Human metapneumovirus in hospitalized children with acute respiratory tract infections in Beijing, China

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Abstract

Background This study aims to described the epidemiology and genotypic diversity of Human metapneumovirus (HMPV) and the impact of SARS-CoV-2 on the prevalence of HMPV in hospitalized children with Acute respiratory tract infections (ARTIs) in Beijing, China. Methods From April 2018 to March 2019 and from September 2020 to August 2021, nasopharyngeal aspirates (NPAs) from hospitalized children with ARTIs in Beijing were collected and subjected to real-time polymerase chain reaction tests for HMPV. Then genotyping, detection of 15 common respiratory viruses and clinical characteristics were analyzed on HMPV positive samples. Results 7.9% (124/1572) enrolled paediatric patients were identified as having HMPV infection, and the majority of children under the age of 5 (78.2%, 92/124), From April 2018 to March 2019. The detection rate of HMPV in spring and winter is significantly higher than that in summer and autumn. The co-infection rate were 37.1% (46/124), the most common co-infected virus were parainfluenza virus type 3 (HPIV-3). The main diagnosis of HMPV infection was pneumonia (92.7%,115/124), most patient have cough and fever. Of 78 HMPV-positive specimens, A2b (82.1%,64/78) were the main epidemic subtypes. .During the COVID-19 outbreak, Among 232 samples, only 4 cases were HMPV-positive. After statistical test, the detection rate of HMPV during the COVID-19 pandemic has decreased significantly compared with that before the epidemic (p=0.001). Conclusions HMPV is an important cause of ARTIs in children under 5 years old. Under the prevention and control of the COVID-19 pandemic, the HMPV infection of hospitalized children with ARTIs has decreased significantly.

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Running title: Epidemiology and Genotypic Diversity of HMPV

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Conflict of Interest statements

The authors declare that there are no conflicts of interest.

Abstract :

Background This study aims to described the epidemiology and genotypic diversity of Human metapneumovirus (HMPV) and the impact of SARS-CoV-2 on the prevalence of HMPV in hospitalized children with Acute respiratory tract infections (ARTIs) in Beijing, China.

Methods From April 2018 to March 2019 and from September 2020 to August 2021, nasopharyngeal aspirates (NPAs) from hospitalized children with ARTIs in Beijing were collected and subjected to real-time polymerase chain reaction tests for HMPV. Then genotyping, detection of 15 common respiratory viruses and clinical characteristics were analyzed on HMPV positive samples.

Results 7.9% (124/1572) enrolled paediatric patients were identified as having HMPV infection, and the majority of children under the age of 5 (78.2%, 92/124), From April 2018 to March 2019. The detection rate of HMPV in spring and winter is significantly higher than that in summer and autumn. The co-infection rate were 37.1% (46/124), the most common co-infected virus were parainfluenza virus type 3 (HPIV-3). The main diagnosis of HMPV infection was pneumonia (92.7%,115/124), most patient have cough and fever. Of 78 HMPV-positive specimens, A2b (82.1%,64/78) were the main epidemic subtypes. Hospitalized children with HMPV genotype A infection had a higher viral load compared to genotype B. During the COVID-19 outbreak, Among 232 samples, only 4 cases were HMPV-positive. After statistical test, the detection rate of HMPV during the COVID-19 pandemic has decreased significantly compared with that before the epidemic (p =0.001).

Conclusions HMPV is an important cause of ARTIs in children under 5 years old. The epidemic peak is generally in winter and spring, and the A2b subtype is the most common. However, under the prevention and control of the COVID-19 pandemic, the HMPV infection of hospitalized children with ARTIs has decreased significantly.

Keywords Acute respiratory tract infections, Human metapneumovirus , Epidemiology

1 Introduction

Acute respiratory tract infections (ARTIs) remain one of the most common major public health threats[1]. According to the data of the main causes of death in the world released by the World Health Organization, Lower Respiratory Tract Infections (LRTIs) ranks fourth in the world and ranks first in low-income countries[2]. Viral infections are the most frequent cause of ARTIs[3]. Since HMPV was first discovered in 2001 in the Netherland and has been detected in other countries, it has been determined to be one of the main pathogens of ARTIs in children, immunocompromised people and the elderly[4,5]. As a member of the Metapneumovirus genus of the Pneumoviridae family[6], HMPV genome is a negative-sense single-stranded RNA molecule, 13Kb long, composed of eight genes encoding nine proteins: 3'-N, P , M, F, M2-1/M2-2, SH, G, L-5'[7]. Based upon the sequence variability of the attachment (G) and fusion (F) surface glycoproteins, HMPV can be divided into four subtypes (A1, A2, B1 and B2). The A2 subgroup is the most genetically heterogeneous of the four subgroups and some studies have suggested its further sub-division into A2a, A2b1 and A2b2(A2c)

