An Outbreak of Severe Respiratory Tract Infection Due to Human Metapneumovirus in a Long-Term Care Facility

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Background. Human metapneumovirus (hMPV) is a newly described paramyxovirus that is mainly associated with bronchiolitis in children. We sought to describe the epidemiological, virological, and histopathological findings associated with a large outbreak of hMPV infection in a long-term care facility.

Methods. An investigation of the outbreak was performed by public health authorities, who used standardized questionnaires to collect relevant clinical information from all residents of the facility. Nasopharyngeal samples were obtained from a subset of patients who had influenza-like illnesses for testing by viral culture and reverse-transcriptase polymerase chain reaction. Lung tissue samples from a patient whose case was fatal were available for molecular, histopathological, and immunohistochemical testing.

Results. A total of 96 (27%) of 364 residents of a long-term care facility presented with respiratory or constitutional symptoms between 1 January 2006 and 15 February 2006. The attack rate in the most affected ward was 72% (31 of 43 patients), which included 4 of the 6 polymerase chain reaction–confirmed cases of hMPV infection. In contrast, viral culture results were positive for hMPV in only 2 of the 5 polymerase chain reaction–positive samples tested. The most reported diagnosis was an upper respiratory tract infection or an influenza-like illness, although 21% of residents in 1 of the 3 wards that had confirmed cases of hMPV infection had lower respiratory tract infections. The fatality rate was 50% (3 of 6 patients) among confirmed cases and 9.4% (9 of 96 patients) among patients with possible cases. A patient with a fatal case had histopathological findings that confirmed the presence of hMPV RNA and proteins in the bronchiolar epithelium of affected lobes. Phylogenetic analysis revealed the presence of 2 distinct strains of hMPV circulating simultaneously on different wards.

Conclusion. hMPV can be associated with important outbreaks of acute respiratory tract infection in elderly institutionalized persons.

Human metapneumovirus (hMPV) is a recently described paramyxovirus with 2 major genotypes (A and B) [1–3]. Several groups have reported that hMPV is a major respiratory pathogen in young children, with clinical features that are indistinguishable from those of another paramyxovirus, the human respiratory syncytial virus (hRSV) [4–7]. In typical respiratory infection seasons, hMPV is responsible for 5%–10% of hospitalizations for acute respiratory tract infection (ARTI)—mainly bronchiolitis and pneumonitis—in children <3 years old [8–11].

There are considerably less data on the burden of hMPV infection in adults. So far, this viral pathogen has been implicated in cases of influenza-like illness in young adults [12], acute exacerbations of chronic obstructive pulmonary disease [13], congestive heart disease [14], and asthma [15], as well as in reports of severe pneumonia among immunocompromised or elderly subjects [4, 16].

However, the potential role of hMPV in causing outbreaks of ARTI in institutions with documented episodes of nosocomial transmission over several gener-
Human Metapneumovirus Outbreak

Table 1. Virological results from patients with acute respiratory tract infection in a long-term care facility, 2006.

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Date of collection</th>
<th>Patient ward</th>
<th>Patient age, years</th>
<th>Viral culture result</th>
<th>Multiplex RT-PCR result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NPS</td>
<td>9 January</td>
<td>C</td>
<td>hRSV</td>
<td>hRSV</td>
</tr>
<tr>
<td>2</td>
<td>NPS</td>
<td>11 January</td>
<td>A</td>
<td>hMPV</td>
<td>hMPV</td>
</tr>
<tr>
<td>3</td>
<td>NPS</td>
<td>12 January</td>
<td>O</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>4</td>
<td>NPS</td>
<td>16 January</td>
<td>O</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>5</td>
<td>NPS</td>
<td>17 January</td>
<td>A</td>
<td>hMPV</td>
<td>hMPV</td>
</tr>
<tr>
<td>6</td>
<td>NPS</td>
<td>17 January</td>
<td>O</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>7</td>
<td>NPS</td>
<td>18 January</td>
<td>O</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>8</td>
<td>NPS</td>
<td>20 January</td>
<td>B</td>
<td>Neg</td>
<td>hMPV</td>
</tr>
<tr>
<td>9</td>
<td>NPA</td>
<td>20 January</td>
<td>A</td>
<td>Neg</td>
<td>hMPV</td>
</tr>
<tr>
<td>10</td>
<td>NPS</td>
<td>20 January</td>
<td>A</td>
<td>Neg</td>
<td>hMPV</td>
</tr>
<tr>
<td>11</td>
<td>NPS</td>
<td>20 January</td>
<td>O</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>12</td>
<td>NPS</td>
<td>20 January</td>
<td>O</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>13</td>
<td>NPA</td>
<td>30 January</td>
<td>D</td>
<td>Neg</td>
<td>hMPV</td>
</tr>
</tbody>
</table>

NOTE. hMPV, human metapneumovirus; hRSV, human respiratory syncytial virus; Neg, negative; NPA, nasopharyngeal aspirate; NPS, nasopharyngeal swab; O, other wards (not wards A–D).

a Specimen collected at another hospital following patient’s transfer.

