

# Human Rhinoviruses Prevalled Among Children in the Setting of Wearing Face Masks in Shanghai, 2020

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## Research Article

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# Abstract

**Background** Human rhinovirus (HRV) is the predominant etiological agent of the common cold in children and adults. A recent study showed that the inhibitory effect of face masks on viral shedding of HRV was less prominent than that on other respiratory viruses. Considering that most Chinese people have worn face masks in public area since the outbreak of coronavirus disease 2019, we aimed to find out whether HRV prevailed among children in 2020 and demonstrate the details of the epidemiological features of HRV under such a special circumstance.

**Methods** We summarized the incidences of various respiratory virus infections in patients who visited the Children's Hospital of Fudan University during 2018~2020, and genotyped HRV positive nasopharyngeal specimens collected from 316 inpatients and 72 outpatients that visited the hospital in 2020.

**Results** There was a major prevalence of HRV among children in the latter half of 2020, with a clear seasonality that HRV-As prevailed in summer while HRV-Cs in autumn. HRV-As were more prone to cause severe lower respiratory tract infections (LRTI), while HRV-Cs were closely associated with childhood wheezing. The predominant genotypes were A11, A28, A47, A82, A101, C40 and C43. Notably, A21, A82 and A101 took up larger proportions in severe cases than in non-severe cases.

**Conclusions** Our findings described a major prevalence of HRVs among children in 2020, which highlight the unique transmitting pattern of HRV and help to narrow the targets for antiviral strategies.

## Background

Human rhinoviruses (HRVs) are the leading cause of upper respiratory tract infections (URTIs) since its first isolation in the 1950s [1]. HRVs also cause pneumonia hospitalization in vulnerable people such as children, the elderly and those with underlying diseases. HRV-associated diseases pose great socio-economic burdens to the country annually [2]. However, given that HRV-infected people are usually manifest self-limited and mild symptoms or even asymptomatic, HRVs have long been afforded little attention and no antivirals or vaccines have been approved for HRVs up to now [3].

HRVs belong to the *Picornaviridae* family, and are single-stranded, positive-sense RNA viruses with a genome of ~7.2 kb [4]. About 100 serotypes which were culturable *in vitro* were classified into HRV-As and HRV-Bs based on the similarity of partial genetic sequences in the 1990s. Afterwards, at the beginning of the 2000s, researchers identified at least 50 more new HRV strains which couldn't be cultured and were classified into a unique species now named as HRV-C [2]. So far, more than 160 HRV genotypes have been identified [5].

After the outbreak of coronavirus disease 2019 (COVID-19), most people have developed the habit of wearing face masks in public area, which prominently hindered the transmission of respiratory pathogens. However, a recently published paper reported that wearing face masks significantly reduced the viral shedding of influenza viruses and coronaviruses (OC43 and NL63), but not HRVs [6], which

aroused our curiosity about the prevalence of HRVs in the setting of wearing face masks. Thus, we conducted this research to find out whether HRV prevailed among children in 2020 and also demonstrate the details of the epidemiological features of HRVs, which helps to expand our knowledge of the viral prevalence and arouse our attention on its unique transmission pattern in such a special background.

## Methods

### Patients and sample collection

We collected a total of 316 HRV-positive inpatients hospitalized in the Children's Hospital of Fudan University in Shanghai from June 2020 to November 2020. All the inpatients were diagnosed with LRTI supported by symptoms and radiographic changes and were defined as HRV positive after routine screening for common respiratory viruses including respiratory syncytial virus (RSV), adenovirus (AdV), influenza A and B viruses (IAV and IBV), parainfluenza virus type 1 (PIV-1), PIV-2, PIV-3, human rhinoviruses (HRV) and human metapneumovirus (MPV) using RT-qPCR or immunofluorescence assays. A total of 703 nasopharyngeal swabs from outpatients with URTI who visited the hospital during June 2020 to November 2020 were collected randomly and screened for HRV by RT-qPCR. The severity-based classification of the patients were performed by experienced clinicians according to the revised World Health Organization severe acute respiratory infection (SARI) case definition. All aspects of the study were approved by the Ethics Committee of the Children's Hospital of Fudan University.

### HRV detection and genotyping

For HRV detection, RNA from respiratory samples were extracted using a magnetic beads-based nucleic acid extraction system NP968-C (Tianlong Technology) according to the manufacturer's instruction. Samples were detected for HRV using a HRV-specific one-step RT-qPCR kit (Land medical).

For genotyping, the extracted RNA were reverse transcribed and amplified using a nested RT-PCR strategy. HRV molecular subtyping was performed using primers targeting the *VP4/VP2* regions (540bp) or *5'NCR* (110bp) of HRVs as reviewed in a previously published paper [7]. The reverse transcription and the first amplification step were performed using a one-step RT-PCR kit (Rui'an Biotechnology). The second amplification step was performed using a Premix Taq kit (Takara). The amplification products were sequenced by Sangon Biotech Co., Ltd., followed by subjection to phylogenetic analysis using MEGA software.