sub-lineages based on sequence data[7-9]. It is well known that these 2 main subgroups are prevalent around the world and that major HMPV subtypes switch every year[10-12]. However, it is questionable whether there is an association between genotype and disease severity.

Since late 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was discovered in Wuhan, China, it has caused a huge economic and health burden worldwide. To prevent the spread of the virus, large-scale non-pharmaceutical interventions have been implemented in China, including working from home, online teaching, mandatory wearing of masks in public places, social distancing, and hand hygiene. Reductions in influenza and respiratory syncytial virus cases under these interventions have been reported in many countries, yet few studies have examined changes in HMPV cases during outbreaks[13-17]. The purpose of this study was to evaluate the epidemiological, clinical, and molecular characteristics of HMPV infections occurring among hospitalized children with ARTIs in China from April 2018–March 2019. In addition, this study explored the impact of the COVID-19 pandemic on HMPV infection.

2 Materials and methods

2.1 specimen collection

From April 2018 to March 2019, 1572 NPAs samples were collected from hospitalized children (aged < 14 years) with ARTIs in Beijing Friendship Hospital, and 233 samples were collected from September 2020 to August 2021. The study protocol was approved by the Ethical Committee of Beijing Friendship Hospital (number: IVDC2018-012). Informed consent has been received from the parent or guardian of the children participating in the study. ARTIs were defined as at least two of the following clinical manifestations during the previous week: fever, cough, nasal obstruction, expectoration, sneeze and dyspnoea. Patients with pneumonia diagnosed by chest radiography were also included, even if they did not exhibit the clinical features described above[18]. Collected samples were stored in virus preservation solution and placed at -80 for further processing.

2.2 Detection of HMPV

Total viral nucleic acids were extracted from 200ul specimen and eluted with 60ul water by using a QIAamp MinElute Kit (Qiagen, Germany) according to the manufacturer's instructions. HMPV detection was performed by using a quantitative real-time reverse transcription PCR (qRT-PCR) assay with a One-step RT-PCR Kit (Ambion, USA). The HMPV qRT-PCR primer set was used as previously described, designed based on the conversed fragment of N gene [19]: HMPV forward primer(5'- TTAARTTACAAAAAAACATGGGAC -3'), reverse primer (5'- AAAGAATATCTTTTCCTTCAGGG -3'), and probe (5'-FAM- AATTACTCATAATCATTTTGACTG -3'-TAMRA). Each 25 μL reaction mixture comprised 12.5 μL of RT-PCR 2×Buffer, 1 μL of each primer (10 pM), 0.3 μL of probe (10pM) , 7.2 μL of Nuclease-Free water, 1μL of 25×RT-PCR Enzyme Mix and 2.0 μL of the nucleic acid components extracted from each sample. The qRT-PCR cycling program was as follows: 45 °C for 10 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, and 60 °C for 45 s. Samples with a cycle threshold (Ct) < 38 were considered positive. Measure of viral load based on a standard curve that has been established in our laboratory[20].

2.3 Detection of viral co-infection in HMPV-positive specimens

The HMPV-positive specimens were subsequently screened for 15 common respiratory viruses.: influenza virus types A, B, and C (IFV A/B/C), parainfluenza virus types 1–4 (HPIV), human coronaviruses HKU1/229E/OC43/NL63 (HCoV), respiratory syncytial virus (RSV), human rhinovirus (HRV), adenovirus (ADV), and human bocavirus (HBoV). All viruses were detected by real-time polymerase chain reaction[21,22], DNA viruses were tested using TaqMan Gene Expression Master Mix (Thermo Fisher, USA), RNA viruses

were tested using AgPath-ID One-Step RT-PCR Kit (Ambion, USA) in accordance the corresponding manufacture's protocols.

