

Prevalence and Clinical Characteristics of Human Metapneumovirus Infections in Hospitalized Infants in Spain

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Summary. Human metapneumovirus (hMPV), a condition recently described in the Netherlands, causes lower respiratory infections, particularly in young children and among the elderly. The objective of this study was to describe the characteristics of hMPV infections in hospitalized infants <2 years of age and to compare them to those of infections caused by respiratory syncytial virus (RSV). A prospective study was conducted on the clinical characteristics of infants admitted to hospital for respiratory infection through 5 years. Simultaneous detection of influenza A, B, and C viruses, RSV, and adenoviruses was performed in clinical samples by multiple reverse transcription nested-PCR assay. The presence of hMPV was tested in all samples using two separate RT-PCR tests. Some respiratory virus was detected in 70.5% of the 1,322 children included in the study. hMPV was found in 101 of the positive nasopharyngeal aspirates (10.8%), and was the most common virus after RSV and rhinovirus. Peak incidence was found in March. Over 80% of children were <12 months. The more common diagnoses were bronchiolitis (49.5%) and recurrent wheezing (45.5%). Fifty-four percent of cases required oxygen therapy and, one percent, assisted ventilation. Thirty percent were co-infections, with clinical characteristics indistinguishable from single infections. Seventy-one hMPV single infections were compared to 88 RSV single infections. hMPV infections were significantly more frequent than RSV in infants older than 6 months ($P=0.04$). Recurrent wheezing was diagnosed more frequently in hMPV patients ($P=0.001$). All other variables tested were similar, in both groups. hMPV was the third most frequent virus after RSV and rhinovirus in infants <2 years of age, hospitalized for respiratory infection, and was associated with bronchiolitis and recurrent wheezing. hMPV predominantly occurred in spring. Co-infections were frequent and clinically similar to single infections and RSV infections. **Pediatr Pulmonol.** 2006; 41:863–871. © 2006 Wiley-Liss, Inc.

Key words: metapneumovirus; respiratory syncytial virus; infants; respiratory infections.

INTRODUCTION

Human metapneumovirus (hMPV), recently described in the Netherlands,¹ is a RNA virus belonging to the *Paramyxoviridae* family, subfamily *Pneumovirinae*, genus *Metapneumovirus*. Although hMPV was described only few years ago, a 100% seroprevalence has been found in samples obtained 50 years ago.¹ It is, therefore, believed it has been circulating in humans for over five decades. The difficulties in isolating this type of virus from cell cultures have, possibly, delayed its detection as a common pathogen in children's respiratory infections. Over the past 4 years, this virus has been identified in patients with respiratory disease in many countries, including Canada,^{2,3} Finland,⁴ United Kingdom,⁵ Spain,^{6,7} the United States,^{8,9} or France.¹⁰

hMPV is genetically similar to avian pneumovirus, particularly serotype C. Two main groups A and B have been identified to date. The phylogenetic studies conducted by the Dutch group¹¹ show a high similarity to the respiratory syncytial virus (RSV), with which it shares

morphological similarities and similar infective capacity and spectrum of disease. Upper and lower respiratory tract infections, from common colds to pneumonia, are attributed to hMPV, with bronchiolitis being the main clinical sign of primary infection.¹⁰ In addition, it seems to play a significant role in recurrent wheezing episodes in the first years of life.⁷

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From an epidemiological point of view, hMPV infection appears seasonal in its behavior, with a pattern similar to that of the RSV infection, though, at present, few prospective studies exist on its year-round behavior.

We had previously reported hMPV infection in 18 infants aged under 2, admitted to hospital throughout 2002/03.⁷ Our objectives were, now, to estimate the relative contribution of hMPV in the hospitalization of infants with acute respiratory tract infection, here, in Spain, and to define the clinical and epidemiological characteristics of hMPV infection as compared to RSV infection, over 5 years.

MATERIALS AND METHODS

Patients and Samples

This was a substudy of an ongoing prospective investigation on respiratory tract infections in children under 2 years of age, funded by Spain's "Fondo de Investigaciones Sanitarias." The study was conducted at the Pediatrics Department of the "Severo Ochoa Hospital" in Madrid, Spain. All children recruited were under the age of 2 and all, consecutively, admitted to our hospital for acute respiratory infections, year-round, between October 2000 and June 2005. The sole exclusion criterion applied was cystic fibrosis. All parents were duly advised, upon admission, that clinical data related to their children might be used for clinical research purposes. Furthermore, in each case, informed verbal consent was obtained from each of the parents or legal guardians.