### Statistical analysis.

Proportions for categorical variables were compared using the  $\chi^2$  test or Fisher's exact test. Independent group *t*-test was used for the comparison of means for continuous variables that were normally distributed. The Mann-Whitney U test was used for continuous variables not normally distributed. All

statistical analyses were performed using GraphPad Prism software. Two-sided *p*-values of less than 0.05 were considered statistically significant.

## Results

### The prevalence of respiratory viruses

We summarized the numbers of patients tested positive for RSV, AdV, IAV, IBV, PIV-1, PIV-2, PIV-3, MPV and HRV in the Children's Hospital of Fudan University from June 2018, when the hospital started the HRV test for patients, to December 2020. Most respiratory viruses were barely detected after the outbreak of COVID-19 (Fig 1A), but HRVs showed a remarkable increase in the middle of 2020 and reached the highest detection rate (30.7%, 94/306) in June 2020 among the three years. There was also a mild increase in HRV infection in September 2020. PIV-3s and RSVs also increased gradually but were much less prominent than HRVs. The proportion of HRVs in the total virus positive cases was 52.5% in 2020, which was much higher than that in 2019 (26.7%) (Fig 2B). Collectively, these data indicated that there was a major prevalence of HRV in the year of 2020.

### Molecular epidemiology of HRVs

A total of 316 nasopharyngeal aspirates from HRV positive inpatients during the epidemics of HRVs in 2020 were collected and 82.3% (260/316) were successfully genotyped. We also randomly collected 703 nasopharyngeal swabs from outpatients with URTI during the same period of the inpatients, among which 10.2% (72/703) were determined as HRV positive and 90.3% (65/72) were successfully genotyped.

The genetic variability of HRV genotypes in our data was very wide, as shown in the phylogenetic tree (Fig 2). A total of 29 HRV-A genotypes, 8 HRV-B genotypes and 22 HRV-C genotypes were detected in the patients. HRV-A was the most frequently detected species both in the inpatients (51.6%, 163/316) and the outpatients (45.8%, 33/72), followed by HRV-C (27.2%, 86/316 for inpatients; 33.3%, 24/72 for outpatients) and HRV-B (3.5%, 11/316 for inpatients; 11.1%, 8/72 for outpatients). The monthly distribution of patients revealed that HRV-As mainly prevailed in the summer (June to August) with A11, A47, A82 and A101 being the most frequent, while HRV-Cs quickly caught up in the autumn and peaked in September represented by C40 and C43. Notably, unlike most HRV-As, A28 mainly prevailed in the autumn (September to November) rather than summer (Fig 3). HRVs infected more males than females both in the inpatients (181 vs. 135) and the outpatients (42 vs. 30). 55.7% of total HRV-positive patients were infants under 1 year of age, and no discernable differences was found in the proportions of HRV species among different age groups (Fig 4A). Also, the predominant genotypes appeared to be similar among children of different ages (Fig 4B).

### HRV genotypes and clinical features

To find out the association between HRV and clinical characteristics, we collected the clinical information of patients including symptoms, co-infections and underlying diseases. Among the three species, HRV-Bs

seemed to infect more females than males (Table 1). Notably, HRV-Cs appeared to be the most frequently detected species in the 46 wheezing patients (60.9%, 28/46). 11.7% (23/196) of the HRV-A positive patients were defined as severe LRTI, which was much higher than HRV-B (0%, 0/19) and HRV-C (4.5%, 5/110), suggesting that HRV-A is more prone to cause severe illness.

Afterwards we classified the patients into three groups according to their disease severity, including the outpatients with URTI, the inpatients with non-severe LRTI or severe LRTI (Table 2). Females were more likely to develop HRV-associated severe LRTI, with 64.5% (20/31) severe cases being girls. In addition to longer hospitalizations, severe LRTI cases were more easily to get cough than the other two groups. Notably, the percentages of underlying diseases decreased in sequence from severe LRTI (87.1%), non-severe LRTI (62.4%) to URTI (33.3%).

The percentage of severe cases in HRV positive cases showed an increase in October and November 2020 (Fig 5A), despite that the detection rate of HRVs displayed a downward trend since September 2020 (Fig 1A), indicating that the prevalence and severity of HRV infections did not correspond completely. The genotypes detected in severe group included A11, A21, A28, A47, A82, A101, C40, C43 and C45, all of which were also detected in non-severe LRTI patients (Fig 5B). Notably, there were three genotypes which made up significantly larger proportions in severe LRTI cases than in non-severe LRTI cases, namely A21 (9.7% vs 0.4%,  $p=0.0004$ ), A82 (16.1% vs. 5.3%,  $p=0.0183$ ) and A101 (22.6% vs. 7.7%,  $p=0.0065$ ). Also, A21 was the only genotype that caused more severe LRTI cases quantitatively than non-severe LRTI cases (3 vs. 1), despite that the total number of the latter was almost ten times of the former.