2.4 HMPV genotyping

The viral RNA of HMPV positive samples was reverse transcribed into cDNA using a SuperScript IV First-Strand Synthesis System (Invitrogen, USA) in accordance with the manufacture's protocols. The conserved sequence of F gene (610bp) was amplified using a nested PCR for genotyping as previously described[23]. The outer primers used were forward 5'- CAATGCAGGTATAACACCAGCAATATC -3' and reverse 5'- GCAACAATTGAACTGATCTTCAGGAAAC -3', and the internal primers were forward 5'- ACATGCCAA-CATCTGCAGGACAAATAAAAC -3' and reverse 5'- ACATGCTGTTCACCTTCAACTTTGC -3'. Nested PCR was conducted in 25 μL volume comprising 2.5 μL of 1 0 × Ex Taq buffer, 2.0 μL of dNTP Mix, 1.0 μL(10 pM) of each primer, 0.4 μL of Ex Taq DNA polymerase, 2.0 μL of viral nucleic acid extract or first nested-PCR product, and 16.1 μL of double-distilled water. The Nested-PCR products were sequenced at Ruibio Co., Ltd (Beijing, China), and a Maximum Likelihood (ML) tree was constructed by the Tamura-Nei model in MEGA 7.0 with 1000 bootstrap replicates. Samples that failed to be amplified were classified as untyped.

2.5 Statistical analysis

SPSS 26.0 software was used for data analysis, and Categorical data were tested using $\chi 2$ test and Fisher's exact test. Wilcoxon's test and independent-samples t-test were used to analyze continuous variables. Two-sided P-value < 0.05 was considered statistically significant.

3 Results

3.1 HMPV epidemiology

From April 2018 to March 2019, there were 900 males and 672 females among the 1572 samples (sex ratio: 1.34:1), and the age range was from 1 day to 14 years of age with a median age of 3 years. The detection rate of HMPV was 7.9% (124/1572), of which 78 were male and 46 were female, with no significant gender difference (p =0.185). As shown in Table 1, HMPV was detected among hospitalized children (< 14 years) with ARTIs at Beijing Friendship Hospital between April 2018 and March 2019, and 78.2% (97/124) of children under 5 years old. The seasonal distribution of HMPV cases in hospitalized children from April 2018 to March 2019 is shown in Figure. 1, and HMPV was detected throughout the year. 34.7%(43/124)cases of HMPV infection were detected in spring, 13.4%(17/124)in summer, 13.4%(17/124)in autumn and 37.9%(47/124)in winter, and there were significant differences in HMPV detection rates in different seasons ($p = 0.000$). From September 2020 to August 2021, A total of 232 hospitalized children with ARTIs were enrolled in this study, among which 134 (57.8%) were male and 98(42.2%) were female (sex ratio: 1.34:1). The detection rate of HMPV was 1.7% (4/232), which was significantly lower than that of the previous year, and the 3 positive cases were children under 5 years old.

3.2 Detection of viral co-infection in HMPV-positive specimens

A total of 124 HMPV-positive samples were detected during the period from April 2018–March 2019, of which 62.9% (78/124) of HMPV single-infected samples and 37.1% (46/124) of HMPV co-infected samples with other respiratory viruses. Among the 46 co-infected cases, there were 32 males and 14 females (sex ratio:2.29:1), with no statistical significance for gender ($p = 0.238$). As shown in Table 2, the most common mixed infection virus was HPIV3 (32.6%,15/46), and the mixed infection rates with HRV, ADV, FLV-A, and RSV were 19.6% (9/46), 17.4% (8/46), 17.4% (8/46), and 10.9% (5/46), respectively. The viral load

of the 124 HMPV positive cases ranged from 14 copies/mL to 4.6×10^6 copies/mL NPA, with no statistical difference in viral load between HMPV mono-infection and coinfection. From September 2020 to August 2021, among the NPAs samples from 232 hospitalized children with ARTIs, 4 were HMPV-infected, with viral loads ranging from 19 copies/mL to 3.16×10^3 copies/mL NPA. 2 cases were combined with other respiratory virus infections, which were mixed with HBOV and HRV respectively.

