary infection (1.99%). The clinical presentations of HPIV-positive children included fever (61.59%), cough (100%), wheezing (37.75%), running nose (5.30%), vomiting (11.26%), diarrhea (21.85%), polypnea (13.25%), and cyanosis (4.64) (Table III).

TABLE II. Clinical Comparison Between HPIV-3 and HPIV-4 Mono-Infection and Co-Infection Groups

	HPIV-3			HPIV-4		
Clinical characteristics	Mono-infection group (n = 61)	Co-infection group (n = 46)	*P*	Mono-infection group (n = 17)	Co-infection group (n = 11)	*P*
≤1 year of age	42 (68.85)	31 (67.39)	0.872[a]	8 (47.05)	4 (36.36)	0.705[b]
Male	39 (63.93)	32 (69.57)	0.542[a]	13 (76.74)	6 (54.54)	0.409[b]
Average days of hospital stay	9.78	8.84	0.225[c]	8.35	11.90	0.075[c]
Underlying disease	11 (18.03)	10 (21.73)	0.633[a]	3 (17.64)	1 (9.09)	1[b]
Fever	35 (57.37)	26 (56.52)	0.929[a]	10 (58.82)	9 (81.81)	0.249[b]
Average duration of fever, days	10.20	10.86	0.790[c]	5.70	7.22	0.473[c]
Wheezing	26 (42.62)	15 (32.61)	0.291[a]	6 (35.29)	4 (36.36)	1[b]
Vomiting	9 (14.75)	3 (6.52)	0.182[a]	1 (5.88)	2 (18.18)	0.543[b]
Diarrhea	13 (21.31)	13 (28.26)	0.407[a]	3 (17.64)	2 (18.18)	1[b]

[a]Indicate χ^2 test.
[b]Indicate Fisher test.
[c]Indicate *t*-test.

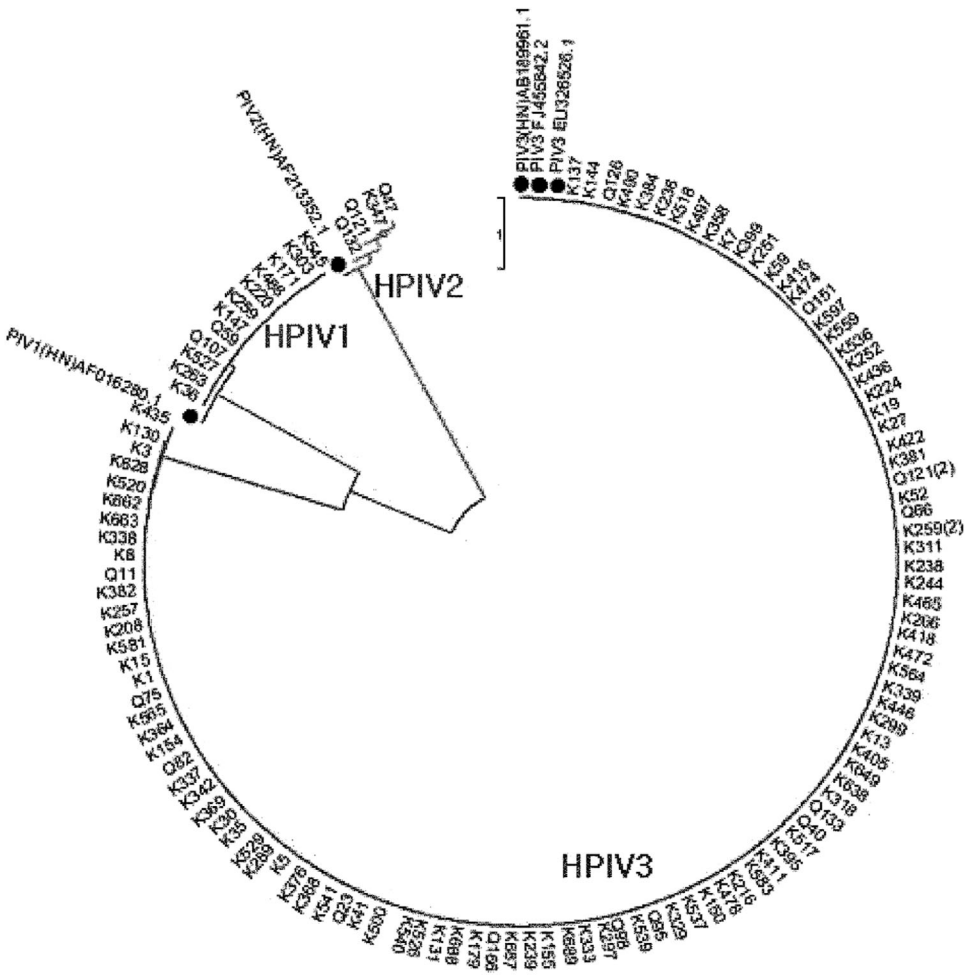

Fig. 2. Evolutionary tree: based on sequences of 317, 203, and 717 nucleotide fragments of the HN gene from HPIV-1, -2, and -3 Changsha strains and reference strains.

