

Human Metapneumovirus Associated With Community-Acquired Pneumonia in Children in Beijing, China

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Community-acquired pneumonia is a major cause of morbidity and mortality in children worldwide. However, few studies have been conducted on the infection of human metapneumovirus (hMPV) associated with pediatric community-acquired pneumonia in China. Nasopharyngeal aspirates were collected between July 2008 and June 2010 from 1,028 children, aged ≤ 16.5 years, who were diagnosed with community-acquired pneumonia in Beijing, China. Reverse-transcriptase polymerase chain reaction was used to screen the samples for hMPV and common respiratory viruses. hMPV was detected in 6.3% of the patients with community-acquired pneumonia. This detection rate is the third highest for a respiratory virus in children with community-acquired pneumonia, after that of rhinovirus (30.9%) and respiratory syncytial virus (30.7%). The detection rate of hMPV in 2008/2009 (42/540, 7.8%) was significantly higher than in 2009/2010 (23/488, 4.7%; $\chi^2 = 4.065$, $P = 0.044$). The hMPV subtypes A2, B1, and B2 were found to co-circulate, with A2 being most prevalent. These results indicate that hMPV plays a substantial role in pediatric community-acquired pneumonia in China. Overall, these findings provide a better understanding of the epidemiological and clinical features of hMPV infections. **J. Med. Virol.** 85:138–143, 2013. © 2012 Wiley Periodicals, Inc.

KEY WORDS: human metapneumovirus; respiratory syncytial virus; community-acquired pneumonia; children; etiology; epidemiology

INTRODUCTION

Community-acquired pneumonia is the major cause of morbidity and mortality in children, especially in developing countries. The disease accounts for approximately two million deaths per year and 20% of all deaths in children [McIntosh, 2002; Sinaniotis and Sinaniotis, 2005]. Due to its significance in public health and social economy development [Murphy et al., 1981; Polverino and Torres Marti, 2011], effective measures are needed to control this disease.

Community-acquired pneumonia can be caused by a variety of microorganisms, including bacteria, mycoplasma, and viruses. With the implementation of bacterial vaccines, such as the *pneumococcal* conjugate vaccine and the *haemophilus influenzae* type b vaccine, to the immunization programs and the use of antibiotics, bacteria-related community-acquired pneumonia has been effectively controlled. However, concern over respiratory viruses, particularly newly identified ones,

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has been rising due to their association with community-acquired pneumonia [Ruuskanen et al., 2011].

Human metapneumovirus (hMPV) is a non-segmented, negative-sense RNA virus and a member of the *Pneumovirinae* subfamily of *Paramyxoviridae* family. It was first isolated from children with acute respiratory tract infections in the Netherlands in 2001 [Van den Hoogen et al., 2001]. The genomic organization of hMPV is similar to that of human respiratory syncytial virus (RSV) [Van den Hoogen et al., 2002], harboring one open reading frame (ORF) for at least eight viral proteins (3'-N-P-M-F-M2-SH-G-L-5') but lacking the non-structural genes, NS1 and NS2. hMPV is divided into four subtypes: A1, A2, B1, and B2 based on the genetic analysis of sequences of hMPV isolates [Bastien et al., 2003; Van den Hoogen et al., 2004; Huck et al., 2006].

Since its discovery, hMPV has been recognized as a major cause of lower respiratory tract infections in children worldwide [Stockton et al., 2002; Boivin et al., 2003; Maggi et al., 2003; Peiris et al., 2003; Esper et al., 2004; Mullins et al., 2004; Williams et al., 2004; García-García et al., 2006; Caracciolo et al., 2008; Kim et al., 2010]. It accounts for 4.1–11.5% of pediatric community-acquired pneumonia cases, depending on geographical location, year, and subjects studied [Lin et al., 2005; Cilla et al., 2008; Mathisen et al., 2010; Wolf et al., 2010; Nascimento-Carvalho et al., 2011]. Although pneumonia is the leading cause of death in children less than 5 years old in China [Rudan et al., 2010], the etiology of pneumonia is not fully understood. Several studies have reported the overall prevalence of hMPV infection in children in China [Ji et al., 2009; Li et al., 2009; Chen et al., 2010; Xiao et al., 2010], but few have investigated the role of hMPV in pediatric community-acquired pneumonia in the country.

