Prevalence of Human Metapneumovirus in Children With Acute Lower Respiratory Infection in Changsha, China

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Human metapneumovirus (hMPV) causes acute respiratory infections in children. The prevalence and clinical characteristics of hMPV were determined in nasopharyngeal aspirates of children in Changsha, China. Reverse transcription-polymerase chain reaction (RT-PCR) or PCR was employed to screen for both hMPV and other common respiratory viruses in 1,165 nasopharyngeal aspirate specimens collected from children with lower respiratory tract infections from September 2007 to August 2008. All PCR products were sequenced, and demographic and clinical data were collected from all patients. Seventy-six of 1,165 (6.5%) specimens were positive for hMPV, of which 85.5% (65/76) occurred in the winter and spring seasons. The hMPV coinfection rate was 57.9% (44/76), and human bocavirus was the most common virus detected in conjunction with hMPV. Phylogenetic analysis revealed that 94.7% of the hMPV detected were of subgroup A2, 5.3% were subgroup B2, and none belonged to either the A1 or B1 subgroups. No significant differences were found in terms of the frequency of diagnosis and clinical signs between either the co- and mono-infection groups, or between patients with and without underlying diseases. It was concluded that hMPV is an important viral pathogen in pediatric patients with lower respiratory tract infections in Changsha. Only hMPV genotypes A2 and B2 were co-circulating in this locality; human bocavirus was the most common coinfecting virus, and coinfection did not affect disease severity.

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KEY WORDS: acute lower respiratory infection; children; hMPV; reverse transcription-polymerase chain reaction

INTRODUCTION

Human metapneumovirus (hMPV) was first identified in 2001 in nasopharyngeal specimens from children with acute respiratory tract illness in the Netherlands [Van Den Hoogen et al., 2001]. This virus has been classified as a member of the genus Metapneumovirus of the subfamily Paramyxoviridae [Van Den Hoogen et al., 2001; Boivin et al., 2002; Bastien et al., 2003]. Subsequently, hMPV was described as a cause of acute respiratory disease in many countries, including Canada, the United States, Australia, Japan, France, Hong Kong, and Korea [Orda’s et al., 2006]. hMPV is recognized as a common cause of respiratory infections, ranging from upper respiratory tract infection to severe lower respiratory tract infection, in individuals of all ages, particularly in infants and children [Boivin et al., 2002; Freymouth et al., 2003; Peiris et al., 2003; Van Den Hoogen et al., 2003; McAdam et al., 2004; Loo et al., 2007]. However, limited data exist for hMPV infection, especially concerning its prevalence, and molecular and clinical characterizations of hMPV in children with lower respiratory tract infections in China, with the exception of a previous study in Lanzhou City [Xiao et al., 2010].

hMPV strains are divided into two main groups, A and B, based on their nucleotide sequences. Each group is subdivided into two sublineages, A1 and A2, and B1 and B2 [Loo et al., 2007]. In addition, parti-
tioning further the sublineage A2 into two genetic clusters designated A2a and A2b has been suggested [Huck et al., 2006], and the relationship of strain differences to clinical features has not been elucidated fully [Schildgen et al., 2005; Agapov et al., 2006; Manoha et al., 2007; Pitoiset et al., 2010]. In this study, 1,165 children with lower respiratory tract infections in Changsha City were screened for hMPV and several other common respiratory viruses, and the epidemiological and clinical features of infection with the various hMPV genotypes were characterized.

The objective of this study was to investigate the prevalence and clinical characteristics of hMPV in Chinese children with lower respiratory tract infections.

**MATERIALS AND METHODS**

**Patients and Specimens**

Nasopharyngeal aspirate samples were collected from 1,165 children with lower respiratory tract infection in the Hunan Province People’s Hospital, China, on 2 days each week from September 2007 to August 2008. All patients were 14 years of age or younger, and informed consent was obtained from their parents/guardians. All patients had symptoms of lower respiratory tract infection on admission. All nasopharyngeal aspirate samples were collected 1–3 days after the onset of lower respiratory tract infection. Demographic data and details of the clinical findings and severity of disease were recorded. The study protocol was approved by the hospital ethics committee.