Nasopharyngeal aspirates (NPA) were taken from each patient upon admission and sent to the Respiratory Virus Laboratory of the National Microbiology Center (ISCIII, Madrid, Spain). Samples were processed within 24 h of collection, for virological study. Indirect immunofluorescence assays and multiplex RT-nested-PCR were carried out on every sample in order to detect RSV, influenza viruses, and adenovirus.¹² In the last 2 years, a second multiplex RT-PCR was also performed to detect parainfluenza viruses, coronaviruses, enteroviruses, and rhinoviruses.¹³

A 200 μ l aliquot was taken from each NPA sample—all the necessary precautions being taken to avoid contamination—and then frozen, at -70°C , until the analysis for hMPV was carried out.

Amplification of Viral Nucleic Acids for Metapneumovirus Detection

All samples were investigated for hMPV using specific amplification methods, regardless of the existence of a previous positive or negative result for other viruses. Detection of hMPV in respiratory secretions from patients was performed using two separate RT-PCR assays designed in two different genes: one gene encoding for the matrix protein (M), and another gene encoding for the

viral polymerase (L), as described elsewhere.¹⁴ Specific primer pairs were designed to amplify highly conserved regions of both *M* and *L* genes. Nucleic acids from 200 μ l aliquots of original samples were extracted as previously described.¹⁵

For reverse transcription and PCR amplification of the *M* gene, a commercial kit (Qiagen One-Step RT-PCR Kit, Qiagen, Valencia, CA) was used. A subsequent half-nested PCR reaction was performed, using a total of 2 μ l from the first reaction products, which were added to the reaction mixture up to a final volume of 50 μ l. The amplified products were analyzed by gel electrophoresis, stained with ethidium bromide, and a 687 bp band was obtained.

The polymerase gene fragment was amplified using L6 and L7 oligonucleotides.¹⁶ The L6 oligonucleotide was labeled at the 5' end with biotin to allow for detection of the PCR product by chemoluminescence. The specific probe designed to be used in the reverse line blot hybridization with the PCR product was 5' end amino-labeled. For the RT-PCR reaction, the Qiagen One Step RT-PCR kit was also used. The resulting amplified and labeled DNA was subjected to membrane hybridization with a specific probe, after which the membrane was washed and finally treated with a streptavidine-peroxidase conjugate (Roche, Indianapolis, IN). The resulting products were detected by chemoluminescence with ECL detection reagents (Amersham Pharmacia Biotech, Buckinghamshire, UK).

After optimizing assay conditions, both assays showed similar sensitivity, equivalent to 0.1 TCID₅₀ of the hMPV NL/1/99 strain, used by way of a positive control. Specificity of both methods was tested using RNA extracted for studying different respiratory viruses: influenza A, B, and C, RSV A and B, parainfluenza 1, 2, 3, and 4, adenoviruses, and enteroviruses.

NPA were only considered positive for the presence of hMPV when a positive result for this virus was obtained in both independent RT-PCR assays.

Sequencing of hMNV *M* Gene

To check the specificity of the results, *M* gene amplified products obtained from selected positive clinical samples were sequenced in an ABI PRISM 3700 DNA Analyzer (Applied Biosystems, Foster City, CA) at the DNA Sequencing Facility, Genomics Unit (National Microbiology Center). The hMNV sequences for *M* gene (462nt) presented in this article have been deposited in the GenBank database under the accession numbers DQ439949-DQ439961.

