

Prevalence of Human Rhinovirus in Children Admitted to Hospital With Acute Lower Respiratory Tract Infections in Changsha, China

Sai-Zhen Zeng,¹ Ni-Guang Xiao,¹ Zhi-Ping Xie,² Guang-Cheng Xie,² Li-Li Zhong,¹ Juan Wang,¹ Han Huang,¹ Bing Zhang,^{1*} and Zhao-Jun Duan²

¹The People's Hospital of Hunan Province, Changsha, China

²National Institute for Viral Disease Control and Prevention, China CDC, Beijing, China

Human rhinovirus (HRV) is a causative agent of acute respiratory tract infections. This study analyzed the prevalence and clinical characteristics of three HRV groups (HRV-A, -B, and -C) among 1,165 children aged 14 years or younger who were hospitalized with acute lower respiratory tract infection in China. PCR or reverse transcription-PCR was performed to detect 14 respiratory viruses in nasopharyngeal aspirates collected from September 2007 to August 2008 in Changsha, China. HRV was detected in 202 (17.3%) of the 1,165 children; 25.3% of the HRV-positive children were 13–36 months of age ($\chi^2 = 22.803$, $P = 0.000$). HRV was detected year round and peaked between September and December. Fifty-three percent of the HRV-positive samples were also positive for other respiratory viruses; respiratory syncytial virus (RSV) was the most common secondary virus. Phylogenetic analysis using the VP4/VP2 region grouped the HRV-positive strains as follows: 101 HRV-A (50.0%), 21 HRV-B (10.4%), and 80 HRV-C (39.6%). HRV-A infections occurred predominantly in spring and autumn, and the peak prevalence of HRV-C was in early winter and late autumn. HRV-B infections were less common in spring ($\chi^2 = 31.914$, $P = 0.000$). No significant difference in clinical severity or presentation was found between patients with HRV single infection and HRV co-detections. Furthermore, the clinical characterizations did not differ among the three HRV species. These results suggest that HRV-C is an important viral agent along with HRV-A and HRV-B and that among hospitalized children with acute lower respiratory tract infection in China, the three HRV genotypes have similar clinical characteristics. **J. Med. Virol.** 86:1983–1989, 2014. © 2014 Wiley Periodicals, Inc.

KEY WORDS: human rhinovirus; acute lower respiratory tract infections; child; China

INTRODUCTION

Acute respiratory tract infection is a major cause of death for children worldwide [Williams et al., 2002]. HRV is one of the most important viral agents of acute respiratory tract infections and has been frequently detected in upper and lower respiratory tract infections using modern molecular detection methods. Previous studies suggest that HRV is associated with an extensive range of human respiratory disorders including the common cold, viral bronchiolitis, exacerbations of asthma, and chronic obstructive pulmonary disease [Gern et al., 1997; Rakes et al., 1999; Seemungal et al., 2000; Johnston et al., 2005; Jackson et al., 2008;]. A recent study also showed that HRV was the most prevalent agent associated with severe bronchiolitis in a population of preterm infants [Miller et al., 2012].

HRV is a small non-enveloped single-stranded RNA virus that is now classified within the genus

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*Correspondence to: Bing Zhang, The People's Hospital of Hunan Province, 61 Jie-Fang West Road, Fu-Rong District, Changsha 410005, China.
E-mail: zhangbing1959@aliyun.com

