Molecular Typing and Epidemiology Profiles of Human Metapneumovirus Infection Among Children With Severe Acute Respiratory Infection in Huzhou, China

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Research

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Abstract

Background: Severe acute respiratory infections (SARI) threaten human health and cause a large number of hospitalized patients every year. However, as one of the most common pathogen that cause acute respiratory tract infection, the molecular epidemiological information relating to hMPV among patients with SARI is limited. Here, we evaluate the epidemiological and molecular characteristics of hMPV infections among children hospitalized patients with SARI from January 2016 to December 2020 in Huzhou, China.

Methods: From January 2016 to December 2020, a total of 1133 nasopharyngeal swabs collected from children inpatients with SARI were screened for hMPV by real-time PCR. All samples that tested positive for hMPV were further typed by sequencing partial sequences of hexon gene. Genotypes of hMPV were confirmed by phylogenetic analysis. Epidemiological data were analyzed using Microsoft Excel 2010 and service solutions (SPSS) 21.0 software.

Results: 56 (4.94%) samples were positive for hMPV, children under 5 years old accounted for 85.71% (48/56) of the infections. Higher activity of hMPV infection could be seen in the period in Spring and Winter. 3 different types of hMPV were identified in hospitalized SARI cases, with hMPV-B1 (42.86 %) was the most prevalent types, followed by HAdV-B2 (35.71 %) and hMPV-A1(21.42 %). The predominant genotypes of hMPV during our study period varied according to surveillance year. Overall, 1 type (hMPV-B1) were detected in 2016, 2 different types(hMPV-B1 and HAdV-A1) were detected in 2017, 3 different types(hMPV-B1, hMPV-B2 and hMPV-A1) were detected in 2018, 2 different types(hMPV-B1 and hMPV-B2) were detected in 2019, 1 type (hMPV-B2) were detected in 2020.

Conclusions: This study revealed the prevalence and the molecular epidemiological characteristics of hMPV infections among children hospitalized patients with SARI in Huzhou from January 2016 to December 2020. The hMPV prevalence is related to age and season. As the most prevalent hMPV types, hMPV-B1 was co-circulating with other types and presented an alternate prevalence pattern.

Background

Human metapneumovirus (hMPV), frst identifed in 2001, is a major viral respiratory pathogen that worldwide reported[1]Since then, scholars around the world have reported its infection2,3hMPV is one of the important pathogens of infant respiratory tract infectionabout half of all children are infected by hMPV before 2 years of age, and most children are infected before 5 years of age1Unfortunately, individuals infected with hMPV usually do not develop lifelong immunity against this virus, and reinfection occurs frequently[1,4]. It is a serious threat to the health of infants under five years old, the elderly and people with immunodeficiency. Global epidemiological studies have shown that most children have been infected with hMPV by the age of five[1]. hMPV infection cannot be distinguished from other respiratory viral infections by its symptoms alone, hMPV can cause upper or lower respiratory tract infections, mainly manifested by cough, expectoration, wheezing, shortness of breath, runny nose,

bronchitis, asthmatic bronchitis, bronchiolitis, pneumonia, et al[5]. hMPV is a non-segmented, negative-stranded RNA virus belonging to the family Pneumoviridae. The viral RNA is approximately 13kb in length, containing eight genes(N,P,M,F,M2,SH,G,and L) coding for nine proteins[5]. The evolutionary analysis of multiple genes showed that hMPV was divided into two different genotypes, A and B, and within each genotype there were two different subtypes (A1, A2 and B1, B2) [6]. The fusion protein expressed by F gene is the main antigen of hMPV and is the most commonly used gene for typing at present [7]. Different subtypes of hMPV can be prevalent at the same time, and the dominant types of hMPV can appear alternately with time. At present, the main prevalent subtypes are A2, B1 and B2 genotypes[8-10]. So far, the correlation between different subtypes of hMPV infection and disease severity has not been determined[11]. Recently, infections of hMPV were increasingly reported in Asian countries[8,12-19], with novel variants of hMPV emerging[18,19], highlighting the risks of hMPV epidemics in these regions. However, there is currently no effective vaccine or antiviral drug for hMPV [20]. Therefore, it is important to understand the prevalence and pathogenesis of hMPV for prevention and treatment. The in-depth analysis of the genomic structure of different genotypes of hMPV is helpful to understand its genetic background and evolutionary status, and provide a molecular basis for epidemiological investigation, prevention and treatment of hMPV.