In this study, the demographics, clinical manifestations, and epidemiological features of hMPV infection in pediatric patients with community-acquired pneumonia in Beijing were evaluated by RT-PCR of hMPV RNA in nasopharyngeal aspirates and analysis of clinical data, using RSV, a well-known agent of pediatric community-acquired pneumonia, as a control. hMPV subtypes associated with community-acquired pneumonia were also assessed.

MATERIALS AND METHODS

Subjects and Clinical Specimens

Nasopharyngeal aspirates (NPAs) were obtained from 1,028 pediatric patients who had a primary diagnosis of community-acquired pneumonia upon admission to the Beijing Children's Hospital from July 2008 through June 2010. The majority of the patients (74.2%) were under 3 years old, although age ranged from 0.3 month to 16.5 years (mean age: 32.02 months, median age: 10 months). The male to female ratio was 1.8. The diagnosis of community-acquired pneumonia followed a guideline issued by the Chinese

Medical Association. In brief, community-acquired pneumonia was clinically defined as the presence of signs and symptoms of pneumonia caused by an infection acquired outside the hospital [The Working Group for Respiratory Diseases of Pediatric Society, 2007; Xiang et al., 2010]. All specimens were stored at -80°C until use.

Demographic, Clinical, and Laboratory Data

Detailed demographic, clinical presentations, and laboratory data were collected from clinical records. Variables studied included age, sex, history of congenital heart disease or pneumonia, presence of clinical signs and symptoms, maximum temperature, respiratory rate, oxygen saturation, white blood cell counting, and C-reactive protein concentrations. The study was performed in compliance with the Human Experimentation Guidelines of the Chinese Ministry of Health.

RT-PCR for hMPV Detection

Viral RNA was extracted from nasopharyngeal aspirates using NucliSENS easyMAG platform (bioMérieux, Marcy l'Etoile, France), following the manufacturer's protocol. To detect and identify the four subtypes of hMPV, specific RT-PCR assays targeting the P gene of hMPV were performed as described previously [Li et al., 2012]. The RT-PCR analysis was carried out using SuperScript III One-step RT-PCR Platinum Taq kit (Invitrogen, Carlsbad, CA). PCR products were analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide. The 240 bp amplicons were cloned into a pMD-18T vector (Takara, Dalian, China) and verified by sequence analysis. hMPV types/sub-types were determined by alignment of the PCR sequences with prototype strains of hMPV, including the isolates of subtype A1 (strain 00-1, GenBank Accession No. AF371337), A2 (strain CAN97-83, GenBank Accession No. AY297749), B1 (strain NL/1/99, GenBank Accession No. AY525843), and B2 (strain CAN98-75, GenBank Accession No. AY297748) by BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and Clustal W which is implemented in the DNASTAR software [Burland, 2000]. An hMPV strain was assigned to a certain subtype when the PCR sequence showed the highest identity to the prototype strain of a specific hMPV subtype based on the results of BLAST and Clustal W.

Detection of Multiple Respiratory Viruses

In addition to hMPV, respiratory viruses including RSV, human influenza virus, human parainfluenza virus, adenovirus, rhinovirus, human coronaviruses (OC43, 229E, NL63, and HKU1), enterovirus, and bocavirus were simultaneously detected using multiplex RT-PCR or single RT-PCR as described previously [Ren et al., 2009; Wang et al., 2011].

TABLE I. Frequency of hMPV Detection in Different Age Groups of Community-Acquired Pneumonia Patients

Age (months)	No. of patients (n = 1,028)	hMPV-A1	hMPV-A2	hMPV-B1	hMPV-B2	hMPV total
≤6	428	0	18 (4.2) ^a	4 (0.9)	8 (1.9)	30 (7.0)
6.1–12	155	0	10 (6.5)	2 (1.3)	3 (1.9)	15 (9.7)
12.1–36	180	0	8 (4.4)	2 (1.1)	5 (2.8)	15 (8.3)
>36.1	265	0	4 (1.5)	0	1 (0.4)	5 (1.9) ^b
Total	1,028	0	40 (3.9)	8 (0.8)	17 (1.7)	65 (6.3)

^aNumbers in parentheses indicate the percentages of the total number of patients who were positive for the indicated hMPV subtype.

^b $\chi^2 = 11.863, P = 0.001$.

Statistical Analysis

The association of demographics and clinical symptoms was compared between different patient groups using χ^2 test or ANOVA, as appropriate. Statistical analysis was performed using SPSS version 16.0 (SPSS Inc., Chicago, IL). P value <0.05 was considered significant.