3.3 Clinical characteristics of HMPV infections

From April 2018 to March 2019, among the 124 children with HMPV infection, 115 (92.7%) were diagnosed with pneumonia, 8 (6.5%) with bronchitis, and 1 with upper respiratory tract infection (0.8%). The main clinical symptoms of children with HMPV infection were cough (95.2%, 118/124) and fever (91.9%, 114/124), and other symptoms included rhinorrhoea (53.2%, 66/124), nasal obstruction (29.8%, 37/124) 124), sneeze (15.3%, 19/124) and Shiver (8.1%, 10/124). There was no statistical difference in the above clinical symptoms between children with HMPV single infection and mixed infection, as shown in Table 3. The hospitalization time of HMPV-infected children was 2-14 days, and the average hospitalization time was 6.35 days. A total of 90 children were discharged within 7 days (72.6%, 90/124). Statistics on the number of children who were hospitalized for more than 7 days showed no significant difference between HMPV combined with other viral infections and HMPV single infection (p =0.320).

3.4 HMPV genotyping and phylogenetic analysis

Nested PCR was used to amplify the conserved sequence (610bp) of the F gene in 128 HMPV-positive samples, and the target fragment was amplified from 78 (63.0%, 78/124) HMPV-positive samples from April 2018 to March 2019. Phylogenetic analysis showed that 64 strains (82.1%, 64/78) belonged to A2b subtype, 9 strains (11.5%, 9/78) B1 subtype and 5 strains (6.4%, 5/78) B2 subtype, A1 and A2a subtype was not found in Figure2. As shown in Figure3, the viral load of A2b subtype samples was significantly higher than that of B type samples (p =0.009), and there was a statistically significant difference in the hospitalization time >7 days between the two subtypes (p =0.031). From September 2020 to August 2021, among the 4 HMPV-infected children, only 1 case was positive for F gene amplification, and was identified as B1 subtype by phylogenetic analysis.

4 Discussion

Since its discovery in 2001, HMPV has been considered an important cause of ARTIs. Some studies have shown that the prevalence of HMPV is different in different regions and years. The detection rate of HMPV in Chinese hospitalized children with ARTIs is about 2%-18.2%[18,24,25], and the detection rate of HMPV in ARTIs inpatients in other countries is 1.2%-20.3%[12,26,27]. In this study, we mainly explored the molecular epidemiology and clinical characteristics of HMPV infection in hospitalized children with ARTIs in Beijing from April 2018 to March 2019 and September 2020 to August 2021, and the impact of the COVID-19 epidemic on HMPV infection. The detection rate of HMPV from April 2018 to March 2019 was 7.9%, and the detection rate of HMPV from September 2020 to August 2021 was 1.7%. A previous study in our laboratory showed that the detection rate of HMPV in hospitalized children from April 2017 to March 2018 was 4.1%[25], which indicated that the detection rate of HMPV in hospitalized children with ARTIs in Beijing from April 2018 to March 2019 was higher than the previous year (p =0.000), while the detection rate of HMPV decreased significantly during the period of the COVID-19 epidemic. It shows that the detection rate of HMPV in hospitalized children with ARTIs has been significantly reduced under the strict prevention and control of the COVID-19 epidemic in China(p =0.001). Mandy Jongbloed et al. reported that despite a 324% increase in HMPV testing during the COVID-19 outbreak in Europe, there was no increase in HMPV incidence[28]. Since the outbreak of the COVID-19, various countries have adopted different prevention and control strategies. A series of measures such as wearing masks, frequent hand washing and disinfection,

delaying the start of school, and maintaining social distance have cut off the transmission of respiratory viruses and reduced HMPV infection. During the COVID-19 outbreak, the number of hospitalized children with ARTIs decreased significantly, and the number of samples collected from September 2020 to August 2021 decreased accordingly. Due to the small number of HMPV-positive samples, the virus mixed infection and clinical characteristics have not been counted yet.

From April 2018 to March 2019, 78.2% of HMPV infections occurred in hospitalized children aged [?]5 years, and 9.1% (16/176) occurred in children aged 3-4 years; There was no significant difference in HMPV detection rate between male and female children(p =0.185), which was consistent with the studies in China and Argentina[25,27] . HMPV infection had typical seasonal distribution characteristics, with the detection rate in winter and spring significantly higher than that in summer and autumn (P =0.000), and the prevalence period of HMPV was concentrated from December to May of the following year, which was consistent with the results of a study in Guangzhou, China[23]. Research shows that HMPV epidemics are typical in early spring, while small peaks in summer appear to be related to local weather conditions[22,27]. In ARTIs cases, HMPV is often co-infected with other respiratory viruses, such as ADV, RSV, HRV and HPIV[25,29,30]. In this study, from April 2018 to March 2019, the proportion of HMPV-positive samples mixed with other viruses was 37.1%, of which HPIV3 had the highest mixed infection rate of 32.6%, which was similar to the findings of Fathima S[27], which may related to the prevalence of HPIV3 in that year. Some studies have concluded that co-infection of HMPV with other respiratory viruses can aggravate clinical symptoms and prolong hospitalization[18,32]. However, in this study, there were no statistically significant differences in viral load, length of hospital stay, cough, fever, nasal obstruction, rhinorrhoea and other clinical symptoms between children with HMPV single infection and mixed infection, which is consistent with the results of previous research in our laboratory[25]. In addition, the relationship between HMPV viral load and clinical symptoms remains unclear[25,33,34], and no association between clinical symptoms and HMPV viral load was found in the study.