Phylogenetic Analysis

Nucleotide sequence alignments were generated with ClustalX 1.81 software. The total length of alignment

was 462nt. Phylogenetic analysis was conducted using MEGA version 3.0 software.¹⁷ The following strains from the database were used for comparisons: AY830147_(BJ1887), AY830146_(BJ1816), AY145271_(CAN00-16), AY145270_(CAN00-15), AY145269_(CAN00-14), AY145268_(CAN00-13), AY145267_(CAN00-12), AY145266_(CAN97-83), AY145265_(CAN97-82), AY145264_(CAN99-81), AY145263_(CAN98-79), AY145262_(CAN98-78), AY145261_(CAN98-77), AY145260_(CAN98-76), AY145259_(CAN98-75), AY145258_(CAN98-74), AY145257_(CAN98-73), AY525843_(NL/1/99), AF371337_(00-1), AY530095_(JPS03-240), AY530094_(JPS03-194), AY530093_(JPS03-187), AY530092_(JPS03-180), AY530091_(JPS03-178), AY530090_(JPS03-176), AY530089_(JPS02-76).

Clinical Assessment and Statistical Analysis

During the hospital stay, and as part of the study, physician filled out a study-questionnaire with the following variables: age, sex, month of admission, clinical diagnosis, history of prematurity and underlying chronic diseases, need for oxygen therapy evaluated through transcutaneous oxygen saturation, axillary temperature $\geq 38^{\circ}\text{C}$, presence of infiltrate/atelectasis in chest X-rays, administration of antibiotic therapy, time of hospital stay, total white blood cell (WBC) count, C-reactive protein (CRP) serum levels, and result of blood culture when performed. In this study, asthma or recurrent wheezing was not considered a chronic underlying disease.

Upper respiratory tract infection (URTI) was diagnosed when rhinorrhea and/or cough was found (in the absence of wheezing, dyspnea, crackling rales, or bronchodilator use), whether with or without fever. All the classic criteria, present in an initial episode of acute onset expiratory dyspnea with previous signs of viral respiratory infection—whether or not this was associated to respiratory distress or pneumonia—were applied in diagnosing *bronchiolitis*.¹⁸ Children with wheezing, breathlessness, and obstruction of the airways, in whom similar episodes had previously been diagnosed and treated by a physician, were diagnosed with *recurrent wheezing*. Cases with both focal infiltrates and consolidation in chest X-rays were, in the absence of wheezing, classified as *pneumonia*.

In order to compare the clinical characteristics associated to hMPV and RSV infections, a random sample of 95 hospitalized infants—aged <2 and documented as RSV-infected—was selected from the same population. Simple random sampling was performed with Excel data analysis function. Cases with dual viral detections were excluded to avoid the potential confounding role of co-infections, and 88 RSV infants were actually selected.

Values are given as percentages for discrete variables, or as mean and standard deviation for continuous variables. Clinical characteristics and laboratory variables were compared applying Student's *t*-test, Mann–Whitney's *U*-test, the χ^2 Test, and Fisher's Exact Test. A two-sided value of $P < 0.05$ was considered statistically significant. All analyses were performed using the Statistical Package for the Social Sciences (SPSS), Version 10.0.

RESULTS

Study Population and Viral Etiologic Agents

The study population consisted of 1,322 hospitalized infants, <2 years of age, with acute respiratory infection. At least 1 respiratory virus was detected in 933 samples (70.5%). Of the positive samples, 599 (64.2%) were RSV, and 101 (10.8%) were hMPV (10.8%, 95% CI: 11.8, 9.8). hMPV accounted for 7.6% of all studied specimens. All other viruses detected were (in descending order of frequency) adenoviruses, influenza viruses, and parainfluenza viruses (Fig. 1). Rhinovirus infections were found in 101 cases (23% of viral detections, from October 2003 to September 2005). Influenza C virus was detected in six cases. Dual or multiple infections were found in 153 cases (16.4%). Thirty of the 101 hMPV infections were co-infections (29.7%).

A higher number of hMPV-positive samples were found in the 2001–02 season as compared to the other four seasons (Fig. 2). The peak number of hMPV infections occurred in March, followed by February, and then April (Fig. 3), distribution patterns remaining similar throughout the 5 years (data not shown).

