

## Impact of human metapneumovirus infection on inpatients and outpatients for the years 2006-2008 in Southern Brazil

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*The human metapneumovirus (hMPV), member of the Paramyxoviridae family, has been reported as an important agent involved with acute respiratory infections (ARIs). The aim of this study is to identify hMPV as the etiological agent of ARIs on inpatients and outpatients in the city of Curitiba, Southern Brazil, and describe clinical data of hMPV subtyping. A retrospective study was performed in 1,572 respiratory samples over a period of three years. hMPV was detected by reverse transcription-polymerase chain reaction and subtyping was performed by nucleotide sequencing. hMPV was present in 61 (3.9%) samples and subtypes A1, A2a, B1 and B2 were detected. The incidence of hMPV was higher in outpatients (5.9%), whose mean age was 19.7 years (range 6 months-75 years old), than in inpatients (3%), whose mean age was 7.6 months (range 1 month-26 years old). The outpatients had upper respiratory tract infections with flu-like symptoms and all hospitalized children had lower respiratory tract infections. A pediatric patient died from complications associated with hMPV A2a infection. hMPV has been reported as a respiratory pathogen in all age groups. No correlation was observed between viral subtype and disease severity in the samples of this study.*

Key words: human metapneumovirus - subtypes - respiratory infection - inpatients - outpatients

Acute respiratory tract infections (ARIs) are an important cause of morbidity and mortality in the world (Alto 2004, Kahn 2006). In Brazil and other countries, viruses have been frequently reported as etiologic agents of ARIs (Alto 2004, Tsuchiya et al. 2005, Costa et al. 2006). Tsuchiya et al. (2005), in a study in Curitiba, state of Paraná (PR), Southern Brazil, identified the most common respiratory viruses [influenza A (FLU A), influenza B (FLU B), respiratory syncytial virus (RSV), adenoviruses (AdV), human parainfluenza viruses 1, 2 and 3 (PIV1, 2 and 3)] as etiologic agents in 30% of the specimens of respiratory tract infection on inpatients and outpatients. However, in a large number of ARIs, the etiologic agent is not determined (Tsuchiya et al. 2005, Thomazelli et al. 2007).

Advances in epidemiologic surveillance and molecular biology have allowed the rapid recognition and identification of several newly emerging respiratory pathogens (Alto 2004, Kahn 2006). The human metapneumovirus (hMPV) was first described in 2001 in the Netherlands and was classified in the Paramyxoviridae family (van den Hoogen et al. 2001). Sequence analysis of isolates identified two main genotypes of hMPV, A and B, with the subtypes A1, A2a, A2b, B1 and B2 (Gray et al. 2006, Huck et al. 2006). In Brazil, the subtypes A1, A2a, B1 and B2 were described circulating during the years 2003-2006 (Carneiro et al. 2009, Oliveira et al. 2009).

hMPV has been associated with respiratory disease in several countries throughout the world (Howe 2002, Bastien et al. 2003, Cuevas et al. 2003, Esper et al. 2003, Freymouth et al. 2003, Madhi et al. 2003, Maggi et al. 2003, Peiris et al. 2003, Ebihara et al. 2004, Mirazo et al. 2005, Gray et al. 2006, Debur et al. 2007). The epidemiological characteristics of hMPV infection include respiratory impairment, particularly in children, elderly and immunocompromised patients (van den Hoogen et al. 2004) with clinical symptoms that cannot be distinguished from those associated with other respiratory diseases caused by virus and may cause infections from upper respiratory tract infections to bronchiolitis and pneumonia (Boivin et al. 2002). Common laboratory methods for the diagnosis of respiratory viruses, such as tissue culture and antigen-based assays, have been shown to have low sensitivity and specificity to detect hMPV. A molecular assay, reverse transcription-polymerase chain reaction (RT-PCR), was the first method selected to detect this virus (MacKay et al. 2003, Calicó et al. 2009).

The aim of this study is to present demographic and clinical data from inpatients and outpatients with respiratory infections caused by hMPV subtypes.

### PATIENTS, MATERIALS AND METHODS

*Samples* - We analyzed nasopharyngeal aspirates (NPAs) from patients with respiratory clinical manifestations admitted to the Teaching Hospital of the Federal University of Paraná (HC-UFPR) and in primary health-care units that were included in the National Influenza Protocol (Influenza Surveillance Program) in Curitiba, between 2006-2008.

