Human metapneumovirus infections in Mexico: epidemiological and clinical characteristics

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The human metapneumovirus (hMPV) is a recently described respiratory RNA virus that mainly affects children. To date there has not been a report that describes the detection of this virus in Mexico. This study was performed to detect hMPV in hospitalized Mexican children with respiratory infections, and describe their epidemiological and clinical characteristics. Nasal wash samples from 558 children younger than 3 years of age with the admission diagnosis of a respiratory tract infection were evaluated. Respiratory viruses were detected in 221 children [respiratory syncytial virus (RSV), 193 (34.6 %); influenza virus, 13 (2.3 %); parainfluenza viruses, 15 (2.7 %)]. Respiratory secretions of 323 children in whom the presence of these viruses was excluded (131 admitted during the 2002–2003 respiratory season and 192 during the 2003–2004 season) were tested for hMPV infection. The hMPV genome was detected in 34 specimens by amplification using real-time RT-PCR. Sequencing of amplicons and phylogenetic analysis indicated the predominance of genotype A hMPV. The months with the highest number of hMPV detections were February and March. During the 2002–2003 season hMPV activity peaked after the RSV season. During the 2003–2004 season hMPV and RSV epidemics occurred simultaneously. The clinical presentation of an hMPV infection was indistinguishable from other respiratory infections. Except for one death, the outcomes of the hMPV infections were good. In this study, among the viruses routinely tested for, hMPV was the second most common agent, after RSV, in children younger than 3 years of age hospitalized with respiratory tract infections.

INTRODUCTION

Respiratory infections are a leading cause of morbidity and mortality in children worldwide. Viral agents are among the most common pathogens affecting the respiratory tract in young children (van Woensel et al., 2003). The human metapneumovirus (hMPV) was described in 2001 for the first time and has since been shown to cause respiratory illness throughout the world (van den Hoogen et al., 2001). Young children and older adults are affected most frequently by this virus. The precise role that hMPV plays in respiratory infections has not been fully established. The virus shows a variable seasonal pattern of infection, but appears to affect children more frequently during winter and spring (Williams et al., 2004; Esper et al., 2003).

To our knowledge the presence and impact of hMPV infections in Mexico have not been reported to date. The aim of this study was to determine and quantify, by a sensitive method, the frequency, and epidemiological and clinical characteristics of hMPV infections among young children hospitalized with acute respiratory tract infections in San Luis Potosí, Mexico.

METHODS

Subjects. Children admitted to the Hospital Central ‘Dr Ignacio Morones Prieto’ with respiratory tract infections since October 2002 have been enrolled onto a programme to determine the presence of viral pathogens. Initial results from this programme have been published
previously (Noyola et al., 2004). Respiratory viral agents identified by direct immunofluorescence assay (DFA) in the programme include influenza A and B, respiratory syncytial virus (RSV), parainfluenza viruses and adenovirus. Infants younger than 36 months of age with a negative DFA test for the above-mentioned viruses were included in the present study, which was approved by the ethics committee at the Hospital Central ‘Dr Ignacio Morones Prieto’.

**Samples.** Nasal wash aspirate specimens were obtained using a sterile feeding catheter as soon as possible after admission from infants presenting with respiratory infections to the hospital. Specimens were maintained at 4 °C and transferred to the Microbiology Laboratory at the School of Medicine of Universidad Autónoma de San Luis Potosí on a daily basis. Samples obtained during weekends were placed on viral transport media and kept refrigerated until processing on Monday mornings. Specimens were processed for DFA detection of common viral respiratory pathogens. The supernatants of the specimens were frozen and stored at −70 °C until further testing. Specimens obtained during a 20 month period encompassing two winter seasons are included in the present report (first season, October 2002 to June 2003; second season, July 2003 to June 2004).

