MAJOR ARTICLE

Prevalence and Clinical Symptoms of Human Metapneumovirus Infection in Hospitalized Patients

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During a 17-month period, we performed retrospective analyses of the prevalence of and clinical symptoms associated with human metapneumovirus (hMPV) infection, among patients in a university hospital in The Netherlands. All available nasal-aspirate, throat-swab, sputum, and bronchoalveolar-lavage samples ($N = 1515$) were tested for hMPV RNA by reverse-transcriptase polymerase chain reaction. hMPV RNA was detected in 7% of samples from patients with respiratory tract illnesses (RTIs) and was the second-most-detected viral pathogen in these patients during the last 2 winter seasons. hMPV was detected primarily in very young children and in immunocompromised individuals. In young children, clinical symptoms associated with hMPV infection were similar to those associated with human respiratory syncytial virus (hRSV) infection, but dyspnea, feeding difficulties, and hypoxemia were reported more frequently in hRSV-infected children. Treatment with antibiotics and corticosteroids was reported more frequently in hMPV-infected children. From these data, we conclude that hMPV is an important pathogen associated with RTI.

Acute respiratory tract illnesses (RTIs) are the most common diseases experienced by people of all ages worldwide [1]. In young children, human respiratory syncytial virus (hRSV) is the most common cause of RTI [1–3]. Recently, a previously unknown pneumovirus was isolated from nasopharyngeal-aspirate samples obtained from children with RTIs in The Netherlands [4]. Those preliminary data indicated that the clinical symptoms of the children were similar to those of patients with RTI caused by hRSV, ranging from mild respiratory problems to severe cough, bronchiolitis, and pneumonia, often accompanied by high fever, myalgia, and vomiting. Some of those patients were hospitalized and required mechanical ventilation. On the basis of the organization of the viral genome and sequence identity to the *Metapneumovirus* avian pneumovirus, also known as turkey rhinotracheitis virus [5, 6], the virus was named human metapneumovirus (hMPV) [4, 7]. As a result, the *Pneumovirus* and *Metapneumovirus* genera within the subfamily *Pneumovirinae* (family *Paramyxoviridae*) now contain the human pathogens hRSV and hMPV, respectively. Serological surveys have indicated that the prevalence of hMPV in the Dutch population is high, because virtually all children tested were seropositive before the age of 6 years [4]. Recently, hMPV was also detected in children, adults, elderly individuals, and immunocompromised individuals with RTI, in Australia, North America, the United Kingdom, and Finland [8–13], indicating that it is a common and ubiquitous human pathogen.

In the present study, all respiratory samples obtained from patients in the university hospital in Rotterdam, over a period of 17 months, were tested for the presence of hMPV RNA by reverse-transcriptase polymerase chain reaction (RT-PCR). We compared the clinical symptoms of hMPV-infected patients with those of patients infected with other respiratory viruses. Our data indicate that, in an academic hospital setting, the prevalence and clinical severity of hMPV infections are slightly lower than those of hRSV infections. Never-

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theless, we conclude that hMPV is an important cause of RTI, primarily in infants and immunocompromised individuals, during the winter months.

SUBJECTS, MATERIALS, AND METHODS

Data collection. Between 28 September 2000 and 16 February 2002, 645 throat-swab, 844 nasopharyngeal-aspirate, 18 sputum, and 297 bronchoalveolar-lavage samples were obtained at the different wards of the university hospital in Rotterdam and were sent to the diagnostic virology laboratory. For 1515 of these samples (573 throat-swab, 661 nasopharyngeal-aspirate, 12 sputum, and 269 bronchoalveolar-lavage samples), sufficient material was available for hMPV testing. Of these samples, 45% were sent specifically for testing for respiratory viruses, including influenza A and B viruses, hRSV, human parainfluenza virus (PIV) types 1–4, adenovirus, and rhinovirus. Routine virological testing for respiratory pathogens was performed by use of a combination of direct immunofluorescence (DIF) on cells present in the respiratory specimen, virus isolation in cell cultures, and immunofluorescence (IF). Cell lines used for virus isolation included human embryonal kidney cells, tertiary monkey kidney cells, Madine Darby canine kidney cells, Vero cells, and Hep-2 cells. After diagnosis of enterovirus infection by virus isolation, IF, and/or DIF, a PCR was performed with rhinovirus-specific primers [14]. Of the throat-swab samples, 622 were obtained from transplant recipients and were submitted for testing for herpes simplex virus (HSV) types 1 and 2, cytomegalovirus, and Epstein-Barr virus and were used here as controls. Throat-swab samples were collected in virus transport media [15], and other samples were stored without virus transport media. All samples were kept at 4°C during processing and subsequently were stored for prolonged periods at −70°C.

