Human metapneumovirus-associated respiratory tract infections in the Republic of Ireland during the influenza season of 2003–2004

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ABSTRACT

Human metapneumovirus (hMPV) is a newly identified paramyxovirus that has been associated with respiratory tract illness in children aged < 5 years, the elderly, and immunocompromised patients. This study determined the frequency of respiratory tract infections (RTIs) associated with hMPV in the Republic of Ireland. Bronchoalveolar lavage (BAL) samples from 168 adult patients and respiratory specimens from 122 children aged < 5 years were collected between September 2003 and May 2004. The virus was detected by reverse-transcription (RT)-PCR using hMPV polymerase (L) and matrix (M)-specific primers in four (2.4% ) of 171 BAL specimens obtained from 168 adults. No other respiratory virus was detected in these specimens, and no hMPV RNA was detected in respiratory specimens from children during the same time period. In all four adult cases, two of whom had underlying disease, hMPV was associated with mild, self-limiting upper RTIs. The most common clinical findings included fever (3/4 patients), cough (4/4) and rhinorrhoea (3/4). No patient died as a result of these RTI episodes. Phylogenetic analysis was performed using the amplified regions of the M and fusion (F) genes of hMPV. The Irish isolates belonged to cluster 1B, and did not show a separate Irish sub-lineage.

Keywords  hMPV, human metapneumovirus, molecular epidemiology, phylogenetics, respiratory tract infections, RT-PCR

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INTRODUCTION

Acute respiratory tract infections (RTIs) remain a leading cause of illness worldwide. They affect individuals of all ages, and are responsible for a significant loss of productive time, as well as being important factors in terms of morbidity and mortality of the elderly population [1]. The viruses associated most frequently with RTIs include influenza virus, parainfluenza virus, human respiratory syncytial virus (hRSV), adenovirus, coronavirus and rhinovirus, but the aetiological agents remain unknown in a substantial proportion of cases.

In 2001, a new paramyxovirus, human metapneumovirus (hMPV), was identified in The Netherlands and linked to acute RTI in children [2]. hMPV has been recognised as the first human pathogen within the genus Metapneumovirus, sub-family Pneumovirinae (family Paramyxoviridae) [2]. Phylogenetic analysis has demonstrated the existence of two genetic subgroups of hMPV, exemplified by the Canadian strains CAN97-83 (genotype 1) and CAN98-75 (genotype 2) [3]. By analogy with hRSV, it was assumed that these distinct genetic lineages would represent different antigenic subgroups. However, it has been shown that the two hMPV genetic groups are highly related antigenically, and that the fusion protein is a major contributor to this antigenic relatedness [4]. No zoonotic source for hMPV is known, despite similarities between hMPV and avian pneumovirus type C [2,5]. Infection appears to be seasonal, and resembles that caused by hRSV in that it occurs primarily, but not exclusively, in the winter months [2,6,7].
The relative importance of hMPV in virus RTIs is still unknown. However, hMPV has been associated with acute RTIs in neonates and children [2,7,8], young and elderly adults [9], and immunocompromised patients [10]. Respiratory illness in these patient groups ranges from upper RTIs to severe bronchiolitis and pneumonia [11]. hMPV is ubiquitous and circulates worldwide. The virus has been identified in Asia [12,13], Australia [14], North America [15–17] and Brazil [18]. RTIs associated with hMPV infection in patients from the Republic of Ireland have never been investigated. In the present study, the frequency of hMPV-associated infection in Irish children and adults during the 2003–2004 influenza season was determined. The clinical features of hMPV-positive patients were reviewed, and phylogenetic analysis based on matrix (M) and fusion (F) gene sequences was performed to evaluate their genetic diversity.

MATERIALS AND METHODS

Specimen collection and testing

Retrospective testing for hMPV was performed on 171 bronchoalveolar lavage (BAL) specimens obtained from 168 adults with RTIs in different wards at St James’s Hospital, Dublin, between September 2003 and May 2004. During the same period, 122 respiratory specimens, comprising 102 nasopharyngeal aspirates (NPAs), 12 BALs and eight sputum samples, were collected from children aged <5 years in Republic of Ireland hospitals. All samples were tested retrospectively for hMPV RNA by reverse-transcription (RT)-PCR (see below). Routine testing for other respiratory viruses included influenza A and B viruses, hRSV, adenovirus, and parainfluenza virus types 1–4. Direct immunofluorescence on cells present in BAL and NPA samples was performed using a commercial respiratory virus screen kit (Imagen; DakoCytomation, Galway, Ireland) with fluorescently-labelled monoclonal antibodies to hRSV, influenza A and B viruses, adenovirus and parainfluenza virus types 1–3. Respiratory virus isolation was performed using the Madin Darby canine kidney, human lung adenocarcinoma (A549), human embryonic lung fibroblast and fetal rhesus monkey kidney cell lines. All BAL and NPA samples were stored at + 4°C during processing, and were then stored at −80°C.