There are five gene subtypes in HMPV, so it is of great significance to explore the relationship between genetic diversity of HMPV and clinical characteristics. In this study, for HMPV samples from April 2018 to March 2019, 78 NPAs samples were successfully amplified by nested PCR amplification of the conserved fragment of the F gene. The phylogenetic analysis showed that 82.1% (64/78) of the prevalent strains belonged to A2b subtype, 11.5% belonged to B1 subtype and 6.4% belonged to B2 subtype, and no A1 and A2a subtypes were found. We found that the viral load of HMPV A2b genotype samples was significantly higher than that of B samples (p =0.009). In addition, there was a statistically significant difference between A2b subtype and B subtype in the number of children with hospitalization time $>$ 7 days, and the hospitalization time of A2b subtype was longer than that of B1 subtype (p =0.030), which suggests that we should pay more attention to A2b subtype in pediatric clinic. From September 2020 to August 2021, only one HMPV case was identified by phylogenetic analysis as subtype B1 infection. From April 2017 to March 2018, the detection rate of B1 subtype (54.5%) were highest, followed by the A2b subtype (40.9%) in the previous study of our laboratory, and no HMPV A1 and A2a subtypes were found[25]. However, in this study, the A2b subtype was the predominant (82.1%), indicating that the main circulating strain of HMPV infection in Beijing in 2017-2019 switched from B1 subtype to A2b subtype. The study by Matsuzaki Y showed that laryngitis is more common in children with HMPV subtype B1 infection, while wheezing is more common in children infected with HMPV subtypes B1 and B2 than in children with subtype A2 infection[35]. Many data suggest that HMPV genotypes are associated with disease severity or clinical manifestations [36,37], but some studies have inconsistent conclusions [24,38], so it is still controversial whether HMPV genotypes are associated with clinical features.

This study was limited by the large decrease in hospitalized children with ARTIs during the COVID-19 outbreak. From September 2020 to August 2021, there were only 4 HMPV positive children of 232 hospitalized children with ARTIs, therefore a detailed epidemiological analysis of HMPV cases during this period was not possible. We will continue to collect respiratory samples from hospitalized children with ARTIs in the subsequent studies, and make a detailed analysis of the impact of SARS-CoV-2 on the prevalence of HMPV.

Data availability

Condensed anonymized data are available from the corresponding author on reasonable request.

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Conflict of Interest statements

The authors declare that there are no conflicts of interest.

Ethical approval

The project was approved by the Ethical Committee of National Institute for Viral Disease Control and Prevention, China CDC, and the committee's reference number is IVDC2018-012. All individuals (or their parents) in the study population were informed about the current study and a written informed consent was obtained from each subject.

Reference

1. Shi T, McLean K, Campbell H, Nair H. Aetiological role of common respiratory viruses in acute lower respiratory infections in children under five years: A systematic review and meta-analysis. *Journal of global health* . 2015;5(1):010408. doi:10.7189/jogh.05.010408

2. WHO. The top 10 causes of death. https://www.who.int/en/news-room/fact-sheets/detail/thetop-10-causes-of-death, accessed May 24, 2018.