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The samples used in this study from the inpatients of HC-UFPR were obtained from those admitted to different clinical units, such as the Hematopoietic Stem Cell Transplantation (HSCT) and Pediatrics (semi-intensive and intensive care) units to treat ARIs. The samples from outpatients were obtained from those treated in the primary healthcare units who presented with “flu-like” clinical manifestations according to the National Influenza Protocol (fever, cough, wheeze, dyspnea, otalgia, myalgia) and the three selection criteria were fever, at least one of the respiratory manifestations and one clinical manifestation.

The NPAs were obtained by aspiration as described by Gardner and McQuillin (1968). The samples were maintained in a transport medium (tryptose phosphate buffer enriched with gelatin) and transported on ice to the virology laboratory. The NPA samples were processed to detect the most common viral antigens, such as RSV, FLU A, FLU B, AdV and PIV, by indirect immunofluorescence assay (IFA) as previously described (Cox et al. 1998), using commercially available monoclonal antibodies (Chemicon International Inc, Temecula, CA). Aliquots of the samples in transport medium were stored at -70°C for use in the RT-PCR assay to detect hMPV.

*Detection of the hMPV N gene* - Samples added to 1 mL of viral transport medium kept at -70°C were used for RNA extraction. Total RNA was extracted using a buffer containing guanidine thiocyanate as previously described (Casas et al. 1995). Five hundred copies of pseudorabies virus (PRV) were added to the extraction buffer as an internal control. Complementary DNA (cDNA) was produced using specific N1 primer (5'-ATGGGGACAAGTGAATAATGTC-3') and the Superscript II RT enzyme (Invitrogen, Life Technologies) incubated for 60 min at 42°C according to manufacturer's instructions. cDNA was amplified to detect a 928 bp conserved region of the nucleoprotein (N) gene (positions 112-1040 nt, based on AY297749 sequence). Two microliters of cDNA were used for a 25 µL reaction containing the following: 2.5 µL 10 x PCR buffer minus Mg (Invitrogen, Life Technologies, Carlsbad, CA), 0.75 µL 50 mM MgCl<sub>2</sub> (Invitrogen, Life Technologies, Carlsbad, CA), 5 µL 2.5 mM dNTPs, 10 pmol PRV1-(5'-ATGACGCCGATGTACTTCTTCTT-3'), 10 pmol PRV1+(5'-CGCGTGGTCTACGGGGACACGGA-3'), 5 pmol N2 (5'-GAGTCTCAGTACACAATAA-3'), 5 pmol N3 (5'-GCATTTCCGAGAACAACAC-3') primers and 1.25 U Taq Polymerase (Invitrogen, Life Technologies, Carlsbad, CA) and the final volume was completed with RNase free distilled water. Cycling conditions were as follows: an initial denaturation step at 94°C for 3 min, followed by 50 cycles at 94°C for 45 s, 50°C for 30 s and 72°C for 1 min and a final extension at 72°C for 10 min. PCR products were analyzed by electrophoresis in a 1% agarose gel stained with ethidium bromide in a 1% agarose gel stained with ethidium bromide (modified from Mirazo et al. 2005). For reference and quality control, water was included as a negative control and a positive control was generated as described in the study by Debur et al. (2007). RT-PCR was performed in duplicate and samples were considered to be positive if both reactions were positive.

*Detection of fusion (F) gene of hMPV* - The positive samples detected by amplification of the N gene were submitted to amplification of the F gene to perform hMPV subtyping by genome sequencing.