Isolation of RNA from clinical specimens and RT-PCR

RNA was extracted from 500 µL of BAL or respiratory specimen using the RNeasy kit (Qiagen, Crawley, UK) according to the manufacturer’s instructions. Virus RNA was amplified in a one-step RT-PCR (Qiagen, Crawley, UK) that contained 5 µL of 5x OneStep RT-PCR buffer (Qiagen), 400 µM each dNTP, 0.6 µM each primer (see below), 1 µL of OneStep RT-PCR enzyme mix (Qiagen) and 5 µL of RNA extract in a final reaction volume of 25 µL. Screening was performed with polymerase (L) gene primers L6 (5'-CATGCCACCTATAAA-AAGTCGAG-3') and L7 (5'-ACCCTACTTCATCGGAA-3'), which amplify a 170-bp fragment from nucleotides 11321–11490 (based on the prototype hMPV Dutch strain 00-1) [2,19]. Screening of the BAL or respiratory specimen cohort was also performed with matrix (M) gene primers hMPV-MF1 (5'-AA-GTGAATGCATCGCCAAAG-3') and hMPV-MR1 (5'-CACA-GACTTGAGTTGGCTAAA-3'), which amplify a 120-bp fragment from nucleotides 2376–2495 from hMPV strain 00-1 [20], to confirm the results of the L-gene RT-PCR. RT-PCR parameters comprised 50°C for 30 min and 95°C for 15 min, followed by 40 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 1 min, with a final extension step at 72°C for 10 min. The RT-PCR products were visualised on a UV transilluminator following agarose gel electrophoresis as described previously [21]. Where necessary, amplicons were purified with a PCR purification kit (Qiagen).

DNA sequencing and phylogenetic analysis

A fragment of the hMPV M gene was amplified with primers hMPV-MR1 (see above) and hMPV-MF2 (5'-ATGGAGTCTC-TATCTAGTAGACA-3'), which amplify a 331-bp fragment from nucleotides 2165–2495 [20]. To compare with the M-gene phylogenetic reconstruction, a 450-bp fragment of the hMPV fusion (F) gene [15] was amplified with primers MPVF1f (5'-CCTTGGACCTTAATGACAGT-3') and MPVF1r (5'-GTCTTGACTACCTTTGCTACCTTTG-3'), corresponding to nucleotides 3704–4153. Automated DNA sequencing was performed on both strands with an ABI Prism 3730XL DNA Analyser (Perkin Elmer-Applied Biosystems, Warrington, UK) as described previously [21]. The nucleotide sequences were aligned with known sequences from GenBank, and multiple-sequence alignments were assembled in MacClade 4 [22]. Modeltest [23] was employed to select the best evolutionary model by means of hierarchical likelihood ratio tests. In addition, the Logdet paralinear model was applied to the F-gene data. Phylogenetic reconstructions and bootstrap analysis (1000 replicates) were carried out in Paup* 4.0 [24] by distance and maximum-likelihood methods.

RESULTS

Clinical and virological findings

In total, 122 respiratory specimens obtained from 122 children aged ≤5 years were negative for hMPV RNA by RT-PCR. Testing for respiratory viruses by direct immunofluorescence and cell culture showed that 12 of these specimens were positive for hRSV, five for influenza A virus, three for parainfluenza virus type 3, and one for parainfluenza virus type 1.

Of 171 BAL samples from 168 adult patients with RTI, four (2.4%) were positive for hMPV RNA. No other respiratory viruses were detected by direct immunofluorescence or cell culture in these four samples. Overall, 91.2% of samples were negative for all viruses tested. The clinical
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Table 1. Clinical features of four adults with human metapneumovirus (hMPV)-associated respiratory tract infection