3. Williams BG, Gouws E, Boschi-Pinto C, Bryce J, Dye C. Estimates of world-wide distribution of child deaths from acute respiratory infections. *The Lancet Infectious diseases* . 2002;2(1):25-32. doi:10.1016/s1473-3099(01)00170-0

4. Jagusic M, Slovic A, Ivancic-Jelecki J, et al. Molecular epidemiology of human respiratory syncytial virus and human metapneumovirus in hospitalized children with acute respiratory infections in Croatia, 2014-2017. *Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases* . 2019;76:104039. doi:10.1016/j.meegid.2019.104039

5. Shafagati N, Williams J. Human metapneumovirus - what we know now.*F1000Research* . 2018;7:135. doi:10.12688/f1000research.12625.1

6. Amarasinghe GK, Bao Y, Basler CF, et al. Taxonomy of the order Mononegavirales: update 2017. *Archives of virology* . 2017;162(8):2493-504. doi:10.1007/s00705-017-3311-7

7. Li J, Ren L, Guo L, et al. Evolutionary dynamics analysis of human metapneumovirus subtype A2: genetic evidence for its dominant epidemic.*PloS one* . 2012;7(3):e34544. doi:10.1371/journal.pone.0034544

8. Chow WZ, Chan YF, Oong XY, et al. Genetic diversity, seasonality and transmission network of human metapneumovirus: identification of a unique sub-lineage of the fusion and attachment genes. *Scientific reports* . 2016;6:27730. doi:10.1038/srep27730

9. Nao N, Saikusa M, Sato K, et al. Recent Molecular Evolution of Human Metapneumovirus (HMPV): Subdivision of HMPV A2b Strains.*Microorganisms* . 2020;8(9):1280. doi:10.3390/microorganisms8091280

10. Mackay IM, Bialasiewicz S, Jacob KC, et al. Genetic diversity of human metapneumovirus over 4 consecutive years in Australia. *The Journal of infectious diseases* . 2006;193(12):1630-3. doi:10.1086/504260

11. Chung JY, Han TH, Kim SW, Hwang ES. Genotype variability of human metapneumovirus, South Korea. *Journal of medical virology* . 2008;80(5):902-5. doi:10.1002/jmv.21129

12. Lim YK, Kweon OJ, Kim HR, Kim TH, Lee MK. Clinical Features, Epidemiology, and Climatic Impact of Genotype-specific Human Metapneumovirus Infections: Long-term Surveillance of Hospitalized Patients in South Korea. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* . 2020;70(12):2683-94. doi:10.1093/cid/ciz697

13. Boelle PY, Souty C, Launay T, et al. Excess cases of influenza-like illnesses synchronous with coronavirus disease (COVID-19) epidemic, France, March 2020. *Euro surveillance : bulletin Europeen sur les maladies transmissibles* . 2020;25(14):2000326. doi:10.2807/1560-7917.Es.2020.25.14.2000326

14. Sakamoto H, Ishikane M, Ueda P. Seasonal Influenza Activity During the SARS-CoV-2 Outbreak in Japan. *Jama* . 2020;323(19):1969-71. doi:10.1001/jama.2020.6173

15. Cheng W, Yu Z, Liu S, et al. Successful interruption of seasonal influenza transmission under the COVID-19 rapid response in Zhejiang Province, China. *Public health* . 2020;189:123-125. doi:10.1016/j.puhe.2020.10.011

16. Agha R, Avner JR. Delayed Seasonal RSV Surge Observed During the COVID-19 Pandemic. *Pediatrics* . 2021;148(3):e2021052089. doi:10.1542/peds.2021-052089

17. Varela FH, Scotta MC, Polese-Bonatto M, et al. Absence of detection of RSV and influenza during the COVID-19 pandemic in a Brazilian cohort: Likely role of lower transmission in the community. *Journal of global health* . 2021;11:05007. doi:10.7189/jogh.11.05007

18. Zhang L, Liu W, Liu D, et al. Epidemiological and clinical features of human metapneumovirus in hospitalised paediatric patients with acute respiratory illness: a cross-sectional study in Southern China, from 2013 to 2016. *BMJ open* . 2018;8(2):e019308. doi:10.1136/bmjopen-2017-019308

19. Maertzdorf J, Wang CK, Brown JB, et al. Real-time reverse transcriptase PCR assay for detection of human metapneumoviruses from all known genetic lineages. *Journal of clinical microbiology* . 2004;42(3):981-6. doi:10.1128/jcm.42.3.981-6.2004

20. Xue P, Zheng W, Zhang M, Zheng L. Development of real-time RT-PCR for human metapneumovirus. *Acta virologica* . 2011;55(3):285-6. doi:10.4149/av_2011_03_285