**Collection and Processing of Nasopharyngeal Aspirate Samples**

All nasopharyngeal aspirate specimens were collected and transported immediately to the laboratory at the National Institute for Viral Disease Control and Prevention, China CDC, and stored at −80°C until required for further testing. Viral DNA and RNA were extracted from 140 μl of each nasopharyngeal aspirate specimen using the QIAamp viral DNA and the QIAamp viral RNA Mini Kits (Qiagen, Shanghai, China) according to the manufacturer’s instructions. cDNA was synthesized using random hexamer primers with the Superscript II RH reverse transcriptase (Invitrogen, Carlsbad, CA).

**Detection of hMPV**

Screening for hMPV was conducted using conventional polymerase chain reaction (PCR) methods. hMPV forward (5’-CCC TTT GTT TCA GGC CAA-3’) and reverse (5’-GCA GCT TCA ACA GTA GCT G-3’) primers, which target the M gene and generate a 416-bp product, were used as described previously [Pujol et al., 2005]. All PCR products were purified using the QIAquick PCR purification kit (Qiagen) and sequenced by SinoGenoMax (Beijing, China). The reaction mix contained 10 pmol of each primer and 1.25 units of EXTaq DNA polymerase (Takara Bio, Tokyo, Japan). Reactions were incubated at 94°C for 8 min, followed by 35 cycles at 94°C for 30 sec, 55°C for 30 sec, and 72°C for 45 sec, followed by a final extension at 72°C for 10 min.

**Screening for Other Respiratory Viruses**

A standard reverse transcription (RT)-PCR was used to screen for respiratory syncytial virus (RSV), human rhinovirus, influenza A virus, influenza B virus, parainfluenza virus, human coronaviruses HKU1 and NL63, and PCR for adenovirus and human bocavirus [Hierholzer et al., 1993; Pujol et al., 2005].

**Nucleotide Sequence Analysis**

All positive sequences were determined and analyzed using the DNASTAR software package. A neighbor-joining tree was constructed using the MEGA software package (version 3.1).

**Clinical Severity Score**

Based on variables reported in previous studies [Caracciolo et al., 2008] a severity index was defined a priori by assigning one point to each of the following: use of supplemental oxygen, duration of hospital stay of more than 7 days, and admission to an intensive care unit (ICU).

**Statistical Analysis**

The significance of differences in rates among various groups was evaluated using the chi-square test, Fisher’s exact test, or Student’s t-test. All analyses were performed using SPSS version 13.0 software (SPSS, Inc., Chicago, IL). P < 0.05 was considered statistically significant.

**RESULTS**

**Patient Characteristics**

In total, 1,165 patients were included; the study sample represented 36.7% of the total of 3,174 admissions for acute lower respiratory disease to the Hunan Province People’s Hospital from September 2007 to August 2008. Patient ages ranged from 3 hr to 156 months with a median of 15.4 months. The majority of patients (97.5%) were 5 years old or younger. The male:female ratio was 1.9:1 (763:402). All subjects were inpatients.

**Detection of hMPV and Other Viral Agents**

At least one respiratory virus was detected in 871 of the 1,165 samples and 76 (6.5%) were positive for hMPV by RT-PCR. hMPV accounted for 8.7% of the total viral agents detected. Forty-four of 76 (57.9%) children who were hMPV-positive were found to be coinfected with other respiratory viruses, including 16 with human bocavirus, 13 with RSV, 10 with human
rhinovirus, 7 with parainfluenza 3 virus, 4 with adenovirus, 3 with influenza B virus, 2 with HCoV-HKU1, and 1 with HCoV-NL63. Human bocavirus was the most common coinfecting virus, accounting for 16/56 (28.6%) coinfections. No differences in coinfection rates were observed between hMPV A and hMPV B (P = 0.106).