**Clinical definitions.** Respiratory infections were defined according to clinical and radiological data. For each child one of the following conditions was established: alveolar pneumonia, interstitial pneumonia, bronchiolitis (with or without associated pneumonia), croup or upper respiratory tract infection. Pneumonia was defined as the presence of infiltrates on chest roentgenogram and/or the presence of rales on physical examination. Infants with pneumonia were categorized as suffering from interstitial pneumonia or alveolar pneumonia according to radiological findings. Bronchiolitis was defined as an acute respiratory illness characterized by wheezing on physical examination and/or roentgenographic evidence of air-trapping with peribronchial thickening. Croup was defined as a respiratory illness characterized by the presence of stridor or hoarseness and no clinical or radiological evidence of pneumonia or bronchiolitis. Infants with respiratory symptoms that did not fulfill any of the previous definitions were considered to suffer an upper respiratory tract infection.

**Molecular assay for hMPV detection**

**RNA isolation.** Supernatants from all available samples that were negative by DFA for the common respiratory disease viruses mentioned above (*n* = 323) were used to isolate virion-associated hMPV RNA with the High Pure RNA isolation kit (Roche Diagnostics) according to the manufacturer’s instructions. The RNA was stored at −70 °C for further analysis.

**cDNA synthesis.** First-strand cDNA was generated from 250 ng of RNA with random-hexamer primers using the TaqMan reverse transcription kit (Applied Biosystems) according to the manufacturer’s instructions. Aliquots of 2 μl of cDNA were used as template for real-time PCR analyses.

**Real-time PCR assays.** The procedure described by Mackay et al. (2003) was the conserved region of hMPV selected for amplion detection in the samples and controls tested. The MPV01.2 sense primer (5'-CACGGTGAATGTCATCT-3') and MPV02.2 antisense primer (5'-CAGTGTGGTATGGG-GCCCTAA-3') were initially used; however, since a fluorescence signal using the SYBR Green detection system was recorded in the blank reagent (without cDNA), probably due to primer-dimer formation, the MPV02.2 primer was replaced by the MPV Ne primer (5'-TATATTATGATTAGGCATCCTG-3') designed by us downstream of the nucleocapsid gene sequence.

The cDNA was amplified using an iCycler iQ instrument (Bio-Rad) as follows: a 50 μl reaction mix was prepared containing 2 μl cDNA, 25 μl SYBR Green 2 × PCR Master Mix (Applied Biosystems) and 0.2 μM of each hMPV primer (MPV01.2 and MPV Ne). The amplification program included an initial denaturation step of 10 min at 94 °C followed by 45 PCR cycles (15 s denaturation at 95 °C, 20 s annealing at 55 °C and 60 s extension at 72 °C). Detailed information on the RT-PCR real-time procedure optimization using the SYBR Green kit for hMPV detection assay is being submitted elsewhere for publication (unpublished data).

The standard curve was obtained using serial dilutions (2.84 × 10^{9} – 2.84 × 10^{-1} copies μl^{-1}) of a plasmid harbouring one copy of the hMPV nucleocapsid gene (249 bp sequence). The plasmid was generated by cloning in a pGEM-Easy vector (Promega) a RT-PCR product of the RNA isolated from the CAN97-83 hMPV reference strain kindly donated by Dr Guy Boivin (Centre de Recherche en Infectiologie, Quebec, Canada).

**Sequencing.** All the positive samples for hMPV were cloned and sent for sequencing to the CINVESTAV-IPN Research Institute in Irapuato, Mexico. Only 26 clones were fully sequenced and used for phylogenetic relationships.

**Statistical analysis.** Demographic and clinical features were compared between hMPV-infected and non-infected children. Categorical variables were compared using the chi-square test with the continuity correction or Fisher’s exact test as appropriate. Continuous variables were compared using the Mann-Whitney U test. All comparisons were two-sided and a *P* value of < 0.05 was considered as significant.