MATERIALS AND METHODS

Epidemiological investigation. In a LTCF located in Quebec City, Canada, reports of respiratory tract infection potentially attributable to influenza virus prompted the collection of respiratory samples for virological testing and an active investigation using a standardized questionnaire to document all cases that occurred between 1 January and 15 February 2006. Residents presenting with ≥1 respiratory symptom (rhinorrhea, sore throat, or cough) or ≥1 constitutional symptom (fever, loss of appetite, fatigue, or myalgia) were included in this study as case patients.

Virological investigation. Nasopharyngeal swab or aspirate samples were tested for the presence of influenza A and B antigens (Binax; Portland, ME), influenza A and B viruses, hRSV, and hMPV RNA using a multiplex real-time RT-PCR assay [13], and for a panel of respiratory viruses (influenza A and B viruses, parainfluenza viruses 1–4, hRSV, hMPV, and adenoviruses) by cell culture using 10 continuous cell lines [8]. The nucleoprotein (N) gene of hMPV-positive samples was subsequently amplified and sequenced for phylogenetic analyses.

Histopathological studies. An autopsy limited to the thoracic cage was performed for an 89-year-old woman from the LTCF who died of pneumonia. Samples from the trachea, 2 main bronchi, and all pulmonary lobes were snap-frozen at −80°C for RT-PCR analysis (including with the above-mentioned multiplex assay and a specific assay for the hMPV N gene [17]) or fixed in 10% formalin for histopathological studies. Tissues were subsequently stained with hematoxylin-eosin or used for antigen detection by immunohistochemical testing with a monoclonal antibody against the hMPV matrix protein (supplied by ViroStat).
### RESULTS

**Epidemiological investigation.** At the beginning of January 2006, residents of a LTCF began to present signs of respiratory infection. The LTCF has a total of 368 beds in 194 rooms (1–6 beds per room) located on 9 wards. The mean age of the residents was 83 years, and 75% of them were female. As part of an initial investigation, 12 nasopharyngeal swab or nasopharyngeal aspirate samples were collected between 9 January and 21 January 2006 from a subset of patients who had respiratory and constitutional symptoms for identification of causative agents. All samples were found to be negative for the presence of influenza A and B antigens, which was consistent with the absence of circulation of these viruses during this period in the Quebec City area. The clinical specimens were then tested for the presence of additional viral agents by conventional viral cultures for a panel of respiratory viruses and by real-time multiplex RT-PCR for influenza A and B viruses, hRSV, and hMPV. These initial tests revealed the presence of hMPV in 5 subjects (4 in ward A and 1 in ward B) and the presence of hRSV in 1 patient residing in a special locked ward (ward C) that houses patients who have advanced dementia and who have no contact with other residents (table 1). Another diagnosis of hMPV infection was subsequently made on 30 January in a patient from another ward (ward D) who had been transferred to an acute-care facility for severe pneumonia. Thus, a total of 6 laboratory-confirmed cases of hMPV infection were found in 3 different wards (A, B, and D). Of note, viral culture results were positive for hMPV in only 2 of the 5 PCR-positive samples tested.

This unusual cluster of hMPV infections in a LTCF prompted an active and detailed investigation by public health authorities initiated on 26 January, using a standardized questionnaire to identify patients who had respiratory or constitutional symptoms that occurred between 1 January and 15 February 2006. Results of the active epidemiological investigation revealed that 96 (27%) of 364 residents from 7 (78%) of 9 wards presented with such symptoms during the 6-week period of observation. Because not all cases were tested, and because of the presence of a confirmed case of hRSV infection, subsequent results are presented as possible cases (including all cases that were identified by the epidemiological study within the institution) and as probable cases (defined as cases occurring in 1 of the 3 wards with laboratory-confirmed cases of hMPV infection [A, B, and D]). Attack rates were 72% (31 of 43 patients), 48% (20 of 42 patients), and 22% (8 of 37 patients) in wards A, B, and D, respectively. The 6 confirmed cases of hMPV infection were in patients who were living in different rooms; 6 of the persons who shared the room with these confirmed case patients also developed ARTI. Epidemic curves suggested >1 generation of cases from 7 January to 7 February 2006, despite installment of droplet and contact precautions on 17 January (figure 1).