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Enterovirus belonging to the family *Picornaviridae*. Classical HRV consists of more than 100 distinct serotypes. Until recently, on the basis of gene sequence analysis, HRV was classified into three species, HRV-A, HRV-B, and the newly designated HRV-C [Lau et al., 2007; McErlean et al., 2007; Bizzintino et al., 2011]. Recent studies have suggested that the illness severity differs among HRV species [Khetsuriani et al., 2008; Calvo et al., 2010] and that HRV-C could be associated with more severe clinical illnesses, including wheezing, lower respiratory infections and asthmatic exacerbations, than HRV-A and HRV-B [Lau et al., 2007; Khetsuriani et al., 2008; Han et al., 2009; Lau et al., 2009; Linsuwanon et al., 2009; Miller et al., 2009; Piralla et al., 2009; Bizzintino et al., 2011]. However, some reports have indicated that HRV-C has been detected in healthy individuals without any acute respiratory symptoms [Calvo et al., 2010] and that there is no difference between the clinical presentations of patients infected with the different HRV species [Iwane et al., 2011]. Therefore, the clinical significance of HRV-C remains controversial and needs to be addressed further.

In the present study, 1,165 children aged 14 years or younger with acute lower respiratory tract infection were included and screened for the three HRV species and other respiratory viruses to explore the impact and the epidemiological and clinical characteristics of HRV-C infections in children with acute lower respiratory tract infection in Changsha, China.

MATERIALS AND METHODS

Patients and Clinical Specimens

Nasopharyngeal aspirates samples were collected from children hospitalized for acute lower respiratory tract infection at The People's Hospital of Hunan province, Changsha, China, during 2 days of each week between September 2007 and August 2008. All enrolled hospitalized patients were 14 years of age or younger and had symptoms of acute lower respiratory tract infection on admission. All nasopharyngeal aspirates were collected within 1–3 days of admission. Demographic data and details of the clinical findings were recorded. Informed consent was obtained from the parents of all children who provided specimens. The study protocol was approved by the hospital ethics committee. All nasopharyngeal aspirates 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 further analysis.

Nucleic Acid Extraction

Total nucleic acids (DNA and RNA) were extracted from 140 μl of each nasopharyngeal aspirates using the QIAamp viral DNA and the QIAamp viral RNA Mini Kits (Qiagen, Shanghai, China) according to the manufacturer's instructions.

Molecular Detection of HRV

A primer pair targeting a 549-bp fragment between the VP4/VP2 region and the 5'-non-coding region was used to amplify HRV. P1: 5'-GGG ACC AAC TAC TTT GGG TGT CCG TGT-3' and P2: 5'-GCA TCI GGY ARY TTC CAC CAC CAN CC-3', as described previously [Savolainen et al., 2002]. The cycling conditions for the PCR were 94°C for 8 min, followed by 35 cycles at 94°C for 45 s, 60°C for 45 s, and 72°C for 45 s, with a final extension at 72°C for 8 min.

Detection of Other Respiratory Viruses

RSV, human metapneumovirus (HMPV), influenza virus (IFVA, IFVB), parainfluenza virus (PIV types 1–3), and human coronaviruses (229E, OC43, NL63, and HKU1) were screened using a standard reverse transcription-PCR technique [Vabret et al., 2001; Bastien et al., 2005; Bellau-Pujol et al., 2005; Vabret et al., 2006]. In addition, adenovirus (AdV) and human bocavirus (HBoV) were screened using PCR methods [Hierholzer et al., 1993; Allander et al., 2005].

Nucleotide Sequence Analysis

All PCR products were purified using the QIAquick PCR purification kit (Qiagen, Shanghai, China) and sequenced by SinoGenoMax (Beijing, China). All positive sequences were determined and analyzed using the DNASTAR software package. Phylogenetic analysis was performed with Mega version 3.1 by using 1,000 bootstrapped replicates and the neighbor-joining algorithm with coxsackievirus (M17711) as the outgroup.

Statistical Analysis

The statistical significance of the differences among variables of groups was evaluated using the Chi-squared test, Fisher's exact test, independent sample *t*-test, Kruskal–Wallis H-test or ANOVA. All analyses were performed using SPSS version 13.0 software (SPSS, Inc., Chicago, IL).

RESULTS

Patient Characteristics

The age of 1,165 enrolled children ranged from 1 day to 156 months, with a median age of 15.4 months. Most of the specimens (1,124/1,165, 96.5%) were collected from patients under 60 months old, and the male to female ratio was 1.9–1 (763:402).