To further investigate the viral load-associated factors, we collected the Ct values of HRV positive samples. It was reported that the patients co-infected with other respiratory viruses showed higher viral loads than those with HRV mono-infection [8], but it is not the case in our data (Fig 6A). Also, the viral loads didn't seem to be correlated with the disease severity (Fig 6B). But HRV-Bs showed lower viral loads (higher Ct values) compared with the other two species (Fig 6C), which was in line with its lowest prevalence and least possibility to cause severe illness.

## Discussion

HRV infections were mainly transmitted via aerosols generated by coughing, sneezing and nose blowing [9], which is supposed to be effectively decreased by face masks. But the major HRV prevalence among children in 2020 indicates a weakened inhibitory effect of face masks [6]. Still, the unique transmitting pattern of HRV which enabled itself to escape from face masks deserves further investigation.

Choi et al. reported an age-dependent infection pattern of HRVs, with HRV-A and HRV-C infections being similarly common in infants, whereas HRV-Cs being detected less often than HRV-As in adolescents [10]. But in our study, the proportions of HRV species were similar among all age groups from infants to adolescents. Zhao et al 's paper based on the respiratory samples of children in Shanghai during 2013-2015 shared some similar findings with ours, such as the age/gender preferences of HRV and the

seasonality of HRV-C [11]. But HRVs were most frequently detected during winter in Zhao's paper but summer in ours. In Zhao's paper, the predominant genotypes included A78, A12, A89, A61, B70, C2, C6, C24 and C16, none of which were the main genotypes in our study. Moreover, we summarized the genotypes in papers focusing on various countries and concluded that the prevailing genotypes changed greatly with time and place [12-14]. But what these papers have in common was that HRV-As and HRV-Cs were the most frequently detected species and usually prevailed alternatively and seasonally. Considering of the substantial genetic diversity of HRVs, long-term and large-population-based studies are needed for a comprehensive understanding of HRV prevalence.

HRV-Cs were more commonly associated with earlier initiation of asthma control medication than the other two species [5, 15-17], which might be attributed to the cellular receptors. HRV-As and HRV-Bs use intercellular adhesion molecule 1(ICAM-1) and the low-density-lipoprotein receptor (LDLR) for viral binding [18-20], while Bochkov et al. later reported that cadherin-related family member 3 (CDHR3), which is a susceptibility locus for wheezing illness and early childhood asthma [21], was closely associated with HRV-C binding and host susceptibility to HRV-Cs, but not for the HRV-A and HRV-B. Hence, anti-childhood wheezing and subsequent asthma strategies should pay more attention to HRV-Cs.

The rates of underlying diseases increased progressively with disease severity, while the viral loads wasn't correlated with the disease severity [22, 23], suggesting that host factors bear important responsibility for the disease severity. Lee et al. reported that the peak prevalence and severity of HRV infections did not correspond, and HRV-As and HRV-Cs were more likely to develop severe LRTI than HRV-Bs [24], which is also the case in our data. A21 was more frequently detected in severe LRTIs than non-severe LRTIs and URTIs in our study, which is in line with the findings of a paper focusing on adults, although they didn't find specific site mutations in the sequences of A21 obtained from severe cases [25]. Whether there are particular A21 mutations that facilitate viral replication and host adaptation, especially in the lower respiratory tract tropism, deserves to be further demonstrated.

There are also some limitations of this paper. Firstly, our data only collected the samples from children in 2020, which makes us fail to compare the epidemiological features of HRV genotypes before and after the outbreak of COVID-19. Also, genetic analysis are needed to figure out whether there are meaningful site mutations in the prevailing HRV genotypes, such as A21, A82 and A101. More efforts are needed for better understanding of the individual and viral factors that contribute to more severe illnesses, so as to reduce the overall burden of respiratory illness.

## Conclusions

Collectively, our findings described the details of the HRV prevalence among children in 2020, which is worthy of our reflection on the distinct transmitting pattern of HRVs. Moreover, our data suggested that the antiviral strategies to reduce HRV-related morbidity in high-risk children should focus on HRV-As and

HRV-Cs. In a word, our findings add to the knowledge of the epidemiological features of HRV among children and underline the necessity to control HRV infection despite of the use of face masks.

## **Abbreviations**

HRV, Human rhinoviruse

URTI, upper respiratory tract infections

LRTI, lower respiratory infection.