RESULTS

Prevalence of hMPV Infection in Pediatric Patients With Community-Acquired Pneumonia

RT-PCR analysis shows that hMPV RNA was detected in 65 (6.3%) pediatric patients with community-acquired pneumonia (40 males and 25 females). The detection rate of hMPV was only lower than that of rhinovirus (30.9%) and respiratory syncytial virus (RSV; 30.7%), but higher than that of other respiratory viruses, including influenza virus (5.7%), adenovirus (5.5%), parainfluenza viruses (5.4%), human coronavirus (5.0%), human bocavirus (3.4%), and enterovirus (2.4%). These data indicate that hMPV plays an important role in pediatric community-acquired pneumonia in Beijing.

hMPV-positive patients ranged in age from 1 month to 6 years (mean age: 16.78 months; median age: 8 months). hMPV was detected at a significantly higher rate in patients aged ≤36 months (7.9%) than in those

aged ≥36.1 months (1.9%; $\chi^2 = 11.863, P = 0.001$). The patients aged ≤36 months represented 74.2% of the subjects studied, but accounted for 92.3% of the hMPV-positive patients (Table I).

Co-infections with other respiratory viruses were detected in 46 of 65 hMPV-positive patients (70.8%). These patients were mainly co-infected with RSV (n = 27, 41.5%) or rhinovirus (n = 25, 38.5%). Other respiratory viruses, including influenza virus, parainfluenza virus, adenovirus, human coronavirus, human bocavirus, and enterovirus were also co-detected (Table II).

Fluctuation of Annual Incidence

The detection rate of hMPV fluctuated between the 2008/2009 and 2009/2010 years (Fig. 1). The detection rate of hMPV in 2008/2009 (42/540, 7.8%) was significantly higher than that of 2009/2010 (23/488, 4.7%; $\chi^2 = 4.065, P = 0.044$). In contrast, the detection rate of RSV did not fluctuate significantly, but remained constant at 32.4% (175/540) and 28.9% (141/488) in 2008/2009 and 2009/2010, respectively ($\chi^2 = 1.487, P = 0.223$).

Co-Circulation of hMPV Subtypes

Nucleotide analysis of the partial P gene showed that 40 (61.5%) patients were positive for hMPV-A2,

TABLE II. Co-Circulation of hMPV and Other Respiratory Viruses in Patients With Community-Acquired Pneumonia

	hMPV-A2 (n = 40)	hMPV-B1 (n = 8)	hMPV-B2 (n = 17)	Total (n = 65)
None	11	1	7	19
Respiratory syncytial virus	18 ^{a,b,c,d,e}	4 ^h	5 ^{i,j}	27
Rhinovirus	13 ^{f,g}	4	8 ^k	25
Influenza virus	2	0	0	2
Parainfluenza virus	6	0	2	8
Human bocavirus	4	0	1	5
Human coronavirus	2	0	0	2
Adenovirus	0	0	1	1
Enterovirus	1	0	0	1

^aIn one patient, rhinovirus and human coronavirus were co-detected.

^bIn two patients, parainfluenza virus was co-detected.

^cIn two patients, parainfluenza virus and human bocavirus were co-detected.

^dIn five patients, rhinovirus was co-detected.

^eIn one patient, enterovirus and parainfluenza virus were also co-detected.

^fIn one patient, human bocavirus virus was also co-detected.

^gIn one patient, human coronavirus was also co-detected.

^hIn one patient, rhinovirus was co-detected.

ⁱIn three patients, rhinovirus was co-detected.

^jIn one patient, parainfluenza virus and rhinovirus were co-detected.

^kIn one patient, adenovirus and human bocavirus were co-detected.

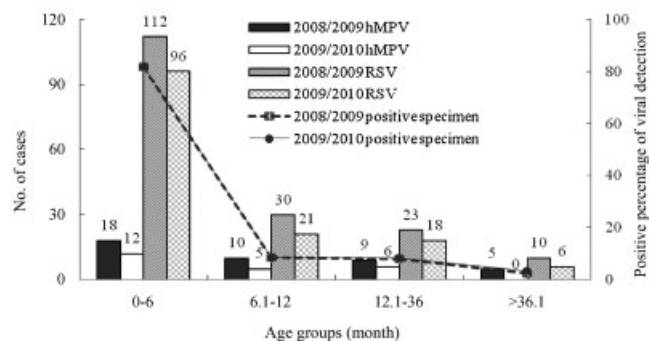


Fig. 1. Annual detection rates of hMPV and RSV in different age groups of pediatric patients with community-acquired pneumonia. Left Y-axis, number of cases positive for hMPV or RSV; right Y-axis, percentage of cases positive for viral detection. The annual detection rates of hMPV and RSV and the annual percentage of viral detection in pediatric community-acquired pneumonia cases are shown.