The genetic evolution and transmission of hMPV are critical to epidemic control but have not been thoroughly studied as yet. Here, we conducted a fve-year study on hMPV in Huzhou, the most populous city in China, from January 2016 to December 2020.

Materials And Methods

Patients and Clinical sample Patients suspected of having acute respiratory tract infections were enrolled according to these criteria[21]: the onset of the disease has a history of fever(> 38°C), accompanied by sore throat, cough, expectoration, or dyspnoea/tachypnoea, and the onset does not exceed 10 days. All the samples of this study were obtained from local SARI surveillance sentinel hospital, the First People's Hospital of Huzhou was responsible for sample collection from surveillance cases. A total of 1133 patients were enrolled from January 2016 to December 2020. A nasopharyngeal flocked swab, nasal wash or combination of nasopharyngeal swab and oropharyngeal swab was collected from each Patients, placed in 3 ml viral transport medium, and stored at −80°C prior to laboratory screening.

RNA extraction and rRT-PCR

Total viral nucleic acids were extracted from 200 µL of each specimen using TIANLONG Ex Viral DNA/RNA Kit (TIANLONG Biotech, Xi□an, China) according to the manufacturer's instructions. Real-time RT-PCR (qPCR) was performed using nucleic acid detection kit for r hMPV (Zhuocheng, Baijin, China) with the ABI Q7 (Applied Biosystems). The reaction was conducted according to the manufacturer's instructions with total volume of 20 µL.

PCR amplifcation of F protein genes

For hMPV positive samples, the viral RNA was reverse transcribed into cDNA using the One Step RNA PCR Kit (TaKaRa Biotechnology Dalian, China, CAT: DRR057A). Then the F genes was amplified using traditional PCR with primers newly designed in this study. Primers used for F gene sequencing were: outer primers set hMPV-F-F1-5'-CAATGCAGGTATAACACCAGCAATATC-3' and hMPV -F-R1 5'-GCAACAATTGAACTGATCTTCAGGAAAC-3', and inner primers set hMPV-F-F2-5'-ACATGCCAACATCTGCAGGACAAATAAAAC-3' and hMPV-F- R2 5'- ACATGCTGTTCACCTTCAACTTTGC-3'. And the amplification conditions as follows: 95˚C for 5 min, followed by 35 cycles of 95˚C for 30 sec, 57˚C for 30sec, and 72°C for 1.5 min, and a final step at 72°C for 5 min. After amplification, 5 μL of the PCR products was visualized by agarose gel electrophoresis. The residual PCR products were purified using a QIA quick PCR purification kit (Qiagen, Leusden, The Netherlands), and the purified products were sequenced directly at both ends with amplification primers by TaKaRa Biotechnology (Dalian, China).

Phylogenetic analysis

Partial nucleotide sequences of hexon gene obtained in this study were compared with the NCBI GenBank database (http://www.ncbi.nlm.nih.gov) by using online BLAST tools to preliminarily determine the genotype. Multiple sequence alignment and phylogenetic analysis were conducted using MEGA software version 6.06. The phylogenetic tree was generated using the neighbor-joining method and bootstrap analysis was performed with 1000 replications.

Statistical analysis

Epidemiological data were analyzed using Microsoft Excel 2010 and service solutions (SPSS) 21.0 software. Statistical differences were determined using the Chi-square test and P-values <0.05 were considered to represent a statistically significant difference.