Detection of HRV and Other Viral Agents

At least one respiratory virus was detected in 871 of the 1,165 samples, and 202 (17.3%) were positive for HRV by PCR. HRV accounted for 23.2% of the total viral agents detected. Other respiratory viruses were detected in 107 of the 202 (53.0%) children who

were HRV positive, including 51 with RSV (25.2%), 26 with HBoV (12.9%), 17 with PIV3 (8.4%), and some other viruses. RSV was the most common additional respiratory virus detected, accounting for 51 of the 107 (47.7%) co-detections. Among the patients with co-detections, 41.1% (83/202) were positive for HRV plus one additional viral agent and 11.9% (24/202) were positive for HRV plus two or three additional viruses; of these 11.9%, 18 patients were positive for three and six patients were positive for four viral agents. No significant difference was found between the patients with HRV single infection and HRV co-detections in their epidemiological characteristics (age, gender, or duration of hospital stay), clinical presentations (cough, wheezing, fever, crackles, rhonchus, supplemental oxygen, underlying illness) or seasonal distribution ($P > 0.05$, Table I).

Epidemiology of HRV Infection

Seventeen point three percent (132/763) of the males and 17.4% (70/402) of the females had HRV detected in this study. Of those with HRV, 65.3% (132/202) were male and 34.7% female. The male to female ratio was not significantly different between the patients with and without HRV infections ($\chi^2 = 0.002$, $P = 0.961$). The ages of the 202 HRV-positive children ranged from 1 day to 156 months (mean age \pm SD, 15.8 ± 17.8 months) and 91.6% (185/202) were ≤ 36 months of age. Children 13–36 months of age had the highest infection rate (25.3%). The

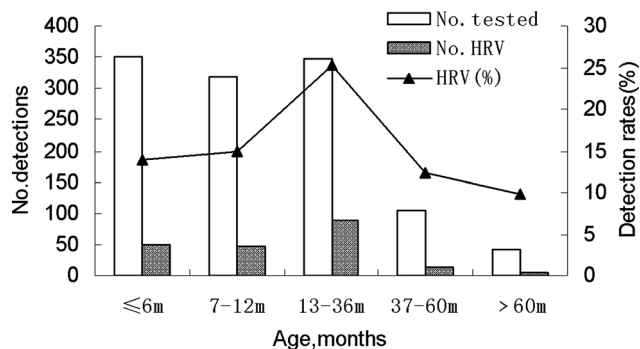


Fig. 1. Age distribution of HRV in children with ALRTIs during a 1-year study period.

HRV infection rates were significantly different between the age groups ($\chi^2 = 22.803$, $P = 0.000$; Fig. 1). HRV could be detected throughout the year; however, the majority of the cases occurred between September and December of 2007 and in April of 2008. The number of positive specimens peaked in November 2007 (36.40%; Fig. 2).

Clinical Characteristics of HRV in Children

Clinical symptom information was available for all of the HRV-positive subjects. The main clinical diagnoses of patients who were HRV-positive included bronchitis (5, 2.5%), bronchiolitis (44, 21.8%), acute asthmatic bronchopneumonia (16, 7.9%), and

TABLE I. Demographic Data and Clinical Symptoms in Children With HRV or HRV-C Single Infections Compared With Co-Detections