RNA extracted as described above was reverse transcribed using the Superscript II RT enzyme (Invitrogen, Life Technologies) and random primer hexamers (Amersham Pharmacia Biotech). cDNA was amplified to detect a 450 bp fragment of the F gene (positions 3728-4168 nt, based on sequence AY297749). Two microliters of cDNA were used for a 25 µL reaction containing the following: 2.5 µL 10 x PCR buffer minus Mg (Invitrogen, Life Technologies, Carlsbad, CA), 0.75 µL of 50 mM MgCl<sub>2</sub> (Invitrogen, Life Technologies, Carlsbad, CA), 4 µL of 2.5 mM dNTPs, 10 pmol of FF1 (5'-CWT-TRGACYTAATGACWGATG-3'), 10 pmol of RR1 (5'-GTCTTCTGTGCTRACTTTG-3') primers (Kaida et al. 2006) and 1.25 U of Taq Polymerase (Invitrogen, Life Technologies, Carlsbad, CA) and the final volume was completed with RNase free distilled water. Cycling conditions were as follows: an initial denaturation step at 94°C for 3 min, followed by 35 cycles at 94°C for 45 s, 55°C for 30 s and 72°C for 45 s and a final extension at 72°C for 10 min. PCR products were analyzed by electrophoresis in a 1% agarose gel stained with ethidium bromide. For reference and quality control, water was included as a negative control and a positive sample was confirmed by N amplification, as described.

*Sequence comparisons and phylogenetic relationship* - Purified PCR products obtained by N and/or F amplification were directly submitted to automated nucleotide sequencing with a BigDye Terminator v3.1 Cycle sequencing kit in an ABI genetic analyser 3130 (Applied Biosystems Inc, CA). The obtained sequences were submitted to GenBank (accessions HM124479-HM124528 and HM173093-HM173096) and were analyzed with complete F and/or N sequences from GenBank database with following accessions: EU857607, EU857582, EU857572, EU857573, EU857548, EU857544, EU857553, EU857551, EU857566 and EU857564 for gene F, as described by Yang et al. (2009), and AY145285, AY145281, AY145280, AY145279, AY145274, AF371337, AY145277, AY525843, EU179266, AY145276, AY530095, AY145272 and AY145276 for gene N, as described by Huck et al. (2006). For outgroups, an avian metapneumovirus type C EF199771 and EF199772 for gene F and AY590688 for gene N were used.

Sequences were aligned with ClustalW software and analyzed by similarity/identity calculations using Bio-Edit Sequence Alignment Editor software, version 7.0.0, and by the bootstrap neighbor-joining method (2,000 replicates) using the complete deletion and Kimura-2 parameters of the MEGA software package, version 4 (Kumar et al. 2004).

*Clinical data* - Demographic and clinical data were obtained from medical records and compared with subtyping results using Excel Microsoft. Statistical analyses were carried out using the Chi-square test or Fisher's exact test and were performed using Graph Pad Prism version 3.00 for Windows, GraphPad Software, San Diego, California, USA. In all of the analyses, a value of  $p < 0.05$  was considered statistically significant.

*Ethics* - The study was approved by the Committee on the Ethics of Research on Human Beings of the HC-UFPR (reference 0035.0.208.000-06).

**RESULTS**

The study was performed with 1,572 NPAs samples collected in the 2006-2008 period. The study involved 445, 527 and 600 samples collected from 2006-2008, respectively. Seven hundred twenty-three samples (723/1572, 46%) from pediatric inpatients, 356 (356/1572, 22.6%) from patients submitted to HSCT and 493 (493/1572, 31.4%) from outpatients were analyzed. A predominance of male patients (592/1079, 54.9%) from all clinical units ( $p = 0.048$ ) was observed in the sample.

An RT-PCR assay for detection of the hMPV N gene was performed on all samples, and 61 (3.9%) positive samples were detected. Fig. 1 shows the clinical unit distribution of the analyzed samples and hMPV positivity. The incidence of hMPV infection was higher in outpatients (29/493, 5.9%) than inpatients (32/1079, 3%) ( $p = 0.0072$ ). The age of the hMPV infected patients varied in the clinical units studied, as shown in Fig. 1. hMPV had a higher incidence in patients less than five years old (39/61, 63.9%) ( $p = 0.0093$ ). Most (36/61, 59%) hMPV infected patients were female ( $p = 0.08$ ).

The incidence by year of hMPV was 3.1% (14/445), 7% (37/527) and 1.7% (10/600), respectively, for 2006, 2007 and 2008 (Table I). Most samples of hMPV detected in 2007 were obtained from outpatients (23/37, 62.2%). Viral co-infections were found to occur mainly in children (12/16, 75%) with the mean age of  $4.8 \pm 3.6$

months. The viruses detected as co-infections were RSV (7/16, 43.7%), FLU A (4/16, 25%), PIV3 (2/16, 12.5%), AdV (2/16, 12.5%) and FLU B (1/16, 6.25%). Only one patient (27 days-old) co-infected with RSV was hospitalized in an intensive care unit for 11 days because of bronchiolitis. Most hospitalized pediatric patients (14/24, 58%) were breastfed until either the time of infection or six months of age. Among the children that were co-infected with other viruses, only the child who was co-infected with AdV had not been breastfed.