DISCUSSION

This study showed that in 771 NPAs from hospitalized children with ALRTIs, the total detection rate of HPIVs was 19.58%, suggesting that HPIVs play an important role in hospitalized children with ALRTIs in this region. The detection rate of HPIV-3 was the highest (13.87%), as previously reported [Laurichesse et al., 1999], followed by HPIV-4 and then HPIV-1 and HPIV-2. The U.S. National Respiratory and Enteric Virus Surveillance System (NREVSS) monitoring from 1990 to 2004 showed that the HPIV4 infection rate did not exceed 0.1% [Fry et al., 2006]. However, serological epidemiological survey data showed that cases of positive HPIV-4 accounted for approximately 3% of all patients with respiratory tract infections and from 50 to 90% in children and adolescents [Hasman et al., 2009]. Studies showed that in adult patients with flu-like or acute respiratory tract disease in the United States, the HPIV4 detection rate was 5.8% [El Feghaly et al., 2010]. Hong Kong Lau et al. [2009] reported that among 2,912 NPAs, with other respiratory viruses being negative, HPIV-4 positives were detected at a rate of 1.2%. In this study, the detection rate of HPIV-4 was 3.63%, which was different from the aforementioned studies.

With regard to the season, reports have indicated that HPIV-1 and HPIV-2 peak in the late fall and early winter season [Laurichesse et al., 1999; Karron and Collins, 2007; Hsieh et al., 2010], but in Taiwan from 2005 to 2007, these strains were sporadic and without epidemic occurrence [Hasman et al., 2009]. In this study, fewer cases with HPIV1 and HPIV2 were found with a scattered distribution, and no significant seasonal peaks were discovered, as previously reported [Counihan et al., 2001; Hasman et al., 2009]. HPIV-3 infections occur in late spring and summer [Laurichesse et al., 1999]. Our study results suggest that HPIV-3 had the highest detection rate in the spring and summer, which is similar to previous reports. Many reports show that the detection rate of HPIV-4 is the highest in the late fall and winter [Laurichesse et al., 1999; Lau et al., 2009], but our study showed no significant seasonal distribution.

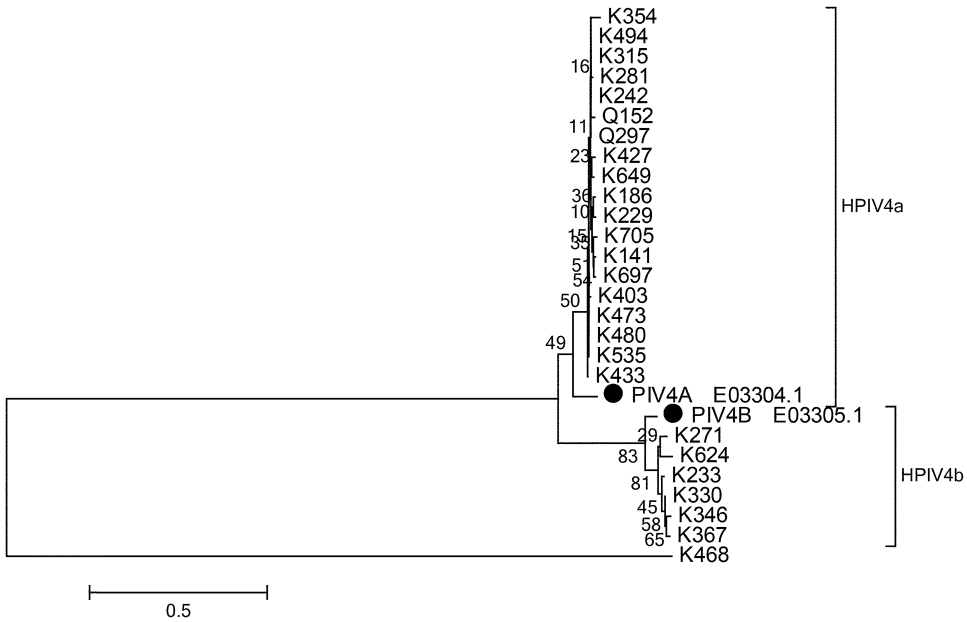

Fig. 3. Phylogenetic analysis of partial p gene sequences of 28 human parainfluenza virus strains from NPA specimens. Phylogenetic trees were constructed by the neighbor-joining method using MEGA 3.1. Viral sequences in marks were generated from the present study; other reference sequences were obtained from GenBank.

Therefore, the seasonal distribution of HPIV infections has geographic differences, and there is a need to conduct epidemiological studies in more regions.