Detection of hMPV RNA by RT-PCR. RNA was isolated from a 40–200-μL sample by use of a High Pure RNA isolation kit (Roche Diagnostics), according to the manufacturer's instructions. RT-PCRs were performed by use of a 1-tube reaction with primers L6 (5′ CAT GCC CAC TAT AAA AGG TCA G 3′) and L7 (5′ CAC CCC AGT CTT TCT TGA AA 3′), amplifying a conserved fragment of 170 nt in the polymerase gene. This RT-PCR was optimized with respect to enzymes, buffer components, and cycling parameters and was found to be 10–100-fold more sensitive than inoculation of tertiary monkey kidney cells with titrated virus stocks (data not shown). Moreover, the assay can detect the genetically diverse hMPV isolates described elsewhere [4]. The L gene was chosen as a target for RT-PCR because of nucleotide-sequence conservation, thereby reducing the chance of missing genetic lineages of hMPV that have not been detected previously. PCRs were performed in a 50-μL volume containing 50 mmol/L Tris Cl (pH 8.5), 50 mmol/L NaCl, 4.5 mmol/L $MgCl_2$, 0.2 μmol/L each primer, 0.6 mmol/L each dNTP, 20 U of RNAsin, 10 U of avian myeloblastosis virus RT, and 5 U of Taq DNA polymerase (all enzymes from Promega). Thermocycling was performed in an MJ PTC-200 apparatus (MJ Research) with the following cycling parameters: 45 min at 42°C and 5 min at 95°C once; 1 min at 95°C, 2 min at 45°C, and 3 min at 72°C repeated 40 times; 10 min at 72°C once; and storage at 4°C. PCR products were analyzed by dot-blot hybridization, as described elsewhere [15], by use of a biotinylated oligonucleotide (5′ CTG TTA ATA TCC CAC ACC AGT GGC ATG C 3′).

Clinical evaluation and statistical analysis. The medical files of hMPV-infected children were scored by pediatricians, as described elsewhere [16]. In brief, scoring lists included demographic data (sex, age, number of children and parents in the family, gestational age, weight at birth, breast-feeding status, and underlying disease of the patient or in the family), clinical symptoms (cough, rhinitis, body temperature, dyspnea, wheezing, feeding difficulties, retractions, respiratory rate, pulse, and cyanosis), laboratory testing (oxygen saturation, pCO_2, hemoglobin, hematocrit, platelet counts, levels of C-reactive protein, and chest X-ray results), and intervention and follow-up (artificial respiration, administration of oxygen, use of bronchodilators, and administration of antibiotics and corticosteroids). The medical files for 25 hRSV-infected children were scored for comparison. For each hMPV-infected child, an hRSV-infected child of the same sex who was hospitalized during the same period (2–3 weeks range) and was closest in age to the hMPV-infected child was selected. Demographic and clinical variables were calculated for the hMPV and the hRSV groups, respectively, and were expressed as point estimates, using percentages for dichotomous variables or means with 95% confidence intervals (CIs) for continuous variables, by use of SPSS for Windows (version 9.0; SPSS). For each variable, the difference between both point estimates with 95% CIs (hMPV vs. hRSV) was calculated by use of the software program Confidence Interval Analysis (version 1.0; BMJ Publishing Group) [17]. If the CI of the difference did not include zero, the corresponding point estimates were regarded as significantly different.