COVID-19, coronavirus disease 2019

RSV, Respiratory syncytial virus

AdV, adenovirus

IAV, influenza A viruse

IBV, influenza B viruse

PIV, parainfluenza virus

MPV, human metapneumovirus

ICAM-1, intercellular adhesion molecule 1

LDLR, low-density-lipoprotein receptor

CDHR3, cadherin-related family member 3

## **Declarations**

### **Ethics approval and consent to participate**

This study and informed consent waiver statement were reviewed and approved by the Institutional Review Board of the Children's Hospital of Fudan University. Consent was not sought from the parent or legal guardians of the children because the specimens we used in this study were left over after routine examination.

### **Consent for publication**

Not applicable

### **Availability of data and materials**

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors have no competing interest.

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### Author Contributions

R. J. and J. X. conceived and designed the experiments; R. J., S. L., L. L., M. X., L. C. and L. S. collected the respiratory samples from patients; R. J. and S. L. collected the clinical data of patients; R. J. and L. L. performed the experiments; R. J., L. L., P. L. and J. X. analyzed the data; R. J. and J. X. wrote the paper. All authors have read and approved the manuscript.

### Acknowledgements

Not applicable

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## Tables

Table 1 The clinical features of patients among the three HRV species

	HRV-A	HRV-B	HRV-C	<i>p</i> -value
	(n=196)	(n=19)	(n=110)	
Male	134(68.4%)	6(31.6%)	82(74.5%)	0.0010*
Age	7m26d(1d-17y)	2m14d(26d-16y)	9m13d(13d-17y)	0.3627
Hospital stays	9.5	10	9	0.8602
Symptom				
Fever	56(28.6%)	9(47.4%)	31(28.2%)	0.2136
Cough	78(39.8%)	9(47.4%)	55(50.0%)	0.2130
Wheeze	15(7.7%)	3(15.8%)	28(25.5%)	0.0001*
Tachypnea	13(6.6%)	0	6(5.5%)	0.4890
Cyanosis	1(0.5%)	0	1(0.9%)	0.8572
Respiratory failure	12(6.1%)	0	5(4.5%)	0.4802
Severe LRTI	23(11.7%)	0	5(4.5%)	0.0382*
Underlying disease	142(72.4%)	12(63.2%)	75(68.2%)	0.5673
Cardiovascular system	40(20.4%)	3(15.8%)	11(10%)	-
Hepatobiliary system	30(15.3%)	2(10.5%)	14(12.7%)	-
Immune deficiency	7(3.6%)	1(5.3%)	3(2.7%)	-
Hemopoietic system	3(1.5%)	0	5(4.5%)	-
Co-infections	28(14.3%)	0	18(16.3%)	0.1672
Klebsiella pneumoniae	4(2%)	0	1(0.9%)	-
Haemophilus influenzae	2(1%)	0	1(0.9%)	-
Pseudomonas aeruginosa	2(1%)	0	1(0.9%)	-
Streptococcus viridans	2(1%)	0	4(3.6%)	-
RSV	2(1%)	0	3(2.7%)	-
PIV	1(0.5%)	0	2(1.8%)	-
AdV	2(1%)	0	1(0.9%)	-
Mycoplasma urinolytica	3(1.5%)	0	0	-
Mycoplasma pneumoniae	2(1%)	0	3(2.7%)	-

\* $P < 0.05$  was considered of significant difference among the three groups.

HRV, human rhinovirus.

LRTI, lower respiratory infection.

RSV, respiratory syncytial virus.

AdV, adenovirus.

Table 2 The clinical characteristics of patients with different disease severity

	URTI (n=72)	Non-severe LRTI (n=285)	Severe LRTI (n=31)	<i>p</i> -value
Age	1y9m(5d to 14y)	7m5d(1d to 17y)	1y6m(3m27d to 7y)	-
Male	42(58.3%)	170(59.6%)	11(35.5%)	0.0350*
Hospital stays	-	9d	19d	0.0007*
Symptom				
Fever	29 (40.3%)	61(21.4%)	6(19.4%)	0.0031*
Cough	12(16.7%)	112(39.3%)	18(58.1%)	<0.0001*
Wheeze	5(6.9%)	34(11.9%)	7(22.6%)	0.0790
Tachypnea	0	9(3.2%)	10(32.3%)	<0.0001*
Cyanosis	0	0	2(6.5%)	<0.0001*
Respiratory failure	0	0	17(54.8%)	<0.0001*
Underlying disease	24(33.3%)	178(62.4%)	27(87.1%)	<0.0001*
Cardiovascular system	2(2.8%)	44(15.4%)	8(25.8%)	-
Hepatobiliary system	1(1.4%)	42(14.7%)	3(9.7%)	-
Immune deficiency	3(4.2%)	6(2.1%)	2(6.5%)	-
Hemopoietic system	4(5.6%)	3(1.1%)	1(3.2%)	-
Co-infection	-	42(14.7%)	4(12.9%)	0.9946
Virus	-	12(4.2%)	2(6.5%)	-
Bacteria	-	19(6.7%)	3(9.7%)	-
Fungi	-	4(1.4%)	2(6.5%)	-
Mycoplasma	-	8(2.8%)	0	-

\* $P < 0.05$  was considered of significant difference among the three groups.