*Genotyping* - Nucleotide sequences were obtained from 51 (51/61, 83.6%) hMPV positive samples. PCR products were selected to submit to partial genome sequencing of the F and/or N gene to determine the hMPV subtype. Thirty-nine samples were sequenced for the F gene and 15 samples were sequenced for the N gene. Only in three samples were both genes sequenced. Eleven (11/14, 78.6%) samples from 2006, 31 (31/37, 83.8%) samples from 2007 and nine (9/10, 90%) samples from 2008 were sequenced.

hMPV subtype A1 was found in five (5/51, 9.8%), A2a in nine (9/51, 17.6%), B1 in 13 (13/51, 25.5%) and B2 in 24 (24/51, 47.1%) samples. Subtypes A1, A2a, B1 and B2 showed, respectively, 95-99%, 96-99%, 92-99% and 94-99% nucleotide similarity with the reference sequences for each of the previously described subtypes (data not shown). These results and the high bootstrap values in the phylogeny tree confirm the detected subtypes (Fig. 2).

*hMPV circulation during the study period* - Fig. 3 shows the distribution of the hMPV positive samples

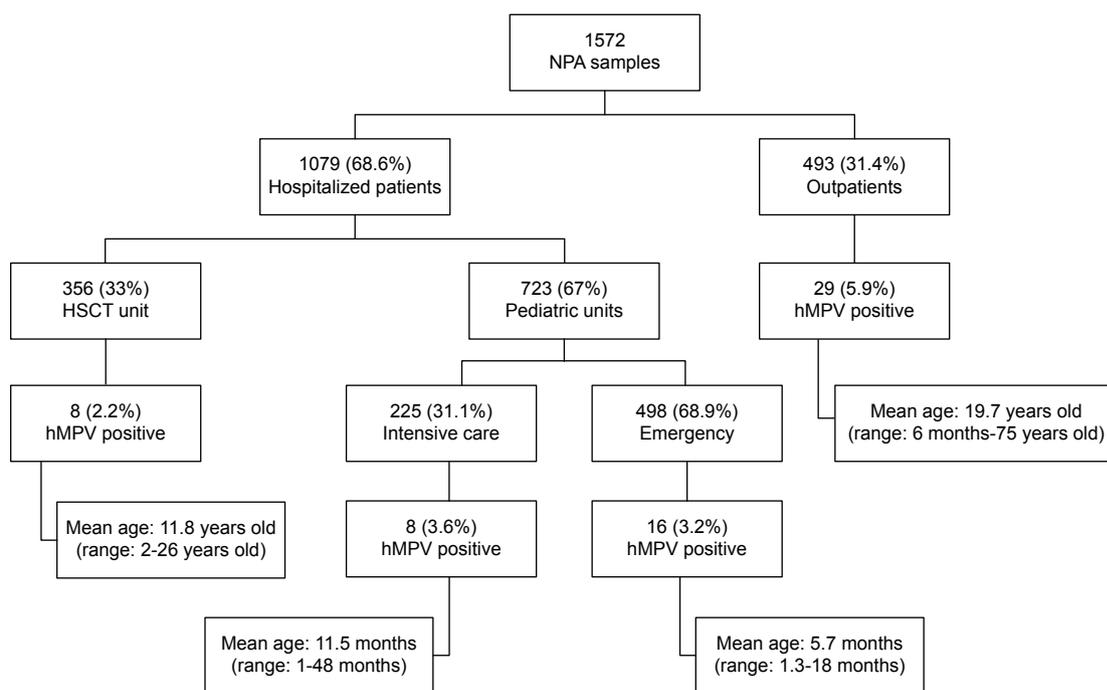


Fig. 1: clinical unit distribution of the nasopharyngeal aspirates (NPA) samples submitted to the virology laboratory for the detection of respiratory viruses; hMPV: human metapneumovirus; HSCT: Haematopoietic Stem Cell Transplantation.