Nucleotide sequence accession numbers

The GenBank accession numbers for sequences obtained in this study are MZ215789-MZ215816.

Results

Epidemic characteristics of hMPV

A total of 1133 nasopharyngeal swab samples were collected from January 2016 to December 2020, and 56 samples were positive for hMPV, with the detection rate of 4.94%(56/1133). As shown in Table 1, among the 56 hMPV-infected patients, 33 (58.93%) were male and 23 (41.07%) were female. No significant difference was observed in males and females in the hMPV-infected cases (P = 0.395). Infection with hMPV was found in all age groups tested(0□, 1□, 3□,5□,7□14). Children under 5 years old accounted for 85.71%(48/56) of the infections. There were no significant differences in hMPV detection rates among different age groups (P = 0.890). The highest detection rate was in the 0□ year age group(6.03%), followed by 1□years (4.43%), 3□ years (4.61%), 5□years (4.76%),7□14 years (4.39%).

In the years 2016–2019, the hMPV detection rate was approximately even, ranging from 4.72% to 5.80%, similar with the rate detected in central and south China. An apparent low frequency of circulation of hMPV in the year 2020 was observed, with a low positive rate of 1.79%. The seasonal distribution showed that hMPV circulated predominantly in the period in Spring and Winter (Fig. 1).And the detection rate in September reached a peak of 8.02%. In contrast, lower activity of hMPV infection were observed during Summer and autumn, when the average detection rate was only 1.16% and 1.96% respectively.

hMPV genotyping and phylogenetic analysis

The F gene sequences of 56 hMPV positive samples confirmed by real-time RT-PCR were amplified and sequenced, 28 samples were successfully sequenced and genotyped by nested-PCR.

Phylogenetic analysis based on partial hexon sequences indicated that 3(A1,B1,B2) different types were identified throughout the study period, see Fig.2. hMPV-B1 (n = 12, 42.86 %) was the most prevalent hMPV types, followed by HAdV-B2 (n = 10, 35.71 %) and hMPV-A1(n = 6, 21.42 %). The predominant genotypes of hMPV during our study period varied according to surveillance year. Overall, 1 type (hMPV-B1) were detected in 2016, 2 different types were detected in 2017, including hMPV-B1 (n = 3) and HAdV-A1 (n = 2), 3 different types were detected in 2018, including hMPV-B1 (n = 1), hMPV-B2 (n = 3) and hMPV-A1 (n = 4), 2 different types were detected in 2019, including hMPV-B1 (n = 2) and hMPV-B2 (n = 5), 1 type (hMPV-B2) were detected in 2020.

Discussion

At present, hMPV has been recognized as one of the important pathogens of respiratory tract infection in the world. The diseases caused by hMPV are not significantly different from other viral infections, ranging from mild upper respiratory tract infection to severe bronchopneumonia. Most of the clinical manifestations were cough, runny nose, fever and wheezing. Hypoxemia occurred in about 1/3 of the patients. Chest X-ray showed local infiltrating shadow of pulmonary lobes or infiltration around hilar lung and peritracheal cuff sign. It is estimated that 4–16% of acute respiratory tract infections are caused by hMPV[22, 23]. The virus has attracted wide attention at home and abroad since it was first identified in 2001. In 2003, Zhu Runan et al [9] first reported the infection of hMPV in China, and then similar reports were successively reported in other places in China [10–12]. However, there has been no research report on the infection of hMPV in Huzhou at present.

The onset of hMPV infection has a certain seasonality, with most reports suggesting that it occurs in winter and spring. In the northern hemisphere peak hMPV disease occurrence is typically in winter and spring months of January to May [24, 25, 26], while in the southern hemisphere peak prevalence is in the spring period of August to September [27]. This study showed that there was a statistically significant difference in the detection rate of hMPV among the positive cases in different months. The main epidemic months were November and January-March, and the epidemic season was winter and spring, which was consistent with the results reported by Jin Yu [13]. In addition, the results of this paper show that the hMPV detection rate was approximately in the years 2016–2019, but an apparent low frequency

of circulation of hMPV in the year 2020, with a low positive rate of 1.79%, which may be related to factors such as the emergence of the local 2019-nCoV in February 2020 in Huzhou, the increased awareness of crowd protection, the reduction in crowd gathering, and the suspension of kindergarten and school.