URTI, upper respiratory infection.

LRTI, lower respiratory infection.

## Figures

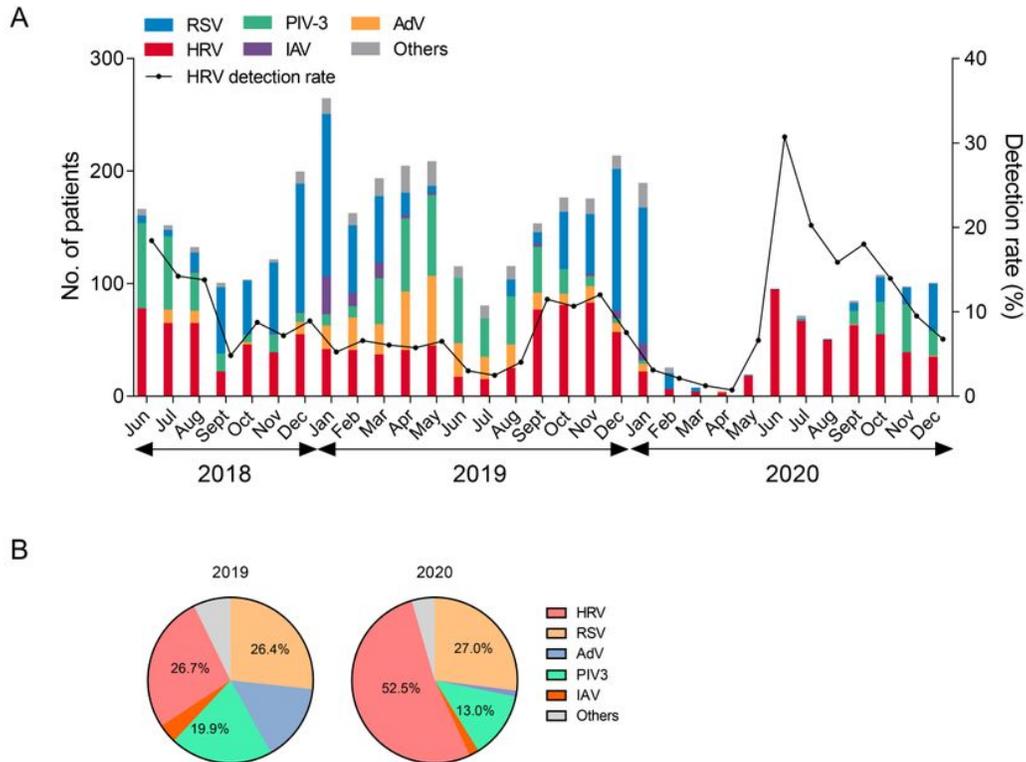
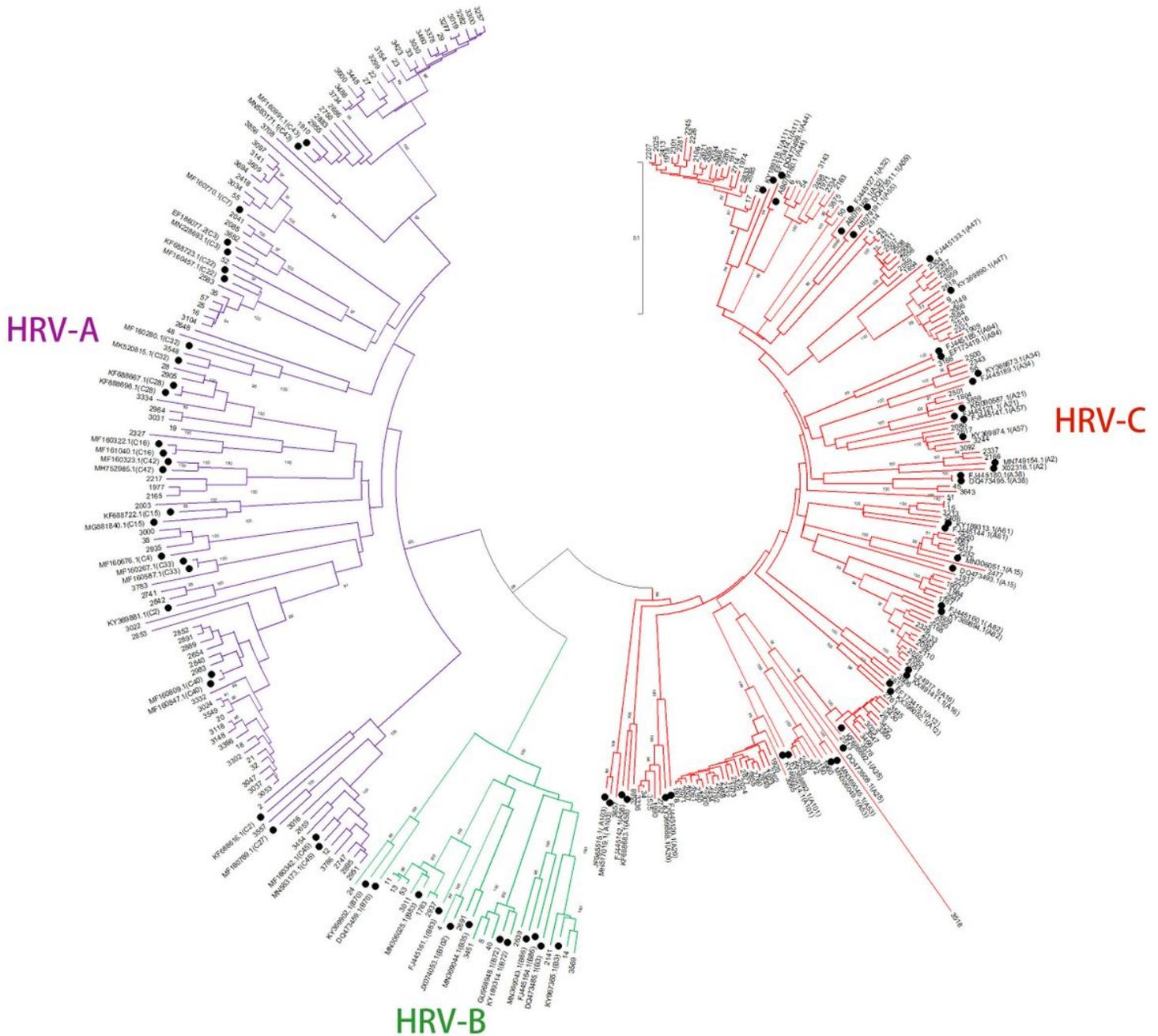