17 (26.2%) for hMPV-B2, and 8 (12.3%) for hMPV-B1 (Table I). No hMPV-A1 was detected. Although hMPV-A2 was the dominant hMPV subtype during the study period, co-circulation with hMPV-B1 and hMPV-B2 was observed. In the study year 2008/2009, hMPV-A2, -B1, and -B2 were co-detected in October, November, December 2008, and March 2009; whereas hMPV-A2 and -B2 were co-detected in February 2009, forming an epidemic peak between late autumn of 2008 and early spring of 2009. In the study year 2009/2010, the co-circulation of hMPV-A2, -B1, and -B2 was observed in January 2010, whereas co-circulation of hMPV-A2 and -B2 was observed between March and June, forming a small epidemic peak in the spring and early summer of 2010 (Fig. 2). No hMPV-positive cases were detected in August and September 2008 or between April and December 2009.

Clinical Manifestations of hMPV Infection

To characterize the clinical features of community-acquired pneumonia caused by hMPV, the clinical

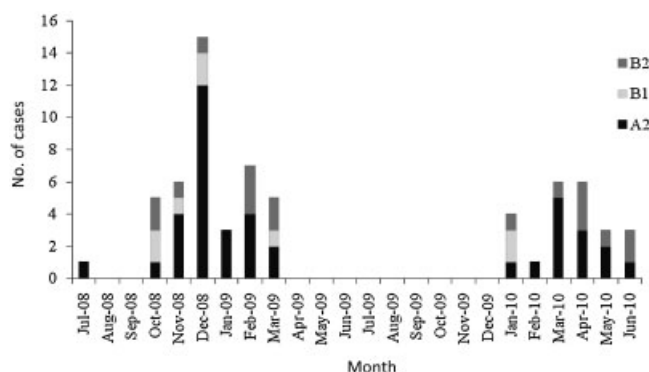


Fig. 2. hMPV subtypes detected in each month during the study period. The subtypes of hMPV (A2, B1, and B2) are represented by different shades, as indicated in the figure key.

manifestations of hMPV-positive and RSV-positive cases were compared. To exclude the interference of co-infections, only the cases with single hMPV or RSV infections were evaluated.

Similar to RSV infection, fever, cough, expectoration, rhinorrhea, wheezing, and swelling of tonsils were the major symptoms observed in hMPV-positive patients with community-acquired pneumonia (Table III). There was no significant difference in major clinical manifestations, gender ratio, the history of pneumonia or congenital heart disease, and laboratory data (e.g., white blood cell counting, hemoglobin, and oxygen saturation) between hMPV-positive and RSV-positive patients. However, the proportion of single hMPV infections was significantly lower (42.1% vs. 68.5%, $\chi^2 = 5.176, P = 0.023$) than that of single RSV infections in children under 6 months old (n = 428).

DISCUSSION

In this study, hMPV subtypes as well as hMPV clinical and epidemiological characteristics associated with pediatric community-acquired pneumonia in Beijing were analyzed. The results show that hMPV plays an important role in pediatric community-acquired pneumonia in Beijing, China, as hMPV infections accounted for 6.3% of the cases from July 2008 through June 2010. hMPV was detected at a lower rate in Beijing than in a study population less than 5 years old in Israel (8.3%) [Wolf et al., 2010] and in a study population less than 3 years old in Spain (11.5%) [Cilla et al., 2008]. However, the hMPV detection rate in Beijing was higher than that reported in Brazil (4.1%) for children younger than 5 years old [Nascimento-Carvalho et al., 2011]. It was also higher than that in Nepal for children less than 3 years old (4.2%) [Mathisen et al., 2010], and that in Taiwan, China for children less than 5 years old (5.2%) [Lin et al., 2005]. These variable rates of hMPV detection may be attributed to variations in climate, location, and the populations studied.