RESULTS

Prevalence of hMPV. Fifty-two samples obtained from 46 patients (28 males and 18 females) tested positive for hMPV by RT-PCR. For 44 of these 46 hMPV-positive patients, the samples had been obtained specifically because of respiratory symptoms, including fever, cough, rhinorrhea, dyspnea, tachypnea, rhinitis, bronchitis, bronchiolitis, and pneumonia (see below). The throat-swab samples obtained from the 2 other

patients were sent for HSV testing, but these patients may also have experienced a mild or subclinical RTI.

Of the 46 hMPV-infected patients, 37 were hospitalized in the wards of the children's hospital, 4 were hospitalized in the nursing rooms of the cancer center, and 5 were hospitalized in other departments of Erasmus Medical Center (including the departments of neurology, hematology, and internal medicine). The age distribution of the patients with RTI who tested positive for hMPV is depicted in figure 1. Most of the hMPV-positive patients were children <2 years old who did not have illnesses other than RTI. Of the hMPV-positive patients who were >5 years old, most had other diseases (e.g., cystic fibrosis, leukemia, and non-Hodgkin lymphoma) or had recently received bone-marrow or kidney transplants. These latter hMPV infections may, therefore, have been associated with host immunosuppression. Although the overall age distribution of hMPV-positive patients was found to be fairly similar to that of hRSV-positive patients in this same cohort (figure 1*A*), hMPV was found significantly less frequently in children <2 months old than was hRSV. Of the 31 hMPV-positive children <2 years old, only 4 (13%) were <2 months old, whereas 43 (35%) of the 122 hRSV-positive children <2 years old were also <2 months old (rate difference, −22.3%; 95% CI, −36.9% to −7.8%).

We obtained, from 4 hMPV-infected patients, >1 sample that tested positive for hMPV RNA. Three of these patients were 2-, 11-, and 16-month-old children, and virus was detected in nasal-aspirate samples obtained from them 6, 3, and 6 days apart, respectively. The fourth patient was a 36-year-old bone marrow–transplant recipient who was recovering from varicella-zoster pneumonia and subsequent infection with influenza B virus; 4 of this patient's respiratory-tract specimens, obtained during a 19-day period, tested positive for hMPV.

hMPV was detected predominantly in samples obtained during the winter months (figure 2). The seasonal distribution of hMPV-positive samples was found to be largely similar to that of hRSV-positive samples, with the peak of virus detection in December and January, in both the 2000–2001 and 2001–2002 winter seasons. hMPV may be slightly less seasonal than hRSV, since the number of hRSV diagnoses in these 2 seasons was higher than the number of hMPV diagnoses.

To compare the effect of hMPV to that of other respiratory viral pathogens more quantitatively, we compared the diagnostic outcome for 685 specimens sent to the diagnostic virology laboratory specifically for respiratory pathogen testing and for which sufficient material was available to complete all tests. The routine testing for respiratory pathogens in our diagnostic virology laboratory included influenza virus types A and B, hRSV, PIV types 1–4, rhinovirus, and adenovirus. hRSV was detected most frequently, in 126 (18%) of 685 samples obtained from patients with RTI, and hMPV was the second-most-detected viral pathogen, in 48 (7%) of 685 samples (figure 3). Thus, from patients with RTI, hMPV was isolated more frequently than PIV (9, 2, 7, and 0 samples were positive for PIV types 1–4, respectively), adenovirus (8 samples), rhinovirus (28 samples), and influenza viruses types A and B (11 and 7 samples, respectively). It is important to note that the sensitivities of detection methods for the range of viral pathogens may be different, making it difficult to compare the contribution of each viral pathogen to the RTI quantitatively. It should also be noted that the influenza virus epidemics in these seasons were milder than those in previous years. From 6 of the hMPV-positive samples, we isolated another respiratory virus: hRSV was isolated from 3 samples, and rhinovirus, influenza A virus, or adenovirus each was isolated from 1 sample. hMPV RNA was detected in only 2 of 622 samples obtained from patients who did not have RTI, suggesting that subclinical hMPV infection is rare in hospitalized patients.