Figure 1

## Figure 1

The prevalence of respiratory viruses from 2018 to 2020. A, Total numbers of patients tested positive for the indicating respiratory viruses and the detection rate of HRVs in the specimens collected from the Children's Hospital of Fudan University during June 2018 to December 2020 were displayed. B, Proportions of different respiratory virus in 2019 and 2020. HRV, human rhinovirus. RSV, respiratory syncytial virus; AdV, adenovirus; IAV, influenza A virus; PIV-3, parainfluenza virus type 1. Others represented for the sum of PIV-1, PIV-2, IBV and human metapneumovirus (MPV).



**Figure 2**

Phylogenetic trees of VP4/VP2 gene sequences of HRVs. Phylogenetic trees were generated from manually trimmed 540 bp fragments using the neighbor-joining method and branch supported with 1000 bootstrap iterations using MEGA software. Bootstrap values were shown on tree nodes. Study sequences were identified by accession number. Reference sequences from GenBank were identified by accession number (HRV type) with black dots. Purple branches, HRV-As; Green branches, HRV-Bs; Red branches, HRV-Cs.

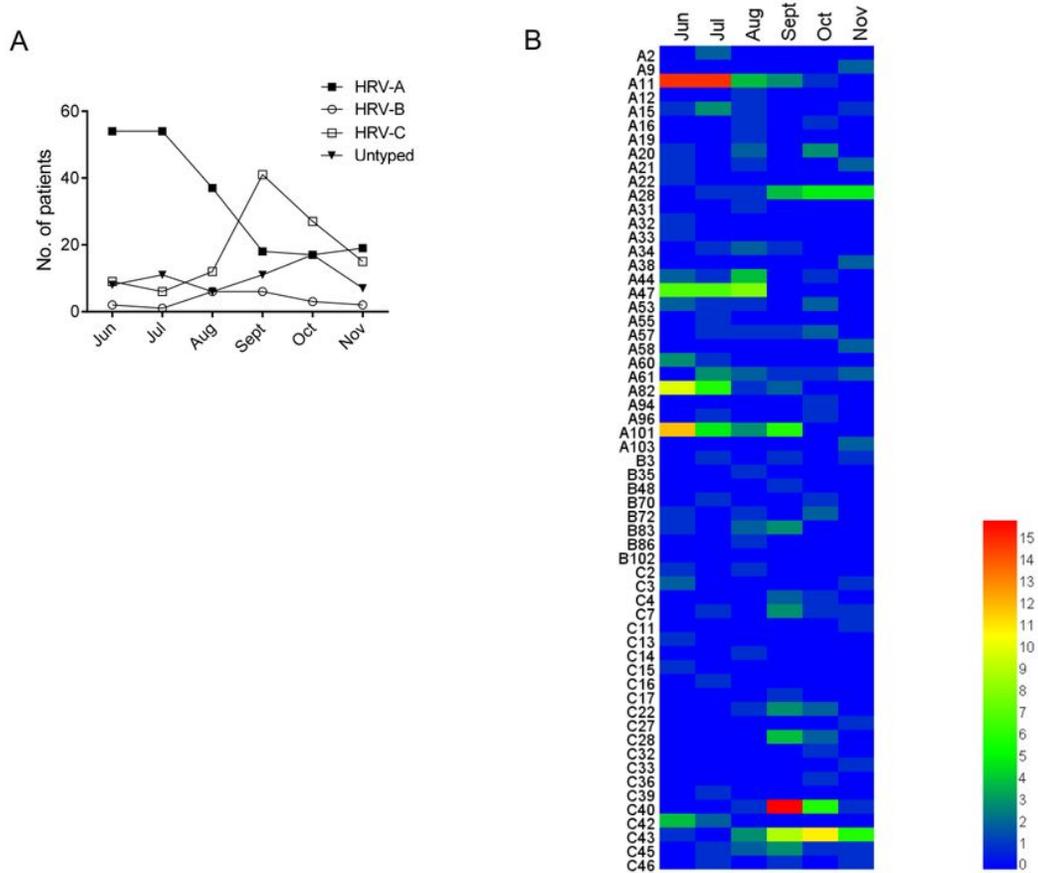


Figure 3

### Figure 3

The seasonality of HRV genotypes. A, The numbers of patients infected with different HRV species were shown by month from June 2020 to November 2020. B, The numbers of patients detected positive for the indicating HRV genotypes were shown by month from June 2020 to November 2020.

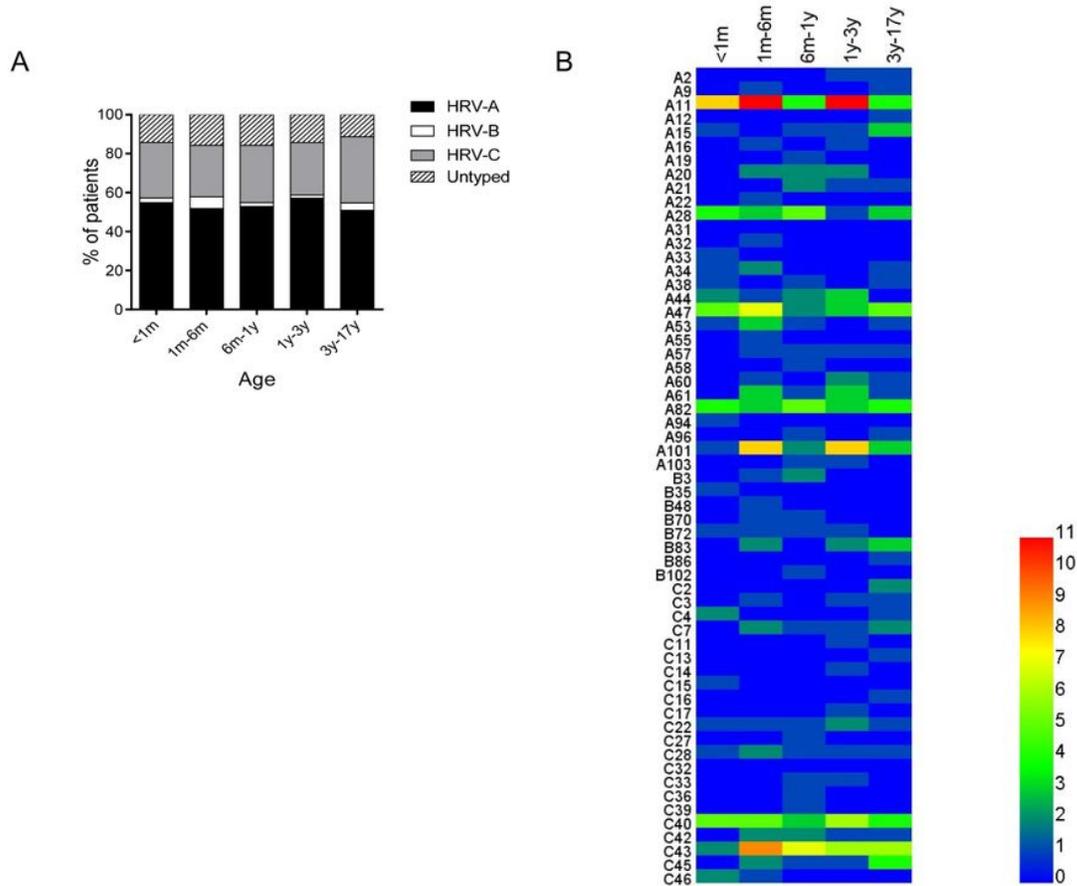


Figure 4

### Figure 4

The distribution of HRV genotypes among patients of different ages. A, The proportions of HRV species in different age groups from infants to adolescents. B, The numbers of patients detected positive for the indicating genotypes were shown by age.