Thirteen positive samples were selected at random and sequenced. The analysis of hMPV *M* gene was performed by comparison with 26 sequences obtained from the database. Selected samples were distributed from 2001, 2002, 2003, and 2004 seasons. Phylogenetic analysis

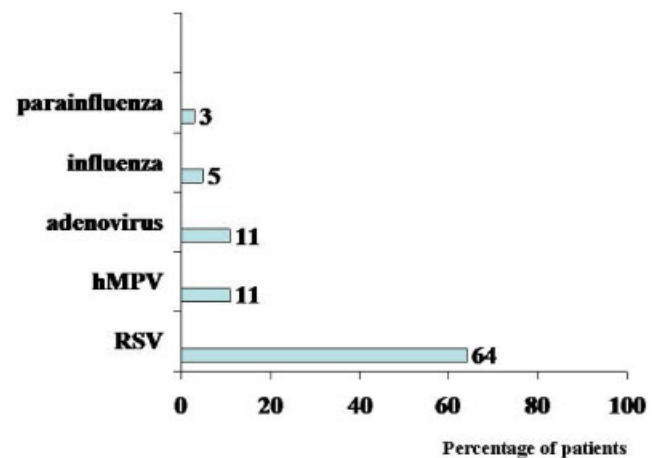


Fig. 1. Comparison of human metapneumovirus (hMPV) detection frequency with other respiratory viral pathogens.

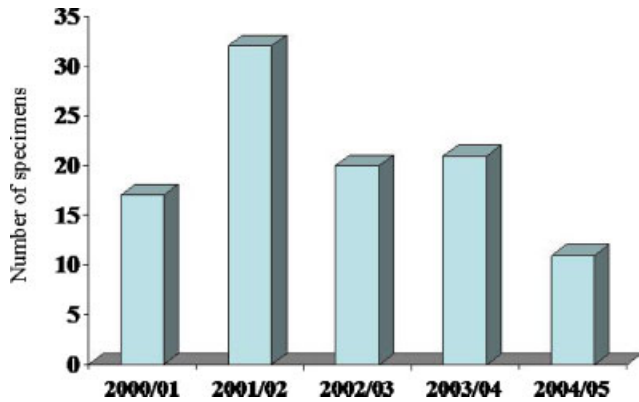


Fig. 2. Nasopharyngeal aspirates for human metapneumovirus at the Severo Ochoa Hospital, October 2000–June 2005.

(Fig. 4) indicated two main groups A and B each divided into two subgroups (A1 and A2, B1 and B2). Bootstrap values were 100 for main groups and >90 for each subgroup. hMPVs from our children belonged into both A and B groups of viruses which cocirculated throughout 2003–2004 season. During epidemics 2001–2002, 2002–2003, and 2003–2004 subgroups A1 and A2 cocirculated.

Clinical Characteristics Associated With hMPV Infection

Clinical characteristics for the 101 infants with hMPV infection are shown in Table 1. Till now, most patients had, otherwise, been in good health. Eight of the 101 patients had underlying medical problems: chromosomopathy $n=3$, congenital heart disease $n=2$ (with Down's syndrome), cerebral palsy $n=1$, epilepsy $n=1$, Prader–Willy syndrome, and bronchopulmonary dysplasia $n=1$.

The most frequent diagnoses were bronchiolitis and recurrent wheezing. Fourteen hMPV infants with recurrent

wheezing had, previously, been admitted for bronchiolitis, in 13 cases, and for recurrent wheezing, in 1 case. Seven of these previous episodes were RSV-infection-related.

Otitis media developed in six children and conjunctivitis in two, although two children showed increased transaminase levels. One child had been admitted to hospital with recurrent wheezing at 6 months and, again, at 15 months. On both occasions, NPA detected hMPV.

Of the chest X-rays obtained in 91 cases, 25.7% showed infiltrate/atelectasis. Serum CRP levels were significantly higher in children with infiltrates as compared to those showing no significant radiological changes ($58.3 \text{ mg/l} \pm 70.9$ vs. $19.7 \text{ mg/l} \pm 28.5$, $P: 0.02$). Blood cultures were negative in all cases, except one case where normal chest X-rays revealed *Streptococcus pneumoniae*-induced bacteremia.

Respiratory infection-related hospital stays went from 4.9 ± 2.6 days. One hMPV-infected infant, with Down's syndrome and interauricular communication, was hospitalized for 60 days due to persistent and severe respiratory repercussion. One child was admitted to the intensive care unit (ICU) and required assisted ventilation. None of the children died.