21. Ma FL, Li DD, Wei TL, Li JS, Zheng LS. Quantitative detection of human Malawi polyomavirus in nasopharyngeal aspirates, sera, and feces in Beijing, China, using real-time TaqMan-based PCR. *Virology journal* . 2017;14(1):152. doi:10.1186/s12985-017-0817-2

22. McLeish NJ, Witteveldt J, Clasper L, et al. Development and assay of RNA transcripts of enterovirus species A to D, rhinovirus species a to C, and human parechovirus: assessment of assay sensitivity and specificity of real-time screening and typing methods. *Journal of clinical microbiology* . 2012;50(9):2910-7. doi:10.1128/jcm.01172-12

23. Yi L, Zou L, Peng J, et al. Epidemiology, evolution and transmission of human metapneumovirus in Guangzhou China, 2013-2017. *Scientific reports* . 2019;9(1):14022. doi:10.1038/s41598-019-50340-8

24. Zhou JY, Peng Y, Peng XY, et al. Human bocavirus and human metapneumovirus in hospitalized children with lower respiratory tract illness in Changsha, China. *Influenza and other respiratory viruses* . 2018;12(2):279-286. doi:10.1111/irv.12535

25. Wang C, Wei T, Ma F, et al. Epidemiology and genotypic diversity of human metapneumovirus in paediatric patients with acute respiratory infection in Beijing, China. *Virology journal* . 2021;18(1):40. doi:10.1186/s12985-021-01508-0

26. Oketch JW, Kamau E, Otieno GP, Otieno JR, Agoti CN, Nokes DJ. Human metapneumovirus prevalence and patterns of subgroup persistence identified through surveillance of pediatric pneumonia hospital admissions in coastal Kenya, 2007-2016. *BMC infectious diseases* . 2019;19(1):757. doi:10.1186/s12879-019-4381-9

27. Rodriguez PE, Frutos MC, Adamo MP, et al. Human Metapneumovirus: Epidemiology and genotype diversity in children and adult patients with respiratory infection in Córdoba, Argentina. *PloS one* . 2020;15(12):e0244093. doi:10.1371/journal.pone.0244093

28. Jongbloed M, Leijte WT, Linssen CFM, van den Hoogen BG, van Gorp ECM, de Kruif MD. Clinical impact of human metapneumovirus infections before and during the COVID-19 pandemic. *Infectious diseases (London, England)* . 2021;53(7):488-97. doi:10.1080/23744235.2021.1887510

29. Pilger DA, Cantarelli VV, Amantea SL, Leistner-Segal S. Detection of human bocavirus and human metapneumovirus by real-time PCR from patients with respiratory symptoms in Southern Brazil. *Memorias do Instituto Oswaldo Cruz* . 2011;106(1):56-60. doi:10.1590/s0074-02762011000100009

30. Greensill J, McNamara PS, Dove W, Flanagan B, Smyth RL, Hart CA. Human metapneumovirus in severe respiratory syncytial virus bronchiolitis. *Emerging infectious diseases* . 2003;9(3):372-5. doi:10.3201/eid0903.020289

31. Fathima S, Lee BE, May-Hadford J, Mukhi S, Drews SJ. Use of an innovative web-based laboratory surveillance platform to analyze mixed infections between human metapneumovirus (hMPV) and other respiratory viruses circulating in Alberta (AB), Canada (2009-2012). *Viruses* . 2012;4(11):2754-65. doi:10.3390/v4112754

32. Xiao NG, Xie ZP, Zhang B, et al. Prevalence and clinical and molecular characterization of human metapneumovirus in children with acute respiratory infection in China. *The Pediatric infectious disease journal* . 2010;29(2):131-4. doi:10.1097/inf.0b013e3181b56009

33. Peng D, Zhao X, Liu E, et al. Analysis of viral load in children infected with human metapneumovirus. *Iranian journal of pediatrics* . 2010;20(4):393-400.