| Lab. no. (patient no.) | Age (years) | Sex | Underlying disease | Presenting symptoms and signs | Radiological findings | Histological findings | Other laboratory findings | Diagnosis |
|-----------------------|-------------|-----|--------------------|-------------------------------|-----------------------|-----------------------|--------------------------|-----------|
| ROI82                 | 72          | Male| Squamous cell carcinoma of lung | Fever, cough, rhinorrhea, wheezing, crepitations | Consolidation and atelectasis of right lower lobe | Bronchial biopsy showed evidence of squamous cell carcinoma | WBC 9/mm³, Sputum—no bacterial growth | Squamous cell carcinoma with upper respiratory tract infection caused by hMPV |
| ROI135                | 48          | Female| Asthma | Cough, rhinorrhea, wheezing | Normal | Bronchial washings showed benign respiratory tract elements with scanty neutrophils | WBC 6/mm³, Sputum—no bacterial growth | Exacerbation of asthma due to hMPV infection |
| ROI48                 | 66          | Male| Chronic obstructive airways disease | Fever, cough, rhinorrhea, hypotension, tachycardia, tachypnoea | Right lower lobe consolidation, left basal consolidation | Not done | WBC 25/mm³, Streptococcus pneumoniae in blood cultures, sputum | Bacterial pneumonia and hMPV upper respiratory tract infection superimposed on chronic obstructive airways disease |
| ROI16                 | 63          | Male| Chronic lymphocytic leukaemia | Fever, rhinorrhea, cough | Normal | Not done | WBC—absolute lymphocyte count < 300/mm³, Sputum—no bacterial growth | hMPV-associated upper respiratory tract infection in immunocompromised patient with lymphocytopenia |

WBC, white blood cell count.

features on admission to St James’s Hospital Dublin of the four adults in whom hMPV RNA was detected are summarised in Table 1. The four patients, aged 48–72 years, were each admitted with symptoms and signs consistent with community-acquired RTI. There was no epidemiological relationship between these patients, with each one presenting at least 6 weeks apart. All four had at least one underlying illness (Table 1). The most common clinical findings were fever (3/4), cough (4/4) and rhinorrhea (3/4). Two of the four patients (Table 1; patients 2 and 4) had clinical presentations consistent with upper RTI, but without radiological evidence of lower respiratory tract involvement. Patient 4 was lymphocytopenic (absolute lymphocyte count < 300/mm³) following treatment with alemtuzumab for refractory chronic lymphocytic leukaemia. Patients 1 and 3 had radiological evidence suggestive of lower RTI. However, patient 1 had right lower-lobe radiological changes, associated with right bronchial squamous cell carcinoma, and a clinical presentation consistent with an upper RTI associated with hMPV, with underlying lung neoplasia. Patient 3 had bilateral pneumonia and sepsis caused by Streptococcus pneumoniae, and required antibiotic treatment and mechanical ventilation in the intensive care unit. No patient died as a result of these episodes of RTI.

Eleven (BAL) specimens from five other patients were positive for other respiratory viruses following routine respiratory virology investigations. Two patients with upper RTI following stem-cell transplantation were positive on direct immunofluorescence and cell culture for influenza A virus. Although these patients became asymptomatic, they remained positive by both methods on repeated testing for influenza A virus for 5 weeks following a 7-day course of treatment with oseltamivir, consistent with asymptomatic shedding of the virus in severely immunocompromised hosts. One patient with underlying asthma was positive by direct immunofluorescence and cell culture for influenza B virus, while two other elderly patients were positive for parainfluenza type 3 virus.

Phylogenetics

In total, 229 hMPV F-gene sequences of the same fragment length as those produced from the Irish isolates (450 bp) were downloaded from the GenBank database. After identical, non-Irish, sequences were removed, 66 sequences remained for analysis. Trees reconstructed using maximum-likelihood or distance methods under the best model of sequence evolution, chosen by Modeltest (HKY + I + G), did not show cluster II to be monophyletic. However, the neighbour-joining tree, drawn using Logdet distances, showed a highly supported monophyletic grouping for this cluster (Fig. 1). Cluster I was monophyletic under all analyses with high bootstrap support, and all hMPV sequences fell into a single cluster with
APV-C as outgroup. All Irish isolates were part of cluster IIB under all analyses, but did not form a separate Irish sub-cluster, although ROI16 was more closely related to NI143 than either was to the other Irish isolates. Three of the four Irish sequences were identical (ROI48, ROI82 and ROI135).

In total, 25 hMPV M-gene sequences from GenBank of the same length as the Irish sequences (287 bp) were included for analysis, including three outgroup sequences. Identical sequences were collapsed (except for the seven Irish isolates), leaving 25 sequences for analysis. The maximum-likelihood phylogeny is shown in Fig. 1, with bootstrap values derived from neighbour-joining. Clusters I and II were clearly separated on this tree, each with high bootstrap support. Irish isolate ROI16 was identical to NI143, while isolates ROI48, ROI82 and ROI135 were also identical. Again, all isolates were within genetic group IIB.