Those hMPV-infected children under 6 months ($n=45$) suffered significantly fewer cases of hypoxia, fever, and radiological alterations than hMPV-infected children over 6 months ($n=56$) (Table 2). Bronchiolitis diagnosis were most frequent among members of the younger age group ($P < 0.001$).

Additional viruses were identified in 30 (29.7%) of the 101 hMPV-positive samples as follows RSV in 7 (23.3%), adenoviruses in 13 (43.5%), influenza virus in 1 (3.5%), and enterovirus in 1 (3.5%). RSV, adenovirus, and hMPV were, simultaneously, detected in two patients.

Clinical characteristics of single virus infections were similar to those of dual infections (Table 3). Patients who

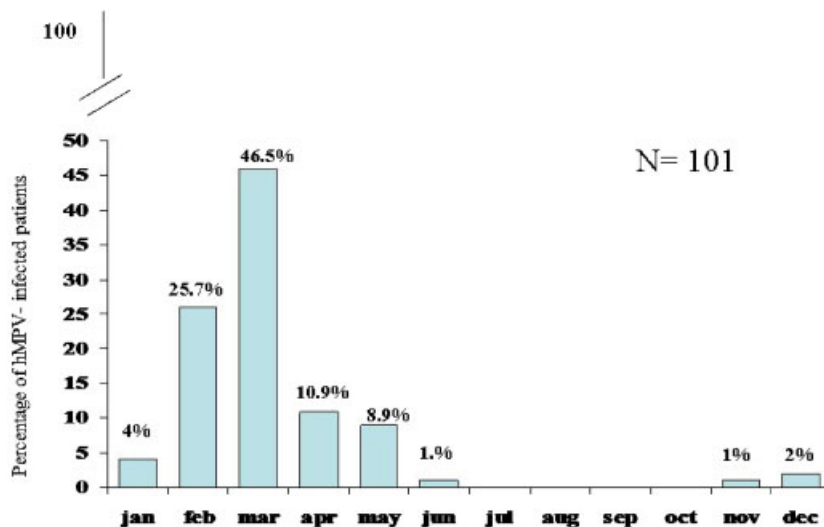


Fig. 3. Monthly distribution of hMPV (dual and single infections) between October 2000 and June 2005.