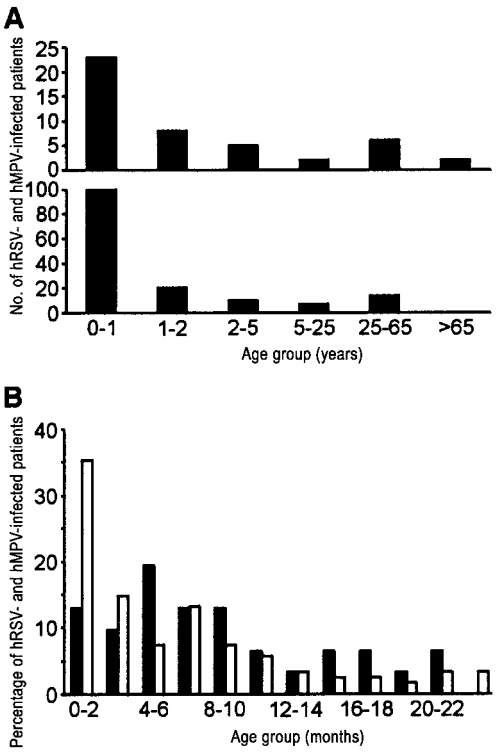

Figure 1. *A,* Age distribution of patients infected with human metapneumovirus (hMPV) *(top)* or human respiratory syncytial virus (hRSV) *(bottom). B,* Age distribution of hMPV-infected *(black bars)* and hRSV-infected *(white bars)* children <2 years old. The proportion of children in each age group was calculated relative to the no. of children <2 years old.

Clinical symptoms. We next examined the medical files of hMPV-infected children. Because the underlying disease for most hMPV-infected adults could have obscured the hMPV-related RTI, we limited these analyses to otherwise healthy children. Medical files were available for 25 of these children, and the medical files for 25 selected hRSV-infected children were used as controls.

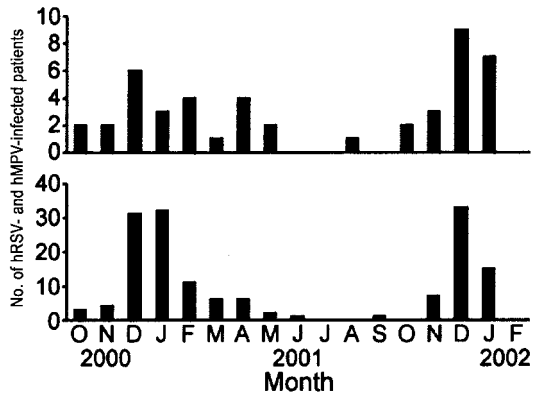

Figure 2. Detection of human metapneumovirus (hMPV) *(top)* and human respiratory syncytial virus (hRSV) *(bottom),* per month, between September 2000 and March 2002.

Demographic data were similar for the hMPV- and hRSV-infected groups (table 1). There were slightly more boys than girls in both groups. Although not statistically significant, the mean age was slightly higher in the hMPV-infected group than in the hRSV-infected group. Virtually all the children in both groups who had siblings were the youngest children in the family, and the household compositions (numbers of children and adults) were similar. The pre- and perinatal data for the 2 groups (e.g., gestational age, birth weight, and breast-feeding status) were also not significantly different. In contrast, history of asthma was more often associated with hMPV than with hRSV infection: 16% of the hMPV-infected patients had asthma and 67% of them had a family member with asthma, whereas none of the hRSV-infected patients had asthma and only 30% of them had a family member with asthma.

The clinical symptoms observed for the hMPV-infected children included cough (72%), rhinitis (80%), fever (61%), dyspnea (28%), wheezing (24%), feeding difficulties (36%), retractions (60%), hyperventilation (42%), tachycardia (23%), and cyanosis (8%). Most of these symptoms were observed with similar frequency in the group of hRSV-infected children. However, dyspnea, feeding difficulties (primarily, decreased intake of food), and hypoxemia were reported significantly more frequently in hRSV-infected children than in hMPV-infected children. Platelet counts, relative white blood cell counts, and levels of C-reactive protein were similar for both groups. For 62% of the children, an X-ray of the lungs revealed atelectasis, hyperinflation, and infiltrates as the most common abnormalities, in both groups of patients.