The clinical presentation of and assignment of medical diagnosis to patients with laboratory-confirmed hMPV infection, probable case patients (i.e., those living on a ward with laboratory-confirmed case patients), and all possible case patients from the LTCF are summarized in table 2. Fever was present in 100% of patients with confirmed cases of hMPV infection, compared with 31% of probable case patients and 36% of all possible case patients within the institution, whereas cough was noted in 67%, 86%, and 87% of patients, respectively. The mean duration of symptoms was 14.5 days (range, 7–22 days) in patients with confirmed cases of hMPV infection, 8.2 days...
Table 3. Characteristics of patients who died during an outbreak of human metapneumovirus (hMPV) infection, 2006.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Patient ward</th>
<th>Patient age, years</th>
<th>Maximum temperature, °C</th>
<th>Date of onset of illness (duration of illness, days)</th>
<th>Underlying condition</th>
<th>Diagnosis</th>
<th>RT-PCR result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>79</td>
<td>38.6</td>
<td>10 January (18)</td>
<td>Cardiac disease, dementia</td>
<td>ILI</td>
<td>hMPV</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>56</td>
<td>39.0</td>
<td>10 January (7)</td>
<td>None</td>
<td>Pneumonia</td>
<td>hMPV</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>67</td>
<td>39.2</td>
<td>17 January (3)</td>
<td>Diabetes, cancer</td>
<td>Lung cancer</td>
<td>NT</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>89</td>
<td>38.9</td>
<td>22 January (15)</td>
<td>Diabetes, dementia</td>
<td>Pneumonia</td>
<td>hMPV</td>
</tr>
<tr>
<td>5</td>
<td>O</td>
<td>88</td>
<td>40.2</td>
<td>10 January (3)</td>
<td>Pulmonary disease, dementia</td>
<td>Pneumonia</td>
<td>NT</td>
</tr>
<tr>
<td>6</td>
<td>O</td>
<td>81</td>
<td>38.6</td>
<td>14 January (6)</td>
<td>Cancer</td>
<td>Aspiration pneumonia</td>
<td>NT</td>
</tr>
<tr>
<td>7</td>
<td>O</td>
<td>84</td>
<td>38.1</td>
<td>30 January (3)</td>
<td>Cardiac disease</td>
<td>Pneumonia</td>
<td>NT</td>
</tr>
<tr>
<td>8</td>
<td>O</td>
<td>84</td>
<td>39.2</td>
<td>5 February (3)</td>
<td>Cardiac disease, dementia</td>
<td>Aspiration pneumonia</td>
<td>NT</td>
</tr>
<tr>
<td>9</td>
<td>C</td>
<td>86</td>
<td>39.8</td>
<td>7 January (11)</td>
<td>Cardiac disease, dementia</td>
<td>Pneumonia</td>
<td>hRSV</td>
</tr>
</tbody>
</table>

NOTE. hMPV, human metapneumovirus; hRSV, human respiratory syncytial virus; ILI, influenza-like illness; NT, not tested; O, other wards (not wards A-D).

(range, 1–22 days) for probable case patients, and 7.9 days (range, 1–22 days) for all possible case patients. The most frequently made diagnoses were an influenza-like illness or an upper respiratory tract infection followed by bronchitis and pneumonia. A diagnosis of bronchitis or pneumonia was made for 50% of patients with confirmed cases of hMPV infection, compared with 21% of probable case patients and 33% of all possible case patients.

During the observation period, 9 (9.4%) of 96 patients in the LTCF died due to respiratory infection, including 3 patients who had confirmed hMPV infection and the single patient who had hRSV infection. The mortality rate among patients with hMPV infection was 50% (3 of 6 patients), whereas it was 9.7% (3 of 31 patients) among probable case patients in ward A (the most affected ward) and 6.8% (4 of 59 patients) among probable case patients who were living on 1 of the 3 wards that had patients with confirmed cases. Most patients (7 [77.8%] of 9 patients) who died received a diagnosis of pneumonia or aspiration pneumonia, and all but 1 had at least 1 underlying disease, including 2 subjects who had advanced cancer (table 3).