	HRV single infection (n = 95), no. (%)	HRV co-detections (n = 107), no. (%)	P-value	HRV-C single infection (n = 38), no. (%)	HRV-C co-detections (n = 42), no. (%)	P-value
Male gender	64 (67.4)	68 (63.6)	0.569	26 (68.4)	30 (71.4)	0.769
Age in months			0.171			0.554
≤ 12	40 (42.1)	57 (53.3)		15 (39.5)	22 (52.4)	
12.1 to ≤ 36	48 (50.5)	40 (37.4)		20 (52.6)	18 (42.8)	
> 36	7 (7.4)	10 (9.3)		3 (7.9)	2 (4.8)	
Median age in months ^b	17.1	14.6	0.307	17.0	12.7	0.157
Average duration of Hospitalization in days ^b	8.0	8.6	0.423	8.2	8.6	0.603
Underlying disease ^a	6 (6.3)	2 (1.9)	0.151	2 (5.3)	0 (0)	0.222
Symptoms and signs						
Cough ^a	92 (96.8)	103 (96.3)	1.000	38 (100)	40 (95.2)	0.495
Wheezing	49 (51.6)	63 (58.9)	0.297	24 (63.2)	25 (59.5)	0.739
Fever	26 (27.4)	29 (27.1)	0.966	11 (28.9)	13 (31.0)	0.845
Rhonchus	52 (54.7)	59 (55.1)	0.954	26 (68.4)	24 (57.1)	0.298
Crackles	75 (78.9)	93 (86.9)	0.131	31 (81.6)	36 (85.7)	0.617
Gastrointestinal symptoms	9 (9.5)	17 (15.9)	0.174	1 (2.6)	6 (14.3)	0.065
Supplemental oxygen	18 (18.9)	13 (12.1)	0.181	7 (18.4)	4 (9.5)	0.249
Seasonal distribute			0.206			0.885
Spring	29 (30.5)	32 (29.9)		9 (23.7)	11 (26.2)	
Summer	9 (9.5)	16 (15.0)		2 (5.3)	3 (7.1)	
Autumn	40 (42.1)	32 (29.9)		14 (36.8)	12 (28.6)	
Winter	17 (17.9)	27 (25.2)		13 (34.2)	16 (38.1)	

^aFisher's exact test.

^bIndependent samples *t*-test.

All the other: Chi-squared test. Seasonal distribution: Spring (March–May), Summer (June–August), Autumn (September–November), Winter (December–February).

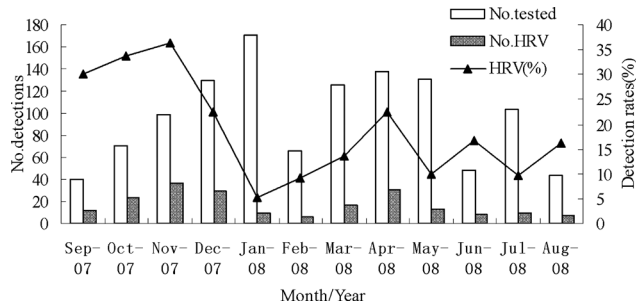


Fig. 2. Seasonal distribution of HRV in children with ALRTIs, September 2007 to October 2008.

pneumonia (137, 67.8%). The most common symptom was cough, which occurred in 195 patients (96.5%). Other clinical presentations included wheezing ($n = 112$, 55.4%), fever ($n = 55$, 27.2%), and gastrointestinal symptoms ($n = 26$, 12.9%). Crackles and rhonchus were common pulmonary symptoms in children with HRV infection. The duration of the hospital stays ranged from 1 to 56 days (mean \pm SD, 8.3 ± 4.8 days).

Phylogenetic Analysis of HRV

Among the 202 HRV strains, phylogenetic analysis of the viral protein VP4/VP2 coding regions indicated that 101 (50.0%) were classified as genetic group A, 21 (10.4%) as genetic group B, and 80 (39.6%) as a separate cluster, HRV-C. The nucleotide and deduced amino acid sequences of the VP4/VP2 gene of 188 HRV specimens were compared with those of HRV strains available at the GenBank site. Outgroup rooting was used for phylogeny with coxsackievirus (M17711). The GenBank accession numbers of the previously published sequences are as follows: HRV-A (DQ473509), HRV-A (DQ473507), HRV-97 (AY040242), HRV-B (EU081787), HRV-C (EF186077), and HRV-C (EF582386; Fig. 3).