**Epidemiology of hMPV**

hMPV was detected in every month except for September and October 2007 and August 2008. The number of positive specimens peaked in March (n = 20; 26.3%) and April (n = 19; 25%; Fig. 1). The age of patients infected with hMPV varied from 20 days to 12 years of age (median, 15.9 months) and 93.4% (71/76) were <20 years of age. Of a subset of 42 children >60 months of age, five (11.9%) acquired hMPV infection (Fig. 2). The male:female ratio of the patients infected with hMPV was 3.5:1 (χ² = 5.300; P = 0.021).

**Clinical Characteristics of hMPV in Children**

Information on clinical symptoms was available for all patients infected with hMPV. The main clinical features of patients infected with hMPV included bronchitis (one, 1.3%), bronchopneumonia (30, 39.5%), bronchiolitis (33, 43.4%), and pneumonia (12, 15.8%). Of the patients infected with hMPV, all (100%) presented with cough, 40 (52.6%) had a fever, 37 (48.7%) had respiratory crepitations, 33 (43.4%) exhibited wheezing, 2 (2.6%) suffered vomiting, and 7 (9.2%) had diarrhea. Five patients with hMPV infection had a fever exceeding 40°C. Duration of stay in hospital ranged from 1 to 26 days (mean, 8.3 days). None of these patients died; however, five required supplemental oxygen and intensive care. Erythrocyte sedimentation rates and C-reactive protein analysis were not performed in all patients infected with hMPV in the study. The majority of patients infected with hMPV had normal erythrocyte sedimentation rates, C-reactive protein and leukocyte counts. Fourteen (20.6%) patients infected with hMPV also had elevated glutamic-pyruvic transaminase, 31 (45.6%) had elevated glutamic-oxaloacetic transaminase, and 46 (73%) had elevated creatine kinase-MB (Table I). Chest radiographs were taken in 57 patients; abnormal infiltrates were noted in 42 (73.7%) subjects (interstitial lung disease, 13 cases; scattered consolidation of the lung, 33 cases; consolidation in lobar distribution, 1 case; pleural effusion, 1 case; single hila of pulmonary swelling, 1 case; and emphysema, 10 cases).

No significant differences were observed in any of the epidemiological characteristics, clinical presentations between the hMPV mono- (group 1) and coinfection groups (group 2), or between the hMPV mono- and human bocavirus coinfection groups (group 3; Table II). Note that a significant difference was observed in the rate of coinfection with RSV between those ≤12 months and those >12 months (P = 0.045). We also found that wheezing was more prevalent in subjects infected with RSV than in those with hMPV mono-infection (69.2% vs. 37.5%), although this difference was not significant (P = 0.053; Table II).

A total of 163 of 1,165 (13.99%) patients with lower respiratory tract infection and 14 of 76 (18.4%) who were hMPV-positive had an underlying illness. No significant difference was observed in the detection rate between these two groups (P = 0.250). A significant difference was detected in the prevalence of diarrhea between the two groups (P = 0.024). However, no
significant difference was found for the duration of hospital stay, age, gender, the majority of clinical diagnoses, and all clinical symptoms (Table III).

**Phylogenetic Analysis of hMPV**

The sequences of positive products shared high homology with standard sequences from GenBank (97–100%). Single nucleotide mutations and nucleotide insertions were found, indicating a slow genetic variation rate. Phylogenetic analyses indicated that the 76 hMPV specimens were classified into the two main genetic lineages, A and B. Seventy-two (94.7%) hMPV strains were group A2, four (5.3%) strains were subgroup B2, and none were either subgroup A1 or B1 (Fig. 3). During the epidemic season, sublineages A2 and B2 co-circulated, with 94.7% (72/76) of the circulating viruses belonging to sublineage A2. Between the A2 and B2 genotype strains, the sequence identities of M gene fragments were 86.06–87.15% and 81.14–82.46%, at the nucleotide and amino acid levels, respectively. The identities within subgroup A2 were 98.91–99.56% and 98.58–99.13%, and within subgroup B2 were 95.91–97.98% and 96.37–97.38%, respectively.