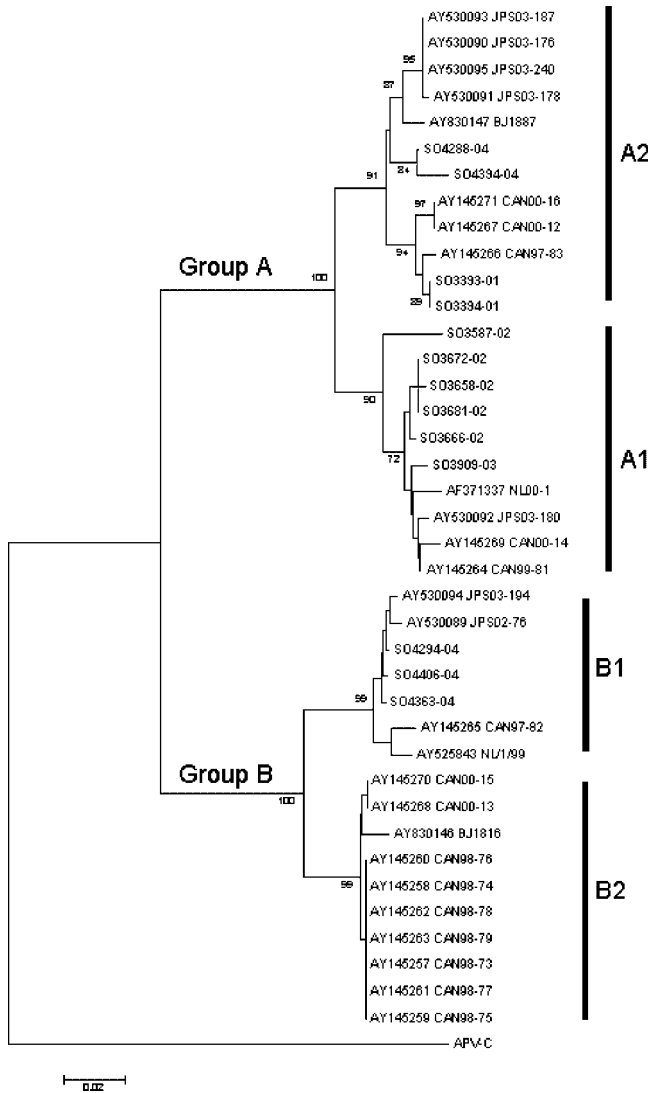


Fig. 4. The set of nucleotide sequences was aligned with Clustal W. Phylogenetic analysis was performed using the Kimura two-parameter model as a model of nucleotide substitution and using the neighbour joining method to reconstruct the phylogenetic tree (MEGA version 3.0 software package). The statistical significance of the phylogenies constructed was estimated by bootstrap analysis with 1,000 pseudoreplicate data sets.

required mechanical ventilation had no co-infection with any other respiratory virus.

Comparison to the RSV-Positive Group

Clinical data from patients with single hMPV infection (n = 71) were compared to those from patients with single RSV infection (n = 88) (Table 4).

Diagnosis of bronchiolitis was significantly more frequent in RSV-infected children (P = 0.001). Recurrent wheezing was the most common diagnosis in the hMPV group, though this was closely followed by bronchiolitis. hMPV infections were, mainly, detected from February to

TABLE 1— Clinical Characteristics Associated With Single hMPV Infections (N = 71)

Clinical feature	N (%)
Age (months) ¹	7.5 ± 5.5
Male	42 (59.2%)
Prematurity	11 (15.5%)
Temperature >37.9°C	45 (63.4%)
Hypoxia (Sat O2 <95%)	41 (57.7%)
Time in hospital (days) ¹	4.9 ± 2.8
Abnormal chest radiograph	18 (25.4%)
Antibiotic treatment 14 (19.7%)	
Diagnosis	
Bronchiolitis	33 (46.5%)
Recurrent wheezing	35 (49.2%)
Pneumonia	2 (2.8%)
Upper respiratory infection	1 (1.4%)

¹Mean ± standard deviation.

May, although RSV was found, more specifically, in December and January. As regards age, children under 1 year were particularly affected by both viruses and, though no significant mean age differences could be established, hMPV infection was found to be more prevalent among those over 6 months (P = 0.04).

No differences were found in any of the other variables evaluated. Two children in the RSV group and one in the hMPV group were admitted to the intensive care unit. Only the hMPV patient required assisted ventilation.

DISCUSSION

This report not only constitutes one of the largest pediatric series to date; it is the largest of its kind to be published in Spain. Our study provides an accurate estimation as to hospitalization proportionality due to acute respiratory hMPV-related infections, among infants under the age of 2 years. Throughout the 5 years of consecutive study, hMPV infections accounted for 7.6% of all respiratory infections and 11% of infections with positive viral detection. hMPV infections were surpassed only by RSV and rhinovirus infections, and are more common than adenovirus, influenza, and parainfluenza infections.

Given the vast methodological diversity employed in previous studies, it is difficult to estimate what the current frequency of hMPV infections might be or, even, what relative significance they bear when compared to infections caused by other respiratory viruses, within the pediatric population. Most studies included hospitalized children; but, although some authors investigated the presence of hMPV in samples from all patients enrolled,^{16,19,21–24} others did so only with the negative samples.^{6,8–10,25,26} Another potentially confounding factor is the different ages of the children studied; for, though hMPV can infect children at any age, its highest incidence

TABLE 2—Clinical Characteristics of Single hMPV Infections in Infants < and > 6 Months

Clinical feature	<6 months N = 32	>6 months N = 39	P	OR
Diagnosis				
Bronchiolitis	23 (74.2%)	10 (27.2%)	<0.001	3.049 (1.594–5.834)
Recurrent wheezing	8 (25.8%)	27 (73%)	<0.001	0.328 (0.171–0.627)
Temperature >37.9°C	15 (46.9%)	30 (77%)	0.009	0.510 (0.310–0.840)
Hypoxia (Sat O ₂ <95%)	14 (43.8%)	27 (69.2%)	0.031	0.569 (0.340–0.953)
Chest X-ray (infiltrate/atelectasis)	4 (13.8%)	14 (41.2%)	0.016	0.400 (0.162–0.987)

is seen in children under the age of 1. Thus, although some series only included children under 2–3 years old,^{6,16,19–21} others included children under 5,^{8,9,26} others, even reported patients whose ages ranged from 0 to 18.^{4,10,23,27} In addition, the seasonality of hMPV infections causes the identification rate of the virus to be very different from some studies to others if complete epidemiological seasons are not studied.