In agreement with the finding of a high number of hRSV-infected patients with hypoxemia, oxygen was administered to significantly more hRSV-infected patients than hMPV-infected patients (64% vs. 36%). In contrast, hMPV-infected patients were treated more often with bronchodilators (36% vs. 24%), antibiotics (60% vs. 12%; statistically significant), and corticosteroids (20% vs. 4%) than were hRSV-infected patients (table 1). The mean duration of hospital stay was similar for both patient groups (6–7 days). It should be noted that, for this latter analysis, 3 hMPV-infected children were excluded because of additional morbidity that was presumably unrelated to the hMPV infection, such as subsequent infection with other pathogens.

DISCUSSION

Since the discovery of hMPV, several studies have provided important information on hMPV epidemiology, clinical symptoms associated with hMPV infection, and the patient groups that are at risk for hMPV infection [4, 8–13]. From these studies, it is now becoming increasingly clear that hMPV is an important human pathogen associated with RTI in young children, immunocompromised individuals, elderly individuals, and, to a lesser extent, other populations. In the present study, we analyzed the relative contribution of hMPV to RTI, in a university hospital setting, and compared the clinical signs with those caused by infection with a related pathogen, hRSV.

In the 2000–2001 and 2001–2002 winter seasons in The Netherlands, hMPV and hRSV were both found primarily in December and January; they were rarely detected in the summer months. It should be noted that, during these years, hMPV infections were temporally distributed somewhat more equally than were hRSV infections. The populations of patients most affected by the 2 viruses—children, immunocompromised individuals, and elderly individuals—were also quite similar, as was the overall age distribution of the infected patients. However, it is interesting to note that hRSV was detected more frequently in very young children than was hMPV; 35% of hRSV-infected children <2 years old were also <2 months old, compared with only 13% of the hMPV-infected children <2 years old. Whether this observed difference is a true reflection

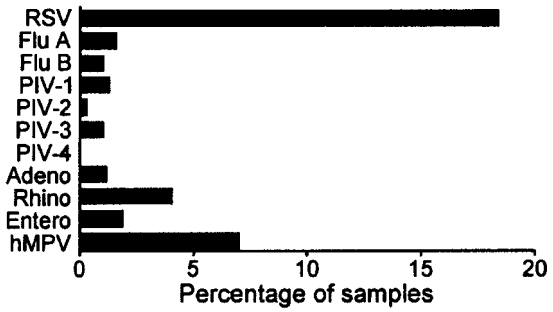

Figure 3. Comparison of the frequency of detection of human metapneumovirus (hMPV) with those of other respiratory viral pathogens, in 685 samples obtained from patients with respiratory tract illness. Adeno, adenovirus; Entero, enterovirus; Flu, influenza virus; PIV, parainfluenza virus; Rhino, rhinovirus; RSV, respiratory syncytial virus.

Table 1. Data from medical files of patients infected with human metapneumovirus (hMPV) and human respiratory syncytial virus (hRSV).