**DISCUSSION**

Of the 1,165 children with lower respiratory tract infection included in this study, 6.5% were hMPV-positive by RT-PCR. A similar incidence was reported in Singapore [Loo et al., 2007], the United States [McAdam et al., 2004], Hong Kong [Peiris et al., 2003], and in Lanzhou City [Xiao et al., 2010], although the findings were different from those of other studies [McAdam et al., 2004; Chung et al., 2006; Bosis et al., 2008; Heikkinen et al., 2008]. Independent of the techniques used, several studies have demonstrated that hMPV infection occurs predominantly early in childhood [Esper et al., 2003; Dollner et al., 2004; McAdam et al., 2004]. By the age of 5 years, >90% of individuals screened have evidence of hMPV infection. In this study, the majority of patients who were hMPV-positive (93.4% /71/76) were less than 5 years old. No significant differences in the age distribution rate were detected, but a study from Chongqing, a city in southwestern China, reported that younger children (less than 6 months old) had the highest rate of hMPV infection [Chen et al., 2010]. Van Den Hoogen et al. [2001] and Bastien et al. [2003] reported that no significant difference in the prevalence of hMPV existed between male and female patients. In this study, the majority of patients infected with hMPV (59/76) were male, and statistical analysis found differences in prevalence between males and females, which indicated that male patients are at a higher risk of hMPV infection. hMPV was detected in each month with the exception of September and October 2007 and August 2008. Positive specimens peaked in March and April, in agreement with findings from the United States [McAdam et al., 2004], Canada [Bastien et al., 2003], and a previous study [Xiao et al., 2010]. However, hMPV was detected year-round in Singapore [Loo et al., 2007] and peaked during the summer months in Hong Kong [Peiris et al., 2003]. Although these data suggest that hMPV may follow varying epidemiologic patterns in different regions, elucidation of the exact epidemiologic characteristics of hMPV infection requires further investigation.

Approximately 58% of patients who were hMPV-positive were coinfected with other respiratory viruses.
| Variable                        | Group 1 (n = 32) | Group 2 (n = 44) | Group 3 (n = 16) | Group 4 (n = 13) | Group 1 vs. Group 2 | Group 1 vs. Group 3 | Group 1 vs. Group 4 |
|--------------------------------|-----------------|-----------------|-----------------|-----------------|-------------------|-------------------|-------------------|
| Age ≤1 year                    |                 |                 |                 |                 |                   |                   |                   |
| 20 (62.5)                      | 30 (68.2)       | 12 (75.0)       | 12 (92.3)       |                 | 0.606a            | 0.386a            | 0.045b            |
| Sex, male (%)                  |                 |                 |                 |                 |                   |                   |                   |
| 24 (75.0)                      | 35 (79.5)       | 14 (87.5)       | 10 (76.9)       |                 | 0.639a            | 0.315a            | 0.607b            |
| Median duration of stay in hospital, days | 8.2             | 8.3             | 8.3             | 9.1             | 0.878c            | 0.878c            | 0.416c            |
| No. of underlying illnesses    |                 |                 |                 |                 |                   |                   |                   |
| 7 (21.9)                       | 7 (15.9)        | 2 (12.5)        | 3 (23.1)        |                 | 0.508a            | 0.433a            | 0.608b            |
| Clinical diagnosis             |                 |                 |                 |                 |                   |                   |                   |
| Bronchopneumonia               | 14 (43.8)       | 16 (36.4)       | 3 (18.8)        | 2 (15.4)        | 0.515a            | 0.088a            | 0.069b            |
| Bronchiolitis                  | 12 (37.5)       | 21 (47.7)       | 9 (56.3)        | 9 (69.2)        | 0.374a            | 0.217a            | 0.053a            |
| Pneumonia                      | 5 (15.6)        | 7 (15.9)        | 4 (25.0)        | 2 (15.4)        | 0.973a            | 0.340b            | 0.680b            |
| Bronchitis                     | 1 (3.1)         | 0               | 0               | 0               | 0.689d            | —                 | —                 |
| Clinical signs                 |                 |                 |                 |                 |                   |                   |                   |
| Fever                          | 18 (56.3)       | 22 (50.0)       | 10 (62.5)       | 5 (38.5)        | 0.590a            | 0.679a            | 0.279a            |
| Median temperature of fever    | 39.1            | 39.1            | 39.1            | 38.7            | 0.919c            | 0.919c            | 0.053c            |
| Median duration of fever, days | 5.4             | 6.4             | 4.4             | 4.8             | 0.149c            | 0.117c            | 0.323c            |
| Cough                          | 32 (100)        | 44 (100)        | 16 (100)        | 13 (100)        | —                 | —                 | —                 |
| Wheeze                         | 12 (37.5)       | 12 (27.3)       | 9 (56.3)        | 9 (69.2)        | 0.344a            | 0.217a            | 0.053a            |
| Vomiting                       | 1 (3.1)         | 1 (2.3)         | 0               | 1 (7.7)         | 0.668a            | —                 | 0.499b            |
| Diarrhea                       | 4 (12.5)        | 3 (6.8)         | 1 (6.3)         | 2 (15.4)        | 0.657d            | 0.454a            | 0.567b            |