Epidemiological and Clinical Characteristics of Three HRV Genotypes

The HRV-A infections occurred predominantly in the spring and autumn (April 2008, 24 samples; October and November 2007, 14 and 15 samples, respectively), and the peak prevalence of HRV-C was in the early winter and late autumn (December and November, 20 and 16 samples, respectively). HRV-B infections rarely occurred in the spring. A significant difference was observed between the seasonal prevalence of HRV-A, HRV-B and HRV-C ($\chi^2 = 31.914$, $P = 0.000$). No significant differences were observed between HRV-A, HRV-B and HRV-C with regard to the epidemiological characteristics (age, gender, or duration of hospital stay), clinical diagnoses (bronchitis, bronchiolitis, acute asthmatic bronchopneumonia, and pneumonia), or other clinical presentations (cough, wheezing, fever, crackles, rhonchus, supple-

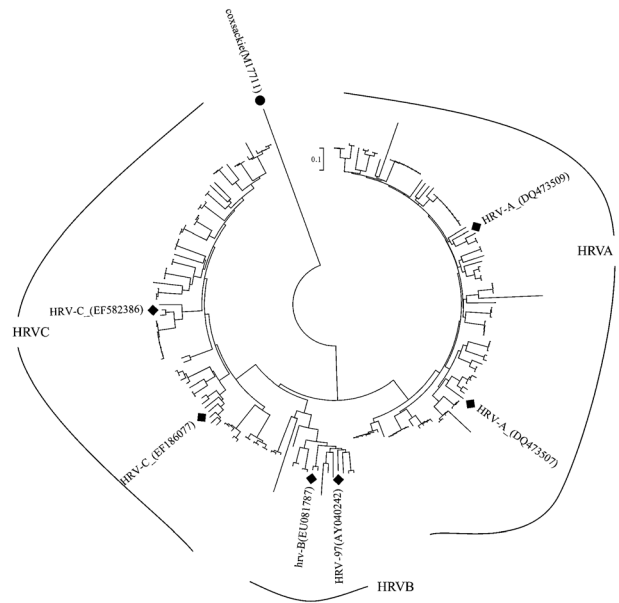


Fig. 3. Phylogenetic analysis of the VP4/VP2 region of the 188 HRV strains from nasopharyngeal aspirates. Phylogenetic trees were constructed by the neighbor-joining method using MEGA3.1 and coxsackievirus (M17711) is indicated as the outgroup. The viral sequences in black solid rhombus were generated from GenBank; other reference sequences were obtained from the present study.

mental oxygen, underlying illness, and co-detection) ($P > 0.05$, Table II). Additionally, no significant differences were found between the epidemiological characteristics or clinical presentations of the patients with HRV-C single infection and patients with HRV-C co-detections ($P > 0.05$, Table I).

DISCUSSION

In the present study, HRV was detected in 202 out of 1,165 (17.3%) nasopharyngeal aspirates collected from The People's Hospital of Hunan province from September 2007 to August 2008. A similar detection rate was reported in Lanzhou, China (13.1%) [Jin et al., 2009]. However, the study rate is lower than 30% detection rate reported in a study of hospitalized pediatric patients diagnosed with acute lower respiratory illness in Thailand [Linsuwanon et al., 2009] and is also lower than the 29.7% detection rate found in a study of hospitalized children <18 years of age in Hong Kong [Lau et al., 2009]. The present study indicates that HRV-A (50.0%) and HRV-C (39.6%) may be more prevalent than HRV-B (10.4%). Similar results have been reported in Hong Kong, Italy, Thailand, and Jordan, where the percentages of HRV-C were 43.8%, 41.1%, 58%, and 26%, respectively [Lau et al., 2009; Linsuwanon et al., 2009; Miller et al., 2009; Piralla et al., 2009]. This shows that HRV-C has been found in patients from various countries and plays an important role in respiratory tract infections worldwide.