Characteristic	hMPV		hRSV		95% CI of difference[b]
	No. of patients	Point estimate[a]	No. of patients	Point estimate[a]	
Demographics					
Sex, % male	25	64.00%	25	56.00%	−19.1 to 35.1
Age, months	25	15.0 (8.3–21.6)	25	11.2 (5.4–16.9)	−4.8 to 12.4
Patient with asthma	25	**16.00%**	25	**0.00%**	1.6–30.4
Asthma in family	9	66.70%	20	30.00%	−0.1 to 73.4
Clinical symptoms and laboratory tests					
Cough	25	72.00%	25	76.00%	−28.3 to 20.3
Rhinitis	25	80.00%	25	72.00%	−15.6 to 31.6
Fever	18	61.10%	23	47.80%	−17.1 to 43.7
Dyspnea	25	**28.00%**	25	**76.00%**	−72.3 to −23.7
Wheezing	25	24.00%	25	32.00%	−32.8 to 16.8
Feeding difficulties	25	**36.00%**	25	**76.00%**	−65.2 to −14.8
Retractions	25	60.00%	25	64.00%	−30.9 to 22.9
Hyperventilation	19	42.10%	24	37.50%	−24.9 to 34.1
Tachycardia	17	23.50%	23	52.20%	−57.3 to 0.1
Cyanosis	25	8.00%	25	20.00%	−30.9 to 7.0
Hypoxemia	19	**47.40%**	22	**81.80%**	−62.1 to −6.8
Hypercapnia	14	42.90%	17	41.20%	−33.2 to 36.6
Decreased hemoglobin	22	22.70%	23	13.00%	−12.6 to 32.0
Decreased hematocrit	21	33.30%	21	19.00%	−12.0 to 40.5
Thrombocytosis	21	9.50%	19	31.60%	−46.4 to 2.3
Illness score[c]	25	**0.38 (0.33–0.44)**	25	**0.50 (0.44–0.56)**	−0.20 to −0.03
C-reactive protein	18	35.9 (2.8–69.1)	20	39.2 (15.7–62.7)	−41.8 to 35.3
Chest X-ray performed	25	60.00%	25	64.00%	−30.9 to 22.9
Atelectasis	15	40.00%	16	18.80%	−10.1 to 52.6
Hyperinflation	15	33.30%	16	43.80%	−44.5 to 23.6
Infiltrate	15	33.30%	16	31.30%	−30.9 to 35.0
Bronchial thickening	15	0.00%	16	12.50%	−28.7 to 3.7
Intervention and follow-up					
Artificial respiration	25	12.00%	25	8.00%	−12.6 to 20.6
Oxygen administration	25	**36.00%**	25	**64.00%**	−54.6 to −1.4
Bronchodilators	25	36.00%	25	24.00%	−13.2 to 37.2
Antibiotics	25	**60.00%**	25	**12.00%**	25.0–71.0
Corticosteroids	25	20.00%	25	4.00%	−1.5 to 33.5
Time in hospital, days[d]	15	6.6 (4.6–8.6)	19	6.2 (4.6–7.7)	−1.9 to 2.8

NOTE. The groups of hMPV- and hRSV-infected children both included 25 patients, but, because some of the files were not completed, the no. of patients used for comparison may be lower. The following dichotomous variables were calculated from continuous variables by use of reference thresholds derived from healthy children: fever (body temperature ≥38.5°C), hyperventilation (respiratory rate >60 or >40 breaths/min for children <1 or >1 year old, respectively), tachycardia (pulse >175, >177, >163, and >143 for children 0–3, 3–6, 6–12 and >12 months old, respectively), hypoxemia (oxygen saturation <95%), hypercapnia (pCO_2 >5.6 kPa), decreased hemoglobin (<8.1 or <6.6 mmol/L for children <1 or >1 month old, respectively), decreased hematocrit (<0.42 or <0.33 L/L for children <1 or >1 month old, respectively), and thrombocytosis (>390×10^9 or >473×10^9 platelets/L for children <1 or >1 month old, respectively). CI, confidence interval.

[a] Point estimates are given as percentages, for dichotomous variables, or as mean (95% CI), for continuous variables.

[b] If the 95% CI did not include 0, the corresponding point estimates were considered to be statistically significant and appear in bold type.

[c] Each of the 15 clinical symptoms above was scored (1 and 0 for presence and absence, respectively), and the sum was divided by the no. of recorded symptoms. Thus, the illness score could range from 0.00 (no symptoms present) to 1.00 (all symptoms present).