Most incidence studies investigating the presence of hMPV, solely in negative samples, reported a lower frequency of hMPV. For instance, 4.4% in children under 5, in an Argentinean series,²⁶ or 6.6% of children—aged 3 months to 12 years—in the French group.¹⁰ In Spain, Vincente et al.⁶ found hMPV in 4.1% of children under 3 years old whose virological study came out negative. Pediatric series, where both positive and negative infant samples were studied, found an hMPV frequency similar to ours.^{19,20,28} In our study, hMPV presence was investigated in 1,322 infants under 2, hospitalized for respiratory infection over 5 years. Overall frequency was 7.6% and 11% of positive samples. These figures could well be an adequate approximation as to the true prevalence of hMPV for this population. This setting's high hMPV frequency reinforces the need for inclusion of

hMPV detection processes in the diagnostic routine for respiratory infections, in hospitalized infants, given that hMPV appears to play a more significant role than adenoviruses and influenza or parainfluenza viruses, in this age group.

As in various other studies, our study-data indicates a higher prevalence of hMPV infections in the late winter months and in spring.^{4,9,20,22} This seasonal distribution seems unlikely to change from 1 year to another, given that, for the five consecutive seasons studied, peak hMPV activity persisted in these same months. Interestingly, in the French,¹⁰ Dutch¹⁶, and Norwegian²³ series, hMPV prevalence occurred mainly in December and January, as did RSV. Given that complete epidemiological seasons were not included in some of these studies, a degree of caution would appear to be in order, when interpreting these results. Potentially, however, this data could reflect the varied nature of hMPV circulation in different countries.

Clinical characteristics in infants with hMPV-positive samples were similar to those previously reported.^{1,4,8,10,16,21–23,25,29,30} (Our data confirms that hMPV-related diseases are more frequent in children <12 months, who, in turn, represent over 80% of our inpatients). Our data suggest that hMPV-related diseases, in infants, are more frequent in those aged <12 months, who, in turn, represent over 80% of our inpatients. From our results, we are able to determine that clinical affection in infants, under 6 months, is less severe than in older children. Most cases were associated with bronchiolitis or recurrent wheezing. Although the study conducted by Rawlinson et al.³¹ attributed a limited role to hMPV in asthma exacerbations in children, other studies found that hMPV was related to asthma exacerbations and wheezing in the pediatric population.^{4,23,27,32,33} Our data clearly indicates that hMPV is associated with recurrent wheezing, certainly in hospitalized children aged <2.

Over half of our patients required oxygen-therapy and relatively long hospital stays. One patient required ICU admission and assisted ventilation. This data, in addition to that which was recently published in several series—reporting hMPV-infected patients who had to be admitted to ICUs and who required assisted ventilation—serves to

TABLE 3—Clinical Characteristics Associated With Hospitalized hMPV Infections (Single vs. Dual Infections)

Clinical feature	Dual infection (N = 30)	Single infection (N = 71)	P
	N (%)	N(%)	
Age (months) ¹	6.8 ± 4.9	7.4 ± 5.5	NS
Male	21 (70%)	42 (59.2%)	NS
Prematurity	6 (20.7%)	11 (15.5%)	NS
Temperature >37.9°C	20 (66.7%)	45 (63.4%)	NS
Hypoxia (SatO ₂ <95%)	15 (50%)	41 (57.7%)	NS
Time in hospital (days) ¹	4.9 ± 1.8	5.6 ± 7.1	NS
Abnormal chest radiograph	8 (26.6%)	18 (25.3%)	NS
Antibiotic treatment	6 (20%)	14 (19.7%)	NS
Diagnosis			
Bronchiolitis	17 (56.6%)	33 (46.4%)	NS
Recurrent wheeze	11 (36.6%)	35 (49.2%)	

%, columns.

¹Mean ± standard deviation.