**Histopathological studies.** An 89-year-old woman from the LTCF (ward D) developed fever (temperature, 39.2°C), cough, and dyspnea on 23 January 2006. This prompted her transfer to an acute care hospital the next day, where a diagnosis of right lung pneumonia (involving the lower and middle lobes) associated with congestive heart failure was made (figure 2). Despite the administration of broad-spectrum antibiotics (ceftriaxone and azithromycin), the patient remained hypoxicemic (oxygen saturation, 89% with 5 L/min of supplemental oxygen by nasal plugs) and eventually died of respiratory failure 14 days after the onset of symptoms. Blood and urine cultures obtained at admission to the acute care hospital were negative for bacteria. No sputum samples could be obtained for bacterial culturing, but a nasopharyngeal aspirate sample collected on 30 January had results positive for hMPV by multiplex RT-PCR. An autopsy limited to the thoracic cage revealed reddened lungs of increased weight (left lung, 610 g; right lung, 715 g) with boggy consistency. Histological evaluation revealed zones of acute and organizing diffuse alveolar damage characterized by hyaline membranes and interstitial fibrosis accompanied focally by vascular fibrin thrombi (figure 3). Peribronchiolar inflammation was also noted in some areas. There was a zone of

Figure 2. Chest radiograph of a patient who died from human metapneumovirus (hMPV) infection. The radiograph shows consolidation of the lower and middle right lobes.
necrosis in the right lower lobe, but no viral inclusions were identified. A specific real-time RT-PCR for the hMPV N gene was positive for samples obtained from the right lower and middle lobes and for the left lower lobe, but was negative for samples from the other lobes, the main bronchi, and the trachea (not shown). RT-PCR was negative for influenza A and B viruses and for hRSV. Immunohistochemical staining of lung slices with a monoclonal antibody directed against the hMPV matrix protein revealed numerous immunoreactive bronchiolar epithelial cells in the same lobes where hMPV was detected by RT-PCR (figure 4). Lung samples were not cultured for viruses and bacteria because of a delay between death and autopsy studies.

**Phylogenetic analysis.** A 753-base pair fragment of the hMPV N gene was amplified and sequenced for molecular analysis. This revealed the presence of 5 related strains of hMPV (99.1%–100% nucleotide and 97.2%–100% amino acid identities) of the B1 genotypes (4 patients on ward A and 1 patient on ward D), as well as 1 divergent strain of the A2 genotype (1 patient on ward B). Nucleotide and amino acid identities between these 2 hMPV clades were 85.1%–85.8% and 92.4%–94.8%, respectively. Phylogenetic analysis of the hMPV strains isolated from patients in the LTCF and in the Quebec City community area during the same period confirmed the circulation of similar strains from the 2 viral lineages (figure 5).

**DISCUSSION**

In this report, we showed that hMPV was responsible for a major outbreak of ARTI with high morbidity and mortality among residents of a LTCF. Over a period of 1 month, approximately one-fourth of the residents of the LTCF became sick; the attack rate was as high as 72% when considering probable cases in the most affected ward. Similarly, the case fatality rate was high among this vulnerable elderly population (10% among all possible case patients within the institution and up to 50% among the laboratory-confirmed case patients, who likely presented with the most-severe symptoms). Our investigation revealed that RT-PCR is much more sensitive than viral culture for the detection of hMPV in respiratory samples from elderly adults. The unexpected detection of 2 clades of hMPV in our investigation illustrates that viruses causing outbreaks in LTCF reflect those that are occurring in the community and highlights the difficulty in preventing their importation unless strict isolation measures are taken. Finally, autopsy findings from a single fatal case confirmed the role of

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**Figure 3.** Histopathological lung studies of a fatal case of human metapneumovirus (hMPV) infection. Lung sections were fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin-eosin. **A,** Normal lung histological findings from a noninfected subject. hMPV lung infection was characterized by diffuse exudative alveolar damage with hyaline membranes (B) and organization (C) accompanied with vascular fibrin thrombi (D). Chronic peribronchial inflammation was also noted (not shown).

**Figure 4.** Immunohistochemical staining of lung specimens for human metapneumovirus (hMPV). Lung sections were fixed in 10% formalin, embedded in paraffin and stained with a monoclonal antibody against the hMPV matrix protein. The cell nuclei were stained blue with Meyer hematoxylin, whereas hMPV-positive cells were stained red. **A,** Epithelial cells from bronchi of a noninfected patient. The presence of hMPV antigens is evident in the cytoplasm of bronchiolar epithelial cells (B and C). HMPV-positive cells were only found in the left lower lobe and in the right lower and middle lobes.
hMPV in viral pneumonia using both RT-PCR and immunohistochemical methods.