Group 1, hMPV mono-infection; Group 2, hMPV coinfection; Group 3, hMPV coinfection with human bocavirus; Group 4, hMPV coinfection with RSV.

*a*χ²-test.

*b*Fisher’s exact test.

*c*Student's *t*-test.

*d*Continuity correction.
in agreement with previous reports [Caracciolo et al., 2008; Cilla et al., 2008; Xiao et al., 2010]. Human bocavirus and RSV were the most common coinfecting viruses (36.4% and 29.5%, respectively). The data regarding the impact of coinfections of human bocavirus and RSV with hMPV on disease severity are conflicting [Greensill et al., 2003; Semple et al., 2005; Caracciolo et al., 2008]. In this study, no significant difference in clinical symptoms, age, sex, or duration of hospital stay between the mono- and coinfection groups was detected (Table II).

Considerable evidence suggests that hMPV is responsible for both upper respiratory tract infection and lower respiratory tract infection in infants and young children [Boivin et al., 2002; Freymouth et al., 2003; Van Den Hoogen et al., 2003]. Indeed, Arabpour et al. [2008] reported a higher prevalence of lower respiratory tract infection in children infected with hMPV. However, the present study encompasses only hospitalized children with lower respiratory tract infection in children infected with hMPV. However, the present study encompasses only hospitalized children with lower respiratory tract infection; therefore, the prevalence of upper respiratory tract infection and lower respiratory tract infection could not be elucidated. Additionally, Williams et al. [2004] reported that one-third of children with hMPV-associated lower respiratory tract infection were diagnosed with concomitant acute otitis media. However, in this study, no acute otitis media occurred in subjects who were infected with hMPV.

Bronchopneumonia and bronchiolitis were the most frequent clinical diagnoses in this study, as has been reported previously [Peiris et al., 2003; Loo et al., 2007; Xiao et al., 2010]. Fever, cough, respiratory crepitations, and wheezing were the most common symptoms of these patients. These symptoms are identical to those reported in children in Canada and Korea [Chung et al., 2006; Caracciolo et al., 2008]. No significant difference was observed in the frequencies of cough, fever, respiratory crepitations, wheezing, vomiting, diarrhea, or duration of stay in hospital between the hMPV mono- and coinfection groups. More than one-half of patients with hMPV had normal erythrocyte sedimentation rates, C-reactive protein and leukocyte counts, and a majority had hepatic and renal injury. Data on whether hMPV infection can induce hepatic and renal injury are limited; therefore, this aspect requires further study. Chest radiographs of the majority of patients who were hMPV-positive showed sporadic consolidation of the lung, 21.1% (12/57) showed interstitial lung disease and emphysema, and only one patient showed consolidation in lobar distribution, pleural effusion, and single hila of pulmonary swelling, which is consistent with a report from Korea [Chung et al., 2006].