DISCUSSION

The present study is the first to document the occurrence of hMPV in the Republic of Ireland. The study confirmed that hMPV infection can occur in adults, including elderly and immunocompromised patients. Since 100% seroprevalence to hMPV, with stable neutralising titres, has been reported in young adults and older individuals [6], the hMPV-associated infections reported in the present study probably reflect reinfection with the virus. The relatively low detection rate (2.4%) of hMPV in this study is not unusual. Thus, three samples from a random selection of 200 NPAs collected during 2001 in an Australian study [14], and nine (2.2%) of 408
nasal and throat swabs tested in the UK [25], were positive by RT-PCR for hMPV RNA.

The clinical features associated with hMPV infection in adults in this study were not sufficiently distinctive to differentiate hMPV from other respiratory viruses. The exacerbation of asthma described here supports the association of hMPV infection with asthma. While viruses such as hRSV and rhinoviruses have been suggested as important triggers of asthma exacerbation in older children and adults, a possible association between hMPV infection and asthma has been indicated [7,13]. The finding in this study warrants further research on this association in Irish patients.

While no other common respiratory virus was identified in the four Irish adults in whom hMPV RNA was detected by RT-PCR, severe pneumococcal sepsis occurred in one patient with chronic obstructive airways disease. Whether hMPV infection predisposed this individual to invasive S. pneumoniae infection is unknown.

hMPV can cause severe RTI in immunocompromised individuals [26], but the present study found only mild illness in an individual with underlying squamous cell carcinoma, and also in a haematology patient with severe lymphocytopenia following treatment with alemtuzumab (Table 1). Both patients presented with upper RTI, but did not develop pneumonia. In other studies, hMPV was the sole pathogen detected in three patients with underlying acute lymphoblastic leukaemia who presented with RTIs and subsequently died [10,11,27]. However, another hMPV-infected immunocompromised child with acute lymphoblastic leukaemia recovered without specific antiviral treatment [13]. These differences in clinical presentation may be related to different biological properties of strains of hMPV. However, while differences in virulence of hRSV subgroups A and B have been reported [28], no data exist to compare the virulence of the two known genetic lineages of hMPV.

Surprisingly, hMPV was not detected in 122 respiratory specimens obtained from children aged ≤5 years who were hospitalised with RTIs. The frequency of hMPV infection in hospitalised children was reported as 5.5% in one study, with hMPV-positive children presenting with mild-to-severe RTIs [13]. Furthermore, other data indicate that hMPV accounts for at least 5–7% of RTIs in hospitalised children [26]. Specimens, including BALs, collected in the present study were obtained in the same period as BALs from Irish adults with hMPV-associated infections. Improper storage of the specimens from children is very unlikely. Retrospective testing for hRSV by RT-PCR using 66 of the stored specimens proved more sensitive than biological methods (18.1% vs. 12%; results not shown). Furthermore, known hMPV-positive NPAVs from a hospital in the UK were used successfully as positive controls, even though the specimens had been frozen and thawed on at least two occasions. An underestimation of the percentage of hMPV-positive samples was observed when only specimens negative for other respiratory viruses were tested for hMPV [17]. It is also possible that a new lineage of hMPV, not amplified by the primers used for RT-PCR, could have caused RTIs in young Irish children. However, phylogenetic analysis of the hMPV isolates from the four adult Irish patients showed that the circulating strains in 2003–2004 belonged to the well-recognised 1B lineage. It is also possible that the occurrence of hMPV shows a seasonal variation, perhaps with sporadic epidemics. Temporal variation in the pattern of hMPV infection has been demonstrated in young and elderly adults, with hMPV illness rates of 1.5% and 7.0% in the winter seasons of 1999–2000 and 2000–2001, respectively [9]. Furthermore, another study [19] showed that the incidence of hMPV infection in infants varied substantially from season to season over a 3-year period, with a positivity rate of 7% in 2001, compared to 40% in 2000 and 2002.

In conclusion, these results provide further evidence of the potential importance of hMPV in RTI in Irish adults of all ages; indeed it may account for a significant proportion of individuals hospitalised with RTI. Further studies of the prevalence, risk factors and temporal pattern of hMPV infection will be needed to determine the overall clinical significance of this virus and its impact on healthcare services.

NUCLEOTIDE SEQUENCE ACCESSION NUMBERS

hMPV ROI16 F gene, AY789626; NI143 F gene, AY789627; ROI48 F gene, AY789628; NI106 F gene, AY789629; NI107 F gene, AY789630; ROI16 M gene, AY789631; ROI48 M gene, AY789632; NI106 M gene, AY789633; NI107 M gene, AY789634.
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