Worldwide, hMPV prevalence in hospital inpatient or community studies, in children or elderly adults, varies widely from as low as 1.7% to as high as 17%, with generally higher prevalence in outpatients compared to inpatients and, also, more in children younger than 5 years compared to older age groups [28,29,30]. In this study, 1133 cases of children with severe acute respiratory tract collected in Huzhou from 2016 to 2020 were tested for hMPV nucleic acid, and 56 cases were detected positive, with a positive rate of 4.94%, indicating that hMPV is indeed one of the important pathogens causing severe acute respiratory tract infection in children in Huzhou. The total number of cases under 5 years old was 84.55%(958/1133), The number of positive cases was 85.71%(48/56)%; No significant difference was found between the two sexes in the infection of the virus, which was consistent with the report of Xu Meijia [14].

Studies have shown that two types of hMPV genotypes A and B can be prevalent together in the same season, and genotype A is the most prevalent. The prevalence pattern of hMPV genotypes in the same region may change continuously in different years. Liu Shiwen reported [31] that there were A2, B1 and B2 genotypes of hMPV prevalent in Jiangxi, among which A2 genotype was the dominant genotype. Our monitoring data showed there are A1, B1 and B2 genotypes of hMPV prevalent in Huzhou area. The B1 genotype strain was the most prevalent types and has been detected every year (except in 2020), followed by B2 and A1, so we speculated that the B1 genotype strain is the main epidemic strain in Huzhou area. However, the genotypes of the endemic strains are different from year to year, and one or several endemic types exist simultaneously every year, there are no type of hMPV presented absolutely predominant during hMPV epidemic seasons.

Our study is limited by a single-site setting, small sample size, and especially the partial genotyping of detected hMPV. Genotyping was only successful for 50%(28/56) of hMPV infection cases. In the future research, we will gradually improve the research content, expand the detection range and quantity of samples, accumulate and analyze data, further evaluate the harm of hMPV-related diseases, and provide more scientific basis for the prevention and control of infection of this virus.

Conclusions

In conclusion, this study revealed the prevalence and molecular epidemiological characteristics of hMPV infections among Pediatric Patients with SARI in Huzhou from January 2016 to December 2020. hMPV is one of the important pathogens causing severe acute respiratory tract infection in children, with most (85.71%) hMPV-positives cases detected among children < 5 years of age. Higher activity of hMPV infection could be seen in spring and autumn season. No type of hMPV presented absolutely predominant during hMPV epidemic seasons, hMPV was co-circulating with other types and presented an

alternate prevalence pattern. Our results provide a reliable scientific basis to better understand the role played by hMPV s in SARI cases, and for the prevention and control of hMPV infection.

Abbreviations

SARI: Severe acute respiratory infections

hMPV: Human metapneumovirus

qPCR: Real-time RT-PCR

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of Huzhou Center for Disease Control and Prevention (20160521). Informed consent for the nasopharyngeal swabs was obtained from the patients or their guardians. This study was part of a routine laboratory-based investigation. No human experimentation was conducted. The only human materials used were nasopharyngeal swabs that had been sent to our laboratory for routine virological diagnosis.

Declarations

The authors declare that they have no competing interests.

Funding

No.

Consent for publication

Not applicable.

Availability of data and materials

The readers interested in using the data may contact the corresponding author.

Authors' contributions

LJ and DSX participated in the design of the study and performed the statistical analysis. DSX and LPC participated in the hMPV detection. XFW and DSX participated in the genomic amplification for genotyping. LPC and LJ participated in the sequence analysis and phylogenetic analysis. LPC drafted the manuscript. All authors read and approved the final manuscript.