TABLE I  
Results of human metapneumovirus (hMPV) detection in Curitiba, Paraná, Southern Brazil, during the 2006-2008 period by clinical units

Virus (n)	Inpatients				Outpatients								
	HSCCT unit		Pediatrics unit		HSCCT unit		Pediatrics unit						
	2006 (119)	2007 (127)	2008 (110)	Total (356)	2006 (208)	2007 (223)	2008 (292)	Total (723)	2006 (118)	2007 (177)	2008 (198)	Total (493)	
hMPV n (%)	4 (3.4)	3 (2.4)	0 (0)	7 <sup>e</sup> (2)	6 (2.9)	8 (3.6)	4 (1.4)	18 <sup>b</sup> (2.5)	3 (2.5)	14 (7.9)	3 (1.5)	20 <sup>c</sup> (4.1)	45 (2.9)
Co-infection <sup>d</sup> n (%)	0 (0)	0 (0)	1 <sup>e</sup> (0.9)	1 (0.3)	1 <sup>f</sup> (0.5)	3 <sup>f,gs,h</sup> (1.3)	2 <sup>f</sup> (0.7)	6 (0.8)	0 (0)	9 <sup>e,f,h,i</sup> (5.1)	0 (0)	9 (1.8)	16 (1)
Other viruses <sup>f</sup> n (%)	21 (17.6)	3 (23.6)	26 (23.6)	77 (21.6)	56 (26.9)	62 (27.8)	105 (35.9)	223 (30.8)	16 (13.6)	28 (15.8)	54 (27.3)	98 (19.9)	398 (25.3)
Negative n (%)	94 (79)	94 (74)	83 (75.4)	271 (76.1)	145 (69.7)	150 (67.3)	181 (62)	476 (65.9)	99 (83.9)	126 (71.2)	141 (71.2)	366 (74.2)	1,113 (70.8)

*a*: one patient less than five years old and six patients greater than five years old; *b*: 11 patients less than six months of age, five patients less than 15 months of age and two patients less than five years old; *c*: seven patients less than five years old, two patients less than 15 years old, 10 patients greater than 15 and less than 65 years old and one patient greater than 65 years old; *d*: to determine co-infection it was utilized results of the indirect immunofluorescence assay (IFA) for detection of others viruses; *e*: hMPV/human parainfluenza viruses 3; *f*: hMPV/respiratory syncytial virus; *g*: hMPV/influenza B; *h*: hMPV/adenoviruses; *i*: hMPV/influenza A; *j*: infections by other viruses were determined using IFA, which was used to investigated the most common viral antigens in our region; HSCT: Haematopoietic Stem Cell Transplantation.