**RESULTS AND DISCUSSION**

During the 20 month study period 558 infants younger than 3 years of age were enrolled for detection of respiratory viral pathogens and had an adequate specimen for laboratory analysis. Common known respiratory viruses were detected in 221 (39.6 %) specimens [RSV in 193 (34.6 %), influenza virus in 13 (2.3 %), parainfluenza type 1 in 3 (0.5 %) and parainfluenza type 3 in 12 (2.2 %)]. No virus was detected in samples from 337 (60.4 %) children. Nasal wash samples from 323 (97.9 %) infants in whom the aetiology of the respiratory infection could not be established were processed for hMPV detection by reverse transcription (RT) followed by real-time PCR. The sensitivity of the PCR was about 20 copies of hMPV DNA as determined by testing a serial dilution of a cloned nucleocapsid gene sequence. The sensitivity achieved is similar to that reported by Côté et al. (2003).

hMPV was detected in 34 samples. The overall frequency for hMPV infection among the 558 children younger than 3 years of age admitted to the hospital was 6.1 %. Reports from other countries have recorded variable frequencies of hMPV infection, ranging from 6-4 % to 20 %, in studies in which only specimens negative for other known respiratory viruses were included (Bastien et al., 2003; Esper et al., 2003; Frenmouch et al., 2003; Mackay et al., 2003; Williams et al., 2004). During the first year of the study (2002–2003) hMPV was detected in eight (3.2 %) children with respiratory infections compared to 26 (4.8 %) during the second year (2003–2004; Table 1). The monthly distribution of hMPV- and RSV-positive samples is shown in Fig. 1. The months with the highest activity of hMPV in our community were
February and March. This seasonal pattern is consistent with previous studies performed in North America, where the highest numbers of hMPV cases were detected between January and April (Esper et al., 2003; Williams et al., 2004).

During the 2002–2003 season hMPV activity was milder and peaked after the RSV season, whereas during the 2003–2004 season hMPV and RSV epidemics occurred simultaneously.

The median age of hMPV-infected children was 10 months, and 19 (55.9%) were male. Previous studies have found that young infants are the main group in which hMPV infections are detected. Van den Hoogen et al. (2003) reported the mean age of infected children to be 15 months, whereas Williams et al. (2004) reported a mean age of 11.6 months. Taking into account all children (558) less than 3 years of age hMPV was

Table 1. Human metapneumovirus and other viruses detected in 558 children less than 3 years of age during two consecutive winter seasons in San Luis Potosí, Mexico

<table>
<thead>
<tr>
<th>Virus</th>
<th>2002–2003 season (n = 249)</th>
<th>2003–2004 season (n = 309)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory syncytial virus</td>
<td>101 (40.6)</td>
<td>92 (29.8)</td>
</tr>
<tr>
<td>Human metapneumovirus</td>
<td>8 (3.2)*</td>
<td>26 (8.4)*</td>
</tr>
<tr>
<td>Influenza A</td>
<td>5 (2.0)</td>
<td>8 (2.6)</td>
</tr>
<tr>
<td>Parainfluenza type 1</td>
<td>0</td>
<td>3 (1.0)</td>
</tr>
<tr>
<td>Parainfluenza type 3</td>
<td>9 (3.6)</td>
<td>3 (1.0)</td>
</tr>
</tbody>
</table>

*Only specimens negative for other respiratory viruses were tested (n = 131 for the 2002–2003 season and n = 192 for the 2003–2004 season).
detected in samples from 4.7% of children younger than 12 months, 9.4% of children aged 12–24 months and 13.2% of children aged 24–36 months (Fig. 2). In contrast, the proportion of children positive for RSV infection tended to decrease in older age groups. Cuevas et al. (2003) also showed that the proportion of children infected with hMPV compared to RSV increased over the first 24 months of life. Ebihara et al. (2004) also reported that the proportion of samples in which the hMPV was detected increased over the first 5 years of life. Contrary to these observations, Maggi et al. (2003) found a decrease in the proportion of infected children as their age increased.