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Table

Table 1

hMPV-positive in Pediatric Patients of different ages and gender with SARI

Variable	Tested SARI cases N (percentage)	hMPV-positive cases N(percentage)	hMPV-negtive cases N (percentage)	Positive rate	χ^2	P
Gender					0.724	0.395
Male	605（53.40）	33（58.93）	572(53.11)	5.45%		
Female	528（46.60）	23（41.07）	505(46.29)	4.36%		
Age (years)					1.126	0.890
0～	315（27.80）	19（33.93）	296（27.48）	6.03%		
1～	361（31.86）	16（28.57）	345（32.03）	4.43%		
3～	282（24.89）	13（23.21）	269（24.98）	4.61%		
5～	84（7.41）	4（7.14）	80（7.42）	4.76%		
7～14	91（8.03）	4（7.14）	87（8.08）	4.39%		
Total	1133	56	1077	4.94%		

Figures

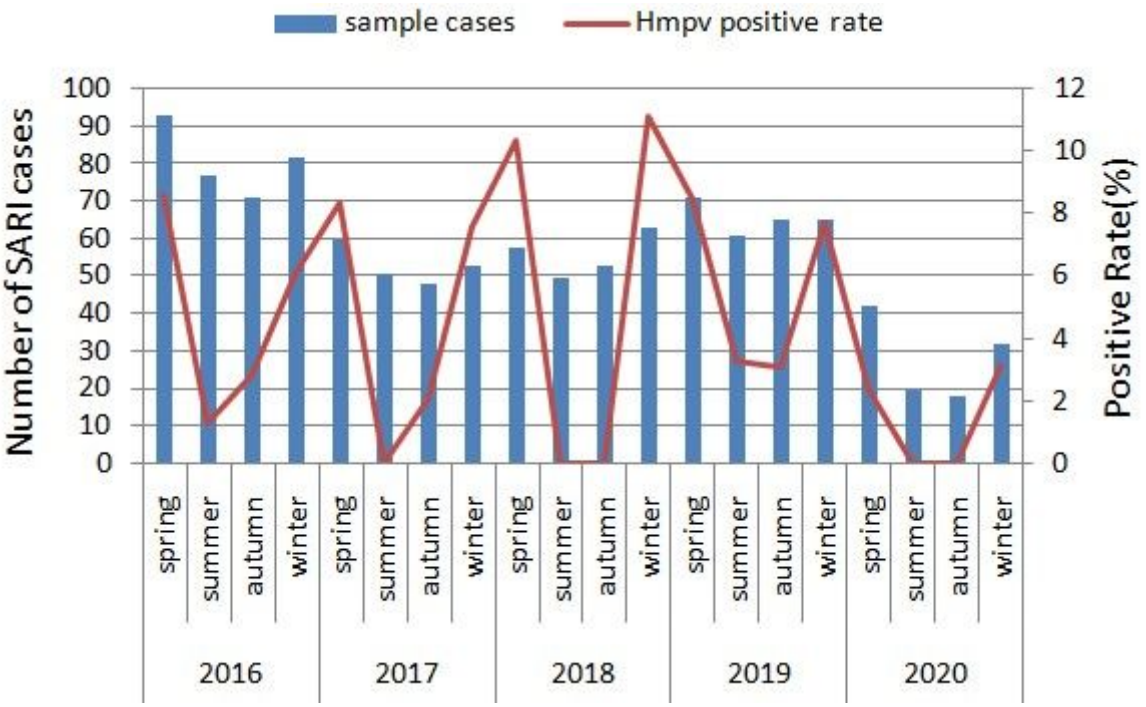

Figure 1

Seasonal distribution of hMPV infections from 2016 to 2020

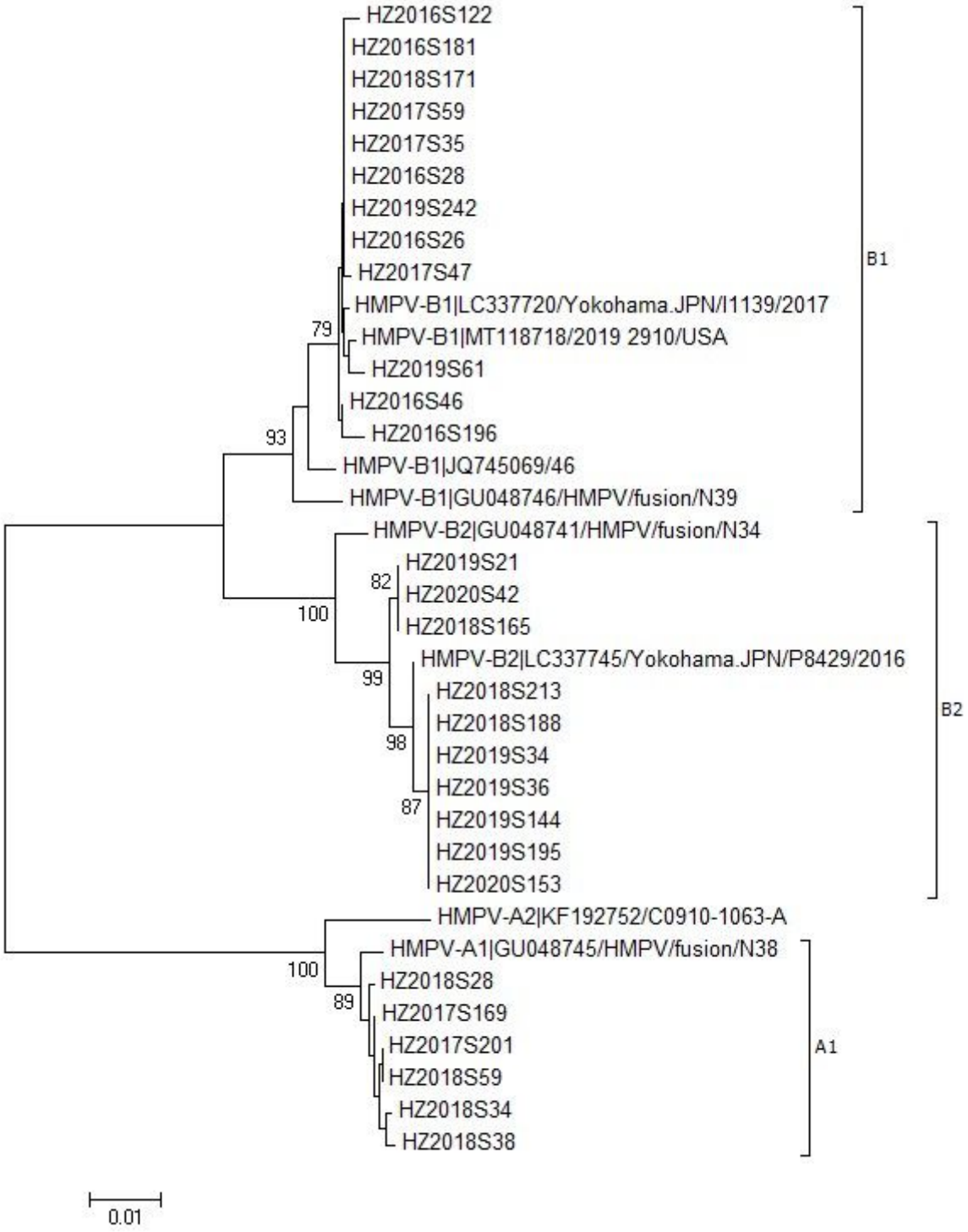

Figure 2

Phylogenetic analyses based on partial hexon sequences of hMPV strains. The trees were generated using the neighbor-joining method, validated by 1000 bootstrap replicates. Bootstrap values ≥ 70% are shown on the branch. hMPV sequences identified in this study are indicated by closed circles.