Despite its recent description in 2001, hMPV is not a novel viral pathogen, because serological studies have indicated the presence of specific antibodies in human serum samples collected in 1958 [1]. Moreover, it appears that hMPV is a ubiquitous pathogen, with seroprevalence rates reaching 100% by the age of 5–10 years [1, 18, 19]. However, because of the presence of multiple viral genotypes and/or waning immunity, reinfection is possible at later ages [3, 13]. One of the reasons that may explain the late description of hMPV is its fastidious growth in conventional cell culture [20]. Despite the use of optimized cell culture conditions in our laboratory, it is interesting to note that the virus could be cultured in only 2 of the 5 respiratory specimens that had positive results with RT-PCR (1 PCR-positive sample was not cultured). Thus, investigation of an outbreak of influenza-like illness would require the use of sensitive molecular methods to rule out the presence of this viral pathogen. The role of rapid antigenic detection methods in that context remains to be determined.

Despite the fact that hMPV infection is now a well-established cause of bronchiolitis and pneumonia in young children [7, 8, 10], its contribution to ARTI in adults has been less studied. We and others have reported that hMPV infection could be responsible for a significant proportion (close to 5%) of hospitalizations for exacerbations of chronic obstructive pulmonary disease and congestive heart disease in adults [13, 14]. Fatal probable cases of hMPV-related pneumonia (determined on the basis of premortem diagnosis) were also reported in elderly subjects who had several underlying diseases and in lung and bone marrow transplant recipients [4, 16, 21]. However, to our knowledge, no large outbreaks of hMPV infection in institutions with documented cases of transmission have been reported prior to this investigation. Our study confirms that hMPV infection is associated with both upper and lower respiratory tract infection in adults and that the severity of this disease is likely to be influenced by both viral factors (genotype, load) and host factors (underlying disease, cytokine response).

Our histopathological investigation of a fatal case of hMPV infection revealed the presence of acute and organizing diffuse alveolar damage. The observed fibrin vascular thrombi were considered to be part of the expected morphological spectrum associated with diffuse alveolar damage. Such findings were reported in a few lung biopsy specimens obtained from hMPV-infected, immunocompromised patients [21]. However, we did not observe the “smudge” cells and viral inclusions that were reported by others [21, 25]. Of note, peribronchiolitis was also observed in postmortem samples obtained from our patient; this feature correlates well with the bronchiolitis and wheezing that is noted clinically in patients who have hMPV infection [7, 26]. Immunohistochemical staining revealed the presence of the hMPV matrix protein in bronchiolar epithelial cells, which is consistent with experimental hMPV infection in macaques reporting viral replication, primarily in ciliated respiratory epithelial cells [27].

Our study has some limitations. First, we only had 6 confirmed cases of hMPV infection in this large outbreak population. In addition, the detection of hRSV in 1 patient from a closed ward whose residents had no contact with individuals from the hMPV-positive wards and the identification of a second hMPV clade show that >1 virus was circulating. Thus, we cannot eliminate the possibility that a subset of case patients were not infected by hMPV and that global percentages for the LTCF are overestimated. Nevertheless, our data from ward A, where 4 of the 31 case patients had laboratory-confirmed hMPV infection and where 4 other case patients were living in the same room as confirmed case patients strongly support the likelihood that other case patients on that ward were also infected with hMPV and that this virus can cause significant outbreaks and deaths in institutionalized elderly patients. Another caveat is the delayed application of the droplet and con-
tact precautions, which may have contributed to the high attack rate and prolonged circulation of the virus in this vulnerable population. Despite the introduction of droplet and contact precautions ~1 week after the identification of the initial cases, the outbreak continued for at least 2 weeks (figure 1). Several reasons may explain the ineffectiveness of these measures, including the circulation of patients between wards, the lack of systematic surveillance of the health care workers who may have transmitted the virus among different wards, and the continued contact with visitors who may have introduced 1 or several strains, as illustrated in our phylogenetic studies.

In conclusion, our investigation illustrates the potential role of hMPV infection in causing outbreaks of ARTI with high fatality rates in residents of LTCFs. The symptoms observed in these cases are indistinguishable from those associated with other more common viral respiratory pathogens, such as influenza viruses and hRSV. Thus, hMPV detection should be actively pursued during influenza virus–negative institutional outbreaks of ARTI that occur during the winter and spring seasons. Our study also highlights the need for developing therapeutic and prophylactic modalities against this viral pathogen.

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