TABLE II. Clinical Characteristics of Patients Infected With Different HRV Species From September 2007 Through August 2008

	HRV-A (n = 101), no. (%)	HRV-B (n = 21), no. (%)	HRV-C (n = 80), no. (%)	χ^2	P-value
Male gender	62 (61.4)	14 (66.7)	56 (70.0)	1.481	0.477
Age in months				5.436	0.245
<12	53 (52.5)	7 (33.3)	37 (46.2)		
12.1 to <36	40 (39.6)	10 (47.6)	38 (47.5)		
>36	8 (7.9)	4 (19.1)	5 (6.3)		
Median age in months ^a	15.1	23.3	14.7	1.901 ^a	0.387
Average duration of hospitalization in days ^b	8.4	7.7	6.7	0.186 ^b	0.830
Coinfection	52 (51.5)	13 (61.9)	42 (52.5)	0.769	0.681
Underlying disease ^c	5 (5.0)	1 (4.8)	2 (2.5)	0.991	0.572
Symptoms and signs					
Cough ^c	98 (97.0)	19 (90.5)	78 (97.5)	2.583 ^c	0.253
Wheezing	51 (50.5)	12 (57.1)	49 (61.3)	2.118	0.347
Fever	26 (25.7)	5 (23.8)	24 (30.0)	0.547	0.761
Rhonchus	49 (48.5)	12 (57.1)	50 (62.5)	3.573	0.168
Crackles	83 (82.2)	18 (85.7)	67 (83.8)	0.187	0.911
Gastrointestinal symptoms	17 (16.8)	2 (9.5)	7 (8.8)	2.834	0.242
Supplemental oxygen	14 (13.9)	6 (28.6)	11 (13.8)	3.156	0.076
Diagnosis ^c				11.286 ^c	0.056
Bronchitis	4 (4.0)	1 (4.8)	0 (0)		
Bronchopneumonia	18 (17.8)	8 (38.1)	18 (22.5)		
Pneumonia	73 (72.3)	9 (42.9)	55 (68.7)		
Acute asthmatic					
Bronchopneumonia	6 (5.9)	3 (14.3)	7 (8.8)		
Seasonal distribute				31.914	0.000
Spring	40 (39.6)	1 (4.8)	20 (25.0)		
Summer	13 (12.9)	7 (33.3)	5 (6.3)		
Autumn	36 (35.6)	10 (47.6)	26 (32.5)		
Winter	12 (11.9)	3 (14.3)	29 (36.2)		

^aKruskal–Wallis H-test.^bANOVA.^cFisher's exact test, All the other: Chi-square test; Seasonal distribution: Spring (March–May), Summer (June–August), Autumn (September–November), Winter (December–February).

The HRV-positive ratio was highest (33.3%) in children <12 months of age in the Hong Kong study [Lau et al., 2009]. Similarly, 64% of the infected children were younger than 6 months in a study in Jordan [Miller et al., 2009]. In addition, in a study in Thailand, most HRV-positive specimens were from children 6 to 23 months of age [Linsuwanon et al., 2009]. In a 21-year study, HRV was more frequently detected in younger children and infants than in older children [Linder et al., 2013]. A recent case-control study found that both the HRV-A and HRV-C detection rates were significantly higher in young children hospitalized for acute respiratory illnesses than in asymptomatic controls [Iwane et al., 2011]. In the present study, 91.6% (185/202) of the HRV-positive individuals were <36 months old, and the highest infection rate was observed in children 13–36 months old (25.3%). Parents of younger children may take children to the doctor more often than parents of older children. In addition, only children hospitalized with acute lower respiratory tract infection were included in the study. Further studies are necessary to determine why age is associated with HRV infections.