[d] Three cases of hMPV infection were excluded from this comparison because of additional morbidity unrelated to the hMPV infection.

of the differences in biological properties of the respective viruses requires further confirmation. During the 2 winter seasons under study, the 2 genetic lineages of hMPV that were identified previously [4] were cocirculating in The Netherlands (data not shown), as has been described recently for other countries [9, 11, 13]. Differences in the pathogenicity of viruses belonging to the hRSV subgroups A and B have been described by some research teams but not by others [18–22]. Unfortunately, at present, our data set is too limited to compare the relative prevalence and pathogenicity of the distinct lineages of hMPV in a similar fashion.

Comparison of the medical files of hMPV-infected children with those of hRSV-infected children revealed that the clinical symptoms associated with these viruses were quite similar. Dyspnea, feeding difficulties, and hypoxemia were recorded more frequently in hRSV-infected children than in hMPV-infected children, but all other recorded symptoms were found at the same frequency in both groups. For each of the children, in both groups, we calculated an illness score, defined as the number of clinical symptoms the children had, divided by the total number of symptoms that were recorded for each child (table 1). This analysis revealed that the hMPV-infected children had, on average, 38% of the recorded symptoms, whereas hRSV-infected children had 50% of the symptoms (statistically significant), suggesting that hRSV may be slightly more pathogenic than hMPV. The higher proportion of children <2 months old in the hRSV group did not have a serious effect on this observation; reanalysis of the data, excluding children <2 months old, still resulted in a statistically significant difference for the illness scores.

It is important to note that subclinical hMPV infections appear to be rare; we detected hMPV RNA in only 2 of 622 samples obtained from patients who did not have RTI. This finding, as well as the observation that hMPV may cause mild RTI in experimentally infected macaques [4], is indicative of hMPV being a causative agent of RTI. In agreement with these observations, we did not detect any other pathogens in the majority of patients with hMPV-related RTI. Three hMPV-infected patients were also positive for hRSV, and 3 were positive for either influenza A virus, adenovirus, or rhinovirus. On the basis of the similar seasonal distribution of hMPV and hRSV infections (figure 2), dual infection with these 2 viruses is not unlikely.

The statistical significance of the difference between the proportion of children with asthma in the hMPV group and that of children with asthma in the hRSV group requires confirmation in larger scale studies in the future. Numerous studies have provided evidence for a link between hRSV infection in early childhood and subsequent manifestations of asthma [23, 24], although this issue is still being studied [25]. The link between hMPV infection and asthma appears to be different from that suggested for hRSV infection. It will be of interest to further elucidate the underlying mechanisms for the possible association between asthma and either hRSV or hMPV infection, in future studies.

It was not surprising that antibiotics and corticosteroids were administered to hMPV-infected children more often than to hRSV-infected children. Because hRSV diagnostics were performed in real time, physicians were able to choose not to give antibiotics and corticosteroids after a positive hRSV diagnosis. However, because, at the time of hospitalization, no etiological agent had been identified in children with RTI associated with hMPV infection, physicians continued treatment with antibiotics and corticosteroids, to control potentially unidentified bacterial infections. This indicates that the inclusion of hMPV in diagnostic testing of patients with RTI may reduce unnecessary use of antibiotics and corticosteroids. Moreover, hMPV diagnostic testing may reduce virus transmission between children in hospital wards, through isolation or alternative measures currently used to limit the spread of hRSV. For a virus that is not easily detected by virus isolation in the laboratory, it will be of great importance that rapid, sensitive, and reproducible diagnostic tests be developed. Our RT-PCR procedure, which is based on the amplification of a conserved sequence in the polymerase gene, proved to be more sensitive than virus isolation and can detect genetically distinct hMPV strains. However, RT-PCRs based on the N gene may take advantage of the transcriptional gradient used by paramyxoviruses and, therefore, may be even more sensitive. In addition, monoclonal antibodies recognizing conserved hMPV epitopes will be useful for rapid virus diagnostics by use of IF or DIF techniques currently used for diagnosing infections with other virus pathogens, including hRSV. It will be important to conduct a wide range of prospective and retrospective studies to obtain a better estimate of the incidence, prevalence, and clinical effect of hMPV in different populations, the full spectrum of hMPV diseases, and risk factors that may be associated with severe hMPV disease.

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