Most articles in the literature report that those infected by HPIVs are mainly infants and that there is no difference in the male and female detection rates [Templeton et al., 2005; Hasman et al., 2009; Lau et al., 2009]. Our data indicated that all of the HPIV-positive individuals were ≤5 years of age (specifically 0–12 months old), which is similar to other reports. The comparison of the male and female detection rates showed no statistically significant difference, as previously reported [Templeton et al., 2005; Hasman et al., 2009; Lau et al., 2009].

Hong Kong Lau et al. [2009] reported that HPIV-4 strains were divided into two genotypes, with HPIV-4a being more common than HPIV-4b. This study showed that the 28 cases of HPIV-4 were divided into these two genotypes, including 20 cases of HPIV-4a (71.43%) and eight cases of HPIV-4b (28.57%), which is consistent with reports from Hong Kong and Australia [Lau et al., 2009]. In most reports, no differences were reported in clinical signs and symptoms or chest X-ray findings between infections due to HPIVs and infections due to other

TABLE III. Clinical Characteristics of Children with HPIV Infections

	$HPIV_S$ (%)				
Parameters	HPIV-1	HPIV-2	HPIV-3	HPIV-4	HPIVs
Clinical symptoms					
Fever	10 (83.33)	3 (75.00)	61 (57.00)	19 (67.85)	93 (61.59)
38.10–39.00°C	3 (30.00)	2 (66.67)	20 (32.78)	6 (26.31)	31 (33.33)
39.00–40.40°C	7 (70.00)	1 (33.33)	40 (65.57)	11 (57.89)	49 (63.44)
≥40.50°C	0	0	1 (1.63)	2 (10.52)	3 (3.23)
Duration of fever, days					
<7	5 (50.00)	3 (100.00)	30 (49.18)	12 (63.15)	50 (53.76)
7–14	5 (50.00)	0	16 (26.22)	6 (31.57)	27 (29.03)
≥14	0	0	15 (24.59)	1 (5.26)	16 (17.20)
Running nose	1 (8.33)	0	4 (3.73)	3 (10.71)	8 (5.30)
Cough	12 (100)	4 (100)	107 (100)	28 (100)	151 (100)
Wheezing	5 (41.67)	1 (25.00)	41 (38.31)	10 (35.71)	57 (37.75)
Vomiting	1 (8.33)	1 (25.00)	12 (11.21)	3 (10.71)	17 (11.26)
Diarrhea	1 (8.33)	1 (25.00)	26 (24.30)	5 (17.86)	33 (21.85)
Polypnea	1 (8.33)	0	14 (13.08)	5 (17.85)	20 (13.25)
Cyanosis	0	0	5 (4.67)	2 (7.14)	7 (4.64)

viruses. Hong Kong's Lau et al. [2009] found that the most common symptoms in HPIV infections included cough, running nose, fever, and wheezing, and that some patients exhibited vomiting, diarrhea and other gastrointestinal symptoms. In the past, HPIV4 was known to cause mild disease and therefore did not attract sufficient attention. However, in recent years, HPIV4 was found to have caused a localized outbreak of pneumonia, bronchiolitis and aseptic meningitis [Vachon et al., 2006; El Feghaly et al., 2010]. This study showed that all patients found with HPIVs had the most common symptoms of HPIV infection, for example, cough, fever, and wheezing. Some patients presented with vomiting and diarrhea and other gastrointestinal symptoms; while others with HPIV-3 and HPIV-4 exhibited polypnea and cyanosis. In most children, rales were heard in lung examination. In terms of clinical diagnosis, bronchial pneumonia was the most common finding. The evidence shows that HPIV was also an important pathogen for children with acute lower respiratory tract infection.

To understand the rate and role of co-infection, we examined each specimen for HPIVs and other common respiratory viruses. Most HPIV-positive patients (60, 39.74%) were co-infected with other viruses, and HPIV-1 had the lowest co-detection rate (16.67%). HRV was the most common additional respiratory virus detected. This study also compared the clinical manifestations between HPIV-3 and HPIV-4 mono-infection and co-infection groups. There was no difference between the two groups in gender, seasonality, mixed infection, fever, wheezing, polypnea, cyanosis or diarrhea. Due to the low number of HPIV-1 and HPIV-2 cases, we did not compare their clinical manifestations in the study.

Due to NPA samples being difficult to collect from healthy individuals, there were no adequate controls in our study. Our conclusions were limited by surveillance that only captured ALRTI cases requiring hospitalization, which missed describing the seasonality and pathogen distribution of the many outpatients and healthy individuals. Thus, additional study is required to define the exact role and molecular and epidemiological characteristics of HPIVs in ALRTIs in different individuals.

In conclusion, this study summarized and analyzed the epidemic and clinical features of HPIV strain types 1–4 in hospitalized children with ALRTIs from March 2010 to February 2011. This is the first report on an epidemic and the clinical features and genotypes of HPIV-4 in hospitalized children with ALRTIs in Changsha, China. Our study also confirmed that HPIVs are very important pathogens in children with ALRTIs in China as a whole. However, our study was limited to 1 year in duration, and additional future investigations should be conducted to provide useful information for HPIV disease treatment.

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