In some studies that included clinical specimens collected throughout the year, HRV-C appears to

show seasonal patterns of infection. In Hong Kong, a subtropical city, infections caused by HRV, including HRV-C, occur throughout the year, although a higher incidence has been observed during the fall and winter months for all three HRV species [Lau et al., 2007, 2009]. One study suggested that HRV, including HRV-C, could be found throughout the year but that it predominates during the rainy season in the pediatric population in Thailand [Linsuwanon et al., 2009]. In Kenya, there was no clear seasonal pattern of occurrence for any species [Onyango et al., 2012], while the number of HRV-C infections peaked in early winter (December) and late spring (April) in Lanzhou, China [Jin et al., 2009]. A recent 21-year study suggested that HRV-C was found most commonly during the winter months [Linder et al., 2013]. In this study, HRV was detected throughout the year and peaked in the winter and spring, and the HRV-C prevalence peaked in early winter and late autumn. These data suggest that HRV may follow different epidemiological patterns in different regions and that different genotypes may present in different seasons.

Other respiratory viruses were co-detected frequently in the HRV-positive patients. In a recent study involving patients from Thailand, other

respiratory viruses were co-detected in approximately 38% of the HRV-positive patients; of these patients, 40% were co-infected with HRV-C and 36% were infected with HRV/RSV [Linsuwanon et al., 2009]. RSV and HRV were the viruses identified most frequently in mixed infections in infants hospitalized with bronchiolitis [Richard et al., 2008]. Of those with HRV in the present study, 53.0% were co-detected with other respiratory viruses and 41.1% with HRV-C. RSV (47.7%) was the most common additional respiratory virus detected in the HRV-positive patients. These findings were in agreement with a report from Thailand. Children with HRV mono-infection versus HRV co-detection with other viruses had similar clinical courses, comparable to those in some US studies [Iwane et al., 2011; Miller et al., 2011]. One study suggested co-infections were not associated with a particular HRV species or with severity [Lauinger et al., 2013]. No significant differences in the clinical severity were found between patients with HRV single infection and those with HRV co-detections. No significant differences in median age, gender, symptomatology (fever, wheezing, cyanosis, and crackles), supplemental oxygen, and time hospitalized were observed between children infected with HRV-C only and those with HRV-C co-detections.

Previous studies have suggested that HRV-C might play a role in severe clinical disease. HRV-C was present in the majority of children with acute asthma and was associated with more severe asthma [Bizzintino et al., 2011]. In another study by Miller et al. [2009], children with HRV-C were more likely to require supplemental oxygen than those with HRV-A. Wheezing episodes were also more common among individuals with HRV-C and HRV-A infection than among those with HRV-B infection [Lau et al., 2009]. It was found that HRV-C was associated with respiratory infections with few symptoms and could trigger apparently life-threatening events in young infants [Calvo et al., 2009]. Another study suggested that HRV-C was associated with more severe disease in children <3 years of age [Lauinger et al., 2013]. However, some reports found no differences in the clinical characteristics among hospitalized enrolled patients positive for HRV-A, HRV-B, or HRV-C, including wheezing [Fry et al., 2011; Iwane et al., 2011]. In the present study, no significant differences were observed in clinical symptoms, signs, and clinical diagnoses including wheezing, supplemental oxygen and acute asthmatic bronchopneumonia among the three HRV genotypes. With the absence of a control group in both the current and previous studies, it is difficult to evaluate the exact role that HRV-C plays in acute lower respiratory tract infection.

The present study showed that HRV-C is an important viral agent, along with HRV-A and HRV-B, in children with acute lower respiratory tract infection in China. HRV was detected throughout the

year and peaked in the winter and spring. The majority of HRV-positive individuals were ≤ 36 months old, and HRV-C was mainly epidemic during early winter and late autumn. The results do not support those of previous studies, which showed differences in the clinical symptoms, signs, and clinical diagnoses among the three HRV genotypes. Additional studies with healthy controls are needed to completely define the epidemiological and clinical characteristics and genetic characterization of HRV-C with HRV-A and HRV-B.

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