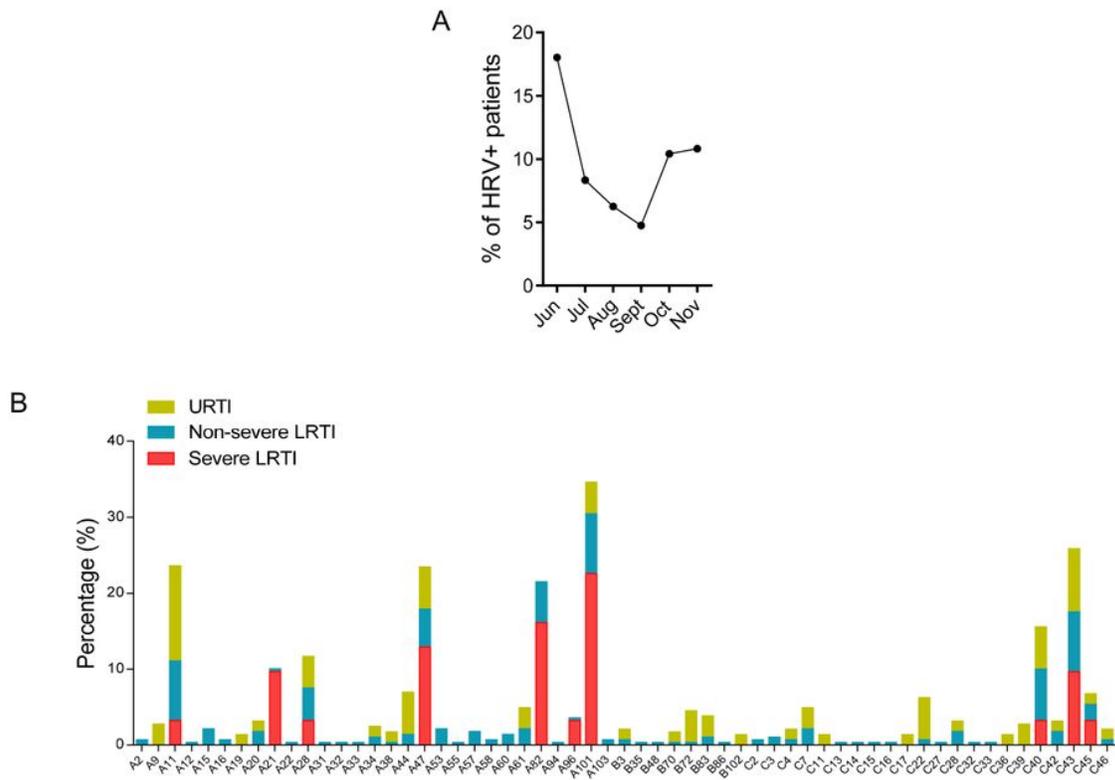


Figure 5

### Figure 5

The diversity of HRV genotypes in patients of different severity. A, The monthly distribution of severe LRTI cases in HRV-positive patients. B, The percentages of different genotypes in patients of URTI, non-severe LRTI and severe LRTI groups respectively. URTI, upper respiratory infection; LRTI, lower respiratory infection.

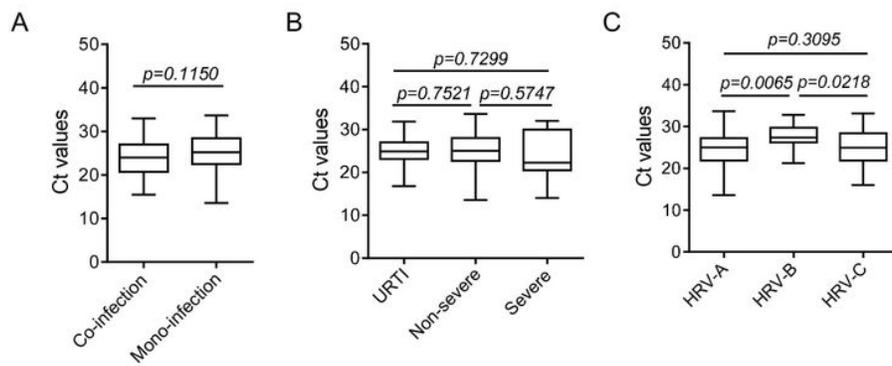


Figure 6

## Figure 6

The Ct values of HRV positive samples. A, The patients infected with HRV only or co-infected with other respiratory pathogens were divided into two groups, and their Ct values were acquired based on RT-qPCR. B, The Ct values of HRV positive samples collected from patients classified based on their disease severity were shown. C, The Ct values of HRV positive samples of different species were shown.  $P < 0.05$  was considered of significant difference.