A majority of patients who were hMPV-positive reportedly had underlying diseases [Boivin et al., 2002; Kaida et al., 2007], and 14 of 76 such patients had at least one underlying disease (Table III). A significant difference in the incidence of diarrhea was observed between subjects who were hMPV-positive with and without underlying illnesses (\( P = 0.024 \)), which suggests that hMPV infection may cause different clinical presentations in patients depending on the underlying conditions. However, no significant difference was detected in the detection rate, age, sex, duration of stay in hospital, the majority of clinical diagnoses, or clinical symptoms between the two groups (Table III). These data suggest that hMPV infection did not aggravate clinical symptoms or contribute to duration of hospital stay in subjects with underlying illnesses. Three subjects had congenital heart disease, which ranked first in terms of underlying diseases. Two each had gastroesophageal reflux and measles. Further study is needed to investigate whether congenital heart disease, gastroesophageal reflux, or measles poses a major risk factor for hMPV infection.

### Table III. Comparison of Clinical Characteristics Between Children Infected With hMPV With and Without Underlying Illness

| Variable                  | With underlying illness (n = 14) | Without underlying illness (n = 62) | P-value |
|---------------------------|----------------------------------|-----------------------------------|---------|
| Age ≤12 months            | 9 (64.3)                         | 41 (66.1)                         | 1.000^c |
| Sex, males (%)            | 11 (78.6)                        | 48 (77.4)                         | 1.000^c |
| Median duration of stay in hospital, days | 8.8                              | 8.3                               | 0.758^b |
| Clinical diagnosis        |                                  |                                   |         |
| Bronchopneumonia          | 3 (21.4)                         | 27 (43.5)                         | 0.126^a |
| Bronchiolitis             | 5 (35.7)                         | 28 (45.2)                         | 0.519^a |
| Pneumonia                 | 5 (35.7)                         | 7 (11.3)                          | 0.063^c |
| Bronchitis                | 1 (7.1)                          | 0                                 | —       |
| Clinical signs            |                                  |                                   |         |
| Fever                     | 8 (57.1)                         | 32 (51.6)                         | 0.708^a |
| Median temp of fever, °C  | 39.0                             | 39.1                              | 0.796^b |
| Median duration of fever, days | 4.6                             | 4.9                              | 0.718^b |
| Cough                     | 14 (100)                         | 62 (100)                          | —       |
| Wheeze                    | 5 (35.7)                         | 28 (45.2)                         | 0.519^a |
| Vomiting                  | 2 (14.3)                         | 0                                 | —       |
| Diarrhea                  | 4 (28.6)                         | 3 (4.8)                           | 0.024^c |

^a x^2-test.
^b Student’s t-test.
^c Continuity correction.
Previous data have suggested that the two hMPV genotypes co-circulate and that different subgroups may predominate from year to year [Peret et al., 2002; Mackay et al., 2004]. In this study, phylogenetic analysis demonstrated the simultaneous existence of two groups (A and B) and two of the four subgroups (A2 and B2). The majority of these strains (94.7%, 72/76) clustered predominantly with group A hMPV, and all belonged to subgroup A2 (100%, 72/72), which is consistent with the work of Boivin et al. [2004]. Moreover, a previous study in Lanzhou City showed that sublineages A1, A2 (A2a and A2b), and B1 co-circulated during the 2006–2007 epidemic, but only A2 circulated during the 2007–2008 epidemic [Xiao et al., 2010]. These data suggest that the circulation pattern of hMPV in China is complex, which poses a challenge for future vaccine development that relies on more molecular epidemiologic studies.

To summarize, the prevalence and clinical characteristics of hMPV in children with lower respiratory tract infection in Changsha, China were described. hMPV was detected in 76 of 1,135 (6.5%) nasopharyngeal aspirate specimens collected. Approximately 58% of subjects infected with hMPV were coinfected with other respiratory viruses, most commonly human bocavirus. The most common symptoms and clinical diagnosis in those infected with hMPV were cough and bronchopneumonia, and the predominant circulating genogroup was subgroup A2. Statistical analysis indicated that male subjects and those less than 5 years of age were at a higher risk of hMPV infection, and coinfection with other respiratory viruses did not affect disease severity.

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Fig. 3. Phylogenetic analysis of the partial M gene sequences of 76 human metapneumovirus strains from nasopharyngeal aspirate specimens. Phylogenetic trees were constructed by the neighbor-joining method using MEGA ver. 3.1. Viral sequences in marks were generated from the present study; other reference sequences were obtained from GenBank. Bootstrap values are shown at each branching point.
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