Similar to previous studies [Lin et al., 2005; Cilla et al., 2008; Wolf et al., 2010], it was found that patients with community-acquired pneumonia positive for hMPV were co-infected with other respiratory viruses; 40% of patients were co-infected with RSV and 21.3% with rhinovirus. A higher rate of co-infection (70.8%) than previously reported was observed in hMPV-positive patients in this study. For instance, the rate of hMPV/RSV co-infection was 22.2% in Israel [Wolf et al., 2010] and 28.2% in Spain [Cilla et al., 2008]. The reason for this difference is unclear, but could be attributed to different detection methods. A direct immunofluorescence assay (DFA) and tissue culture methods were used in the study in Israel [Wolf et al., 2010]; whereas a nested-PCR or PCR method different from the RT-PCR method employed here was used in the study in Spain [Cilla et al., 2008].

TABLE III. Comparison of Demographics, Medical History, Clinical Presentation, and Laboratory Findings in Children With hMPV and RSV Infection

	hMPV single infection (n = 19) ^a	RSV single infection (n = 149)
Age (months)		
≤6 ^b	8 (42.1)	102 (68.5)
>6.1	11 (57.9)	47 (31.5)
Gender (male)	10 (52.6)	100 (67.1)
History of pneumonia	2 (10.5)	6 (4)
History of congenital heart disease	3 (15.8)	25 (16.8)
Clinical presentations		
Temperature ≥ 38.5°C	10 (52.6)	35 (23.5)
Cough	19 (100)	148 (99.3)
Expectoration	18 (94.7)	145 (97.3)
Rhinorrhea	8 (42.1)	71 (47.7)
Wheezing	6 (31.6)	78 (52.3)
Swelling of tonsils	6 (31.6)	46 (30.9)
Sneezing	4 (21.1)	65 (43.6)
Chill	1 (5.3)	16 (10.7)
Diarrhea	0	11 (7.4)
Rash	0	4 (2.7)
Laboratory findings		
White blood cell (WBC; ×10 ⁹ /L) ^c	9.7 ± 4.8	11.7 ± 7.0 (n = 147)
Pulse rate (times/min) ^c	142.8 ± 27.7	155 ± 25.4 (n = 146)
Hemoglobin (Hb; g/L) ^c	113.8 ± 14.2	111.4 ± 18.4 (n = 145)
Platelet (×10 ⁹ /L) ^c		
Oxygen saturation (SpO ₂ ; <93%)	8 (57.1, n = 14)	67 (47.9, n = 140)
C-reactive protein (>8 mg/L)	3 (21.4, n = 14)	27 (19.6, n = 138)

^aNumbers in parentheses indicate percentage.

^b $\chi^2 = 5.176$, $P = 0.023$.

^cData are expressed as mean ± standard deviation.

In this study, detection of bacteria in respiratory samples was not carried out, because such detection cannot be used as evidence for infections in respiratory samples [Bogaert et al., 2004]. Although there might have been a few community-acquired pneumonia cases in this study co-infected with bacterial pathogens, these exceptions should not affect the major findings described here.

Based on sequence analysis of the partial P gene of hMPV, three hMPV subtypes (A2, B1, and B2) were detected during the study period. Consistent with a previous study [Cilla et al., 2008], hMPV-A2 was the most frequently detected subtype in pediatric patients with community-acquired pneumonia. hMPV-A1 was not detected in this study. hMPV-A1 was also not found in patients with other lower respiratory tract infections (data not shown). These results agree with those of previous reports from Tianjin, China [Li et al., 2009] and from Korea [Chung et al., 2008]. In contrast, in Taiwan, China (between February 2006 and February 2009) [Wang et al., 2008] and in Brazil (between January 2003 and December 2006) [Oliveira et al., 2009], all four hMPV subtypes were detected in patients with lower respiratory tract infections. In another report from Gansu Province, China (between December 2006 and November 2008), hMPV-B2 was not detected [Xiao et al., 2010]; whereas in Chongqing, China (between April 2006 and March 2008), hMPV-A1 and hMPV-B1 were not detected [Chen et al., 2010]. Taken together with findings in this study, these reports suggest that the prevalence of

hMPV subtypes varies among different geographical locations. Long term surveillance of hMPV infections are needed to better understand the distribution of hMPV subtypes, to prevent possible outbreaks, and to inform vaccine development.

In summary, the detection rate of hMPV ranked third in patients with community-acquired pneumonia and similar clinical manifestation was observed between hMPV-positive and RSV-positive patients. These findings indicate that hMPV plays a significant role in pediatric community-acquired pneumonia in China. The findings also provide a better understanding of the epidemiological and clinical features of hMPV infections.

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