TABLE 4—Clinical Characteristics of Patients With Single Human Metapneumovirus (hMPV) Versus Respiratory Syncytial Virus (RSV) Infections

Clinical characteristic	hMPV	RSV	P	OR
	(N = 71)	(N = 88)		
Age (months) ¹	7.4 ± 5.5	6.0 ± 5.2	NS	
Age <6 months	32 (45.1%)	54 (61.4%)	0.04	0.696 (0.492–0.987)
Male	42 (59.2%)	53 (60.2%)	NS	
Prematurity	11 (15.5%)	10 (11.4%)	NS	
Temperature >37.9°C	45 (63.4%)	47 (53.4%)	NS	
Hypoxia (SatO ₂ <95%)	41(57.7%)	54 (61.4%)	NS	
Time in hospital (days) ¹	4.9 ± 2.8	4.9 ± 2.2	NS	
Chest radiograph				
Infiltrate/atelectasis	18 (25.4%)	31 (35.2%)		
Normal	45 (63.4%)	54 (61.4%)	NS	
Not done	8(11.3%)	3 (3.4%)		
Antibiotic treatment	14 (19.7%)	12 (13.6%)	NS	
Diagnosis				
Bronchiolitis	33 (46.5%)	63 (71.6%)	0.001	0.550 (0.390–0.775)
Recurrent wheeze	35 (49.2%)	21 (23.9%)		

¹Mean ± standard deviation.

illustrate the pathogenetic role of this virus, in the pediatric population.^{24,33–35} In discovering a patient with contracted two hMPV infections, in two different seasons, and requiring hospital admission on both occasions, we learn that hMPV infections may not confer permanent immunity, or that viruses from different seasons belong to different lineages, as it is shown in Figure 4.

Our work, as with most studies published to date, was not designed to study mild or asymptomatic hMPV infections. However, the results of both Van den Hoogen et al.^{1,16} and Williams et al.²⁵ suggest that subclinical or asymptomatic infections are, indeed, very rare in young children.

Whereas data from other reports suggest that dual infection with hMPV and other respiratory viruses is rare,^{6,8,19} the high co-infection rate found in our patients (29.7%) appears to suggest quite the opposite, the co-infection rate being among the highest reported to date, after the series by Greensill et al.³⁶, Maggi et al.²⁰, and Cuevas et al.²¹ The seasonal distribution overlap between hMPV and RSV could well explain the high frequency of co-infections. True hMPV-frequency may be underestimated if its presence is studied only in samples testing negative for other respiratory viruses. This is probably one of the most important factors in explaining the varied hMPV-incidence rates seen in very similar populations.^{6,7}

The role of hMPV as a concomitant pathogen in these dual infections has not yet been fully established. Some studies, such as those by Greensill et al.,³⁶ König et al.³⁴, and Semple et al.³⁷ suggest greater severity of co-infections. However, other series did not report severity differences between co-infections and single infections.^{25,28} In our series, there were no significant differences in diagnosis, oxygen-therapy requirements,

length of hospital stay, fever frequency, antibiotic therapy, or in radiological changes, between co-infections and single infections. All of which would appear to suggest that the presence of hMPV in the setting of another viral respiratory infection does not make the condition more severe. However, the role of co-infections in the most severe disease could not clearly be evaluated in our study, seeing as only one patient required assisted ventilation.

A comparative analysis of clinical characteristics in hMPV and RSV single infections suggests that hMPV infections are more frequent in infants over 6 months, compared to RSV. Almost all children suffered fewer respiratory infections, yet recurrent wheezing was diagnosed significantly more frequently in the hMPV group, whereas bronchiolitis was the most common diagnosis in the RSV group. The severity of the disease associated with both viruses was similar, and no differences could be shown either in terms of length of hospital stay, oxygen-therapy requirements, and fever frequency, nor in the patient's prematurity or underlying disease history.^{19,30,38}

In conclusion, our study supports both the epidemic nature of hMPV infection and its significant role as a major pathogen in RTI, in infants under the age of 2. Results obtained to date, lead us to recommending that hMPV be taken into account in the differential diagnosis of acute respiratory infections in hospitalized infants, principally, to ensure that they are distinguished from respiratory syncytial-virus infections.

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