Symptoms reported in children with hMPV infection included respiratory distress (100%), cough (97.1%), rhinorrhea (75.8%), fever (73.5%), vomiting (34.4%) and diarrhoea (25.8%). An underlying disorder was observed in nine (26.5%) children, including prematurity, congenital heart disease, neurological disorders, malignancies and congenital malformations. Twenty-seven children required supplementary oxygen administration. No statistically significant differences in any of these clinical features were observed when children with hMPV infections were compared to children in whom no viral agent was identified.

The most common clinical presentation for hospitalized infants with hMPV infection was bronchiolitis with or without pneumonia (64.7%), followed by interstitial pneumonia (26.5%), alveolar pneumonia (5.9%) and croup (2.9%). Previous studies have reported bronchiolitis to be the main clinical syndrome associated with hMPV infection in hospitalized young children (Boivin et al., 2003; Dollner et al., 2004; Esper et al., 2003; Freymouth et al., 2003). Pneumonia and asthma exacerbations have also been reported as frequently associated manifestations. The role that hMPV plays among children presenting with asthma exacerbations is not fully understood. The proportion of children with hMPV infections that have been diagnosed with asthma varies in different reports (up to 19%) (Bosis et al., 2005; McAdam et al., 2004; Peiris et al., 2003; van den Hoogen et al., 2003; Williams et al., 2004). However, Rawlinson et al. (2003) detected the presence of hMPV in only 1-6% of asthmatic children presenting with an acute exacerbation. None of the children in whom we detected hMPV had a prior diagnosis of asthma.

A complete blood count was available from 30 children infected with hMPV and 241 children without identification of any viral infection. There were no significant differences in the blood counts between hMPV-infected and non-infected children. Wide variability in white blood cell counts and lymphocyte or neutrophil predominance, as well as platelet counts were observed in both groups.

The clinical outcomes for children infected with hMPV were good in general. However, one child required admission to the intensive care unit and eventually died; he was a 1-year-old infant with developmental delay and seizure disorder secondary to herpetic encephalitis. On admission he was in respiratory distress and required mechanical ventilation. He had a prolonged stay in the intensive care unit and died 44 days after admission.

Whether hMPV infections are more or less severe than those caused by RSV is not clear (Boivin et al., 2003; Cuevas et al., 2003; Peiris et al., 2003; Viazov et al., 2003). Some authors have found milder clinical courses than those of patients with RSV, whereas others have noted no differences or have reported longer hospitalizations in children with hMPV infections compared to those infected with RSV (Cuevas et al., 2003; Peiris et al., 2003; Viazov et al., 2003). In some reports none of the children infected with hMPV required admission to the intensive care unit, others indicate that 15–25% of children required this (Boivin et al., 2003; Hamelin et al., 2004; Mullins et al., 2004). In one report, a high frequency of hMPV and RSV coinfection was found in patients with severe bronchiolitis requiring mechanical ventilation (Greensill et al., 2003).

As for the real-time RT-PCR assay, the set of primers used in the present work was able to detect both A and B hMPV genotypes. The phylogenetic relationships (Fig. 3) show that 23 out of 26 samples sequenced were grouped in the A genotype lineage, whereas only three were grouped in the B genotype. A recent publication of Mackay et al. (2004) suggests that primers for the phosphoprotein gene (P) should be used in order to detect the actual B genotype frequencies in positive samples since these primers show higher sensitivity to the B genotype; we plan to evaluate this assay in future studies.

In conclusion, hMPV was detected in respiratory secretions of 6.1% of infants less than 3 years of age hospitalized with respiratory infections. After RSV, it was the second most common viral agent identified in these children. The majority of cases presented in February and March, and both genotype A and B types circulated in Mexico between 2002 and 2004. Clinical presentation was similar to other respiratory infections. To our knowledge this is the first study to date to report the frequency of hMPV infections in Mexico detected by real-time RT-PCR assay.

Fig. 2. Samples positive for human metapneumovirus and respiratory syncytial virus according to subjects’ age. Bars: dark grey, hMPV; light grey, RSV.
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