34. Bosis S, Esposito S, Osterhaus AD, et al. Association between high nasopharyngeal viral load and disease severity in children with human metapneumovirus infection. *journal of clinical virology : the official publication of the Pan American Society for Clinical Virology* . 2008;42(3):286-90. doi:10.1016/j.jcv.2008.03.029

35. Matsuzaki Y, Itagaki T, Abiko C, Aoki Y, Suto A, Mizuta K. Clinical impact of human metapneumovirus genotypes and genotype-specific seroprevalence in Yamagata, Japan. *Journal of medical virology* . 2008;80(6):1084-9. doi:10.1002/jmv.21194

36. Papenburg J, Carbonneau J, Isabel S, et al. Genetic diversity and molecular evolution of the major human metapneumovirus surface glycoproteins over a decade. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology* . 2013;58(3):541-7. doi:10.1016/j.jcv.2013.08.029

37. Vicente D, Montes M, Cilla G, Perez-Yarza EG, Perez-Trallero E. Differences in clinical severity between genotype A and genotype B human metapneumovirus infection in children. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* . 2006;42(12):e111-3. doi:10.1086/504378

38. Wei HY, Tsao KC, Huang CG, Huang YC, Lin TY. Clinical features of different genotypes/genogroups of human metapneumovirus in hospitalized children. *Journal of microbiology, immunology, and infection* . 2013;46(5):352-7. doi:10.1016/j.jmii.2012.07.007

TABLE1 HMPV infections in children of different ages and gender with ARTIs

Variable	Number of patients	Number of HMPV positive children	Prevalence of HMPV (%)
Gender			
Male	900	78	8.7
Female	672	46	6.8
Age(years)			
[?]1	420	29	6.9

Variable	Number of patients	Number of HMPV positive children	Prevalence of HMPV (%)
>1to[?]2	204	18	8.8
>2to[?]3	328	29	8.8
>3to[?]4	176	16	9.1
>4to[?]5	91	5	5.5
>5to[?]7	145	11	7.6
>7to[?]14	208	16	7.7
Total	1572	124	7.9

TABLE2 Co-detection of HMPV and other respiratory viruses

Groups	Virus composition	No. (%)
2018.4-2019.3 2 Virus (n=37)	HMPV+HPIV3	13(28.3)
	HMPV+RV	6(13.0)
	HMPV+IFV-A	6(13.0)
	HMPV+RSV	4(8.7)
	HMPV+ADV	4(8.7)
	HMPV+ IFV-B	1(2.2)
	HMPV+ IFV-C	1(2.2)
	HMPV+HCoV-OC43	1(2.2)
	HMPV+HPIV4	1(2.2)
3 Virus (n=7)	HMPV+ADV+HBOV	1(2.2)
	HMPV+ADV+IFV-C	1(2.2)
	HMPV+HBOV+RV	1(2.2)
	HMPV+HBOV+RSV	1(2.2)
	HMPV+RV+HPIV4	1(2.2)
	HMPV+IFV-A+IFV-B	1(2.2)
	HMPV+ HCoV-OC43+HPIV3	1(2.2)
4 Virus (n=2)	HMPV+ADV+RV+HPIV3	1(2.2)
	HMPV+ADV+HBOV+IFVA	1(2.2)
2020.9-2021.8 2 Virus(n=2)	HMPV+RV	1(50)
	HMPV+HBOV	1(50)

TABLE3 Clinical features among HMPV-positive hospitalized children in 2018-2019

Clinical characteristics	Single infection NO. (%) (n = 78)	Co-infection NO. (%) (n = 46)	Total NO. (%) (n = 124)	p
Fever ([?] 38)	71(91)	43(95.7)	114(91.9)	0.886[b]
Convulsion	2(2.6)	1(2.2)	3(2.4)	1.000[b]
Shiver	6(7.7)	4(8.7)	10(8.1)	1.000[b]
Cough	74(94.9)	44(95.7)	118(95.7)	1.000[b]
Nasal obstruction	21(26.9)	16(34.8)	37(29.8)	0.355[a]
Rhinorrhea	44(56.4)	22(47.8)	66(53.2)	0.355[a]
Sneeze	12(15.4)	7(15.2)	19(15.3)	0.980[a]
Vomit	4(5.1)	4(8.7)	8(6.5)	0.687[b]
Diarrhea	4(5.1)	0	4(3.2)	0.296[c]
Hospitalization>7d	19(24.4)	15(32.6)	34(27.4)	0.320[a]

Note: a χ2-test; b Continuity correction; c Fisher's exact test.

Figure legends

FIGURE 1 Seasonal distribution of HMPV in children with ARTIs from April 2018 to March 2019

FIGURE 2 Phylogenetic relationships of the strains detected in HMPV-infected children.

The reference strain is marked with a *'andthestrainsanalysedinthisstudyaremarkedwitha'*

FIGURE 3 HMPV viral loads in NPA from children with ARTIs with different genotype from April 2018 to March 2019