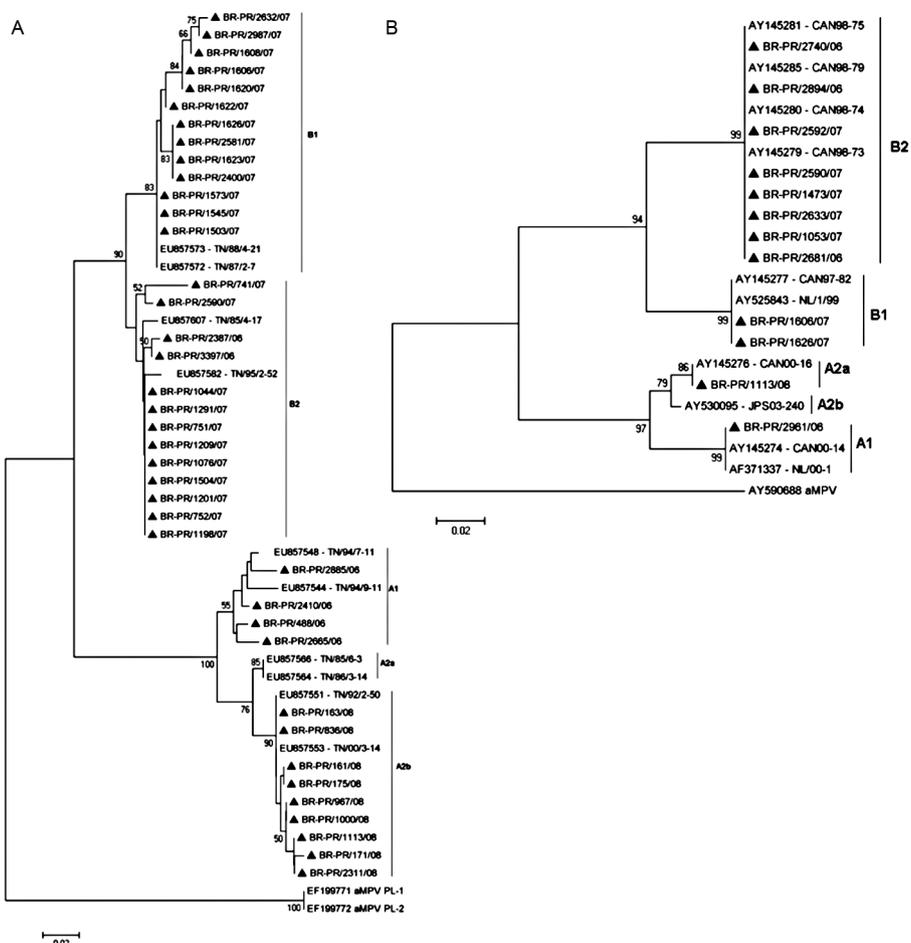


Fig. 2: a neighbor-joining tree resulting from the sequence analysis of human metapneumovirus from Curitiba, Paraná, during the 2006-2008 period and sequences retrieved from GenBank (see Patients, Materials and Methods) by MEGA version 4. A: partial fusion gene; B: partial nucleoprotein gene. The sequences of this study are dotted with a black triangle and named BR-PR followed by the number of the samples and year of the sample collection. Only bootstrap values higher than 50% are shown.

throughout the entire three year study period. It was found that hMPV circulated throughout the year with two annual peaks, one in the autumn and another in the late winter and spring. A higher incidence of hMPV (18/37, 48.6%) was observed in May and June 2007. All genotypes (A and B) of hMPV were detected in the three years of the study. In 2006 and 2007, two hMPV subtypes co-circulated per year (A1/B2 and B1/B2, respectively). In 2008, only one subtype was detected (A2a). Subtype B2 showed higher circulation than other subtypes in the study period. The most common subtype within a one year period was found in both inpatients and outpatients.

**Clinical findings** - The respiratory symptoms frequently reported in pediatric inpatients were: dyspnea (90.9%), cough (86.3%), tachypnea (77.3%), fever (54.5%), wheeze (54.5%), cyanosis (50%), vomiting (50%), apnea (22.7%), rhinorrhea (18.2%) and diarrhea (9.1%). Pulse oximetry and pCO<sub>2</sub> mean values at the moment the patients were admitted to hospital were 81.1 ± 11.6% and 39.6 ± 13.8 mmHg, respectively. Oxygen therapy was used in 20 (90.9%) hMPV infected patients for an average of 8.2 ± 6.6 days. Mechan-

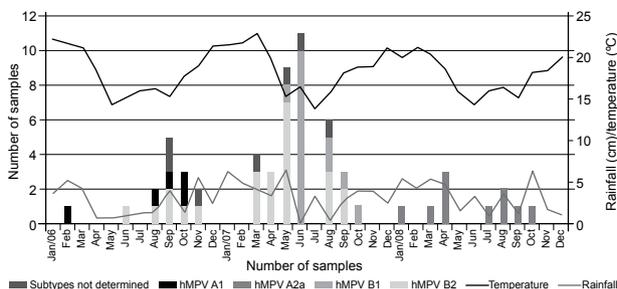


Fig. 3: distribution of human metapneumovirus (hMPV) positive samples and its relationship with the average daytime temperature and rainfall from January 2006-December 2008 in Curitiba, Paraná, Southern Brazil.

cal ventilation was necessary in five (22.7%) patients for an average of 9.5 ± 1.0 days. Antibiotic therapy was used in 12 (54.5%) patients for an average of 10.5 ± 6.8 days. Bronchodilator therapy was used in 16 (72.7%) patients for

an average of  $5.5 \pm 2.7$  days. Corticoid therapy was used in eight (36.4%) patients for an average of  $5.0 \pm 3.8$  days. The most common diagnoses given were bronchiolitis (31.8%), bronchopneumonia (18.2%), trachibronchitis (18.2%), laryngotracheobronchitis (9.1%), pneumonia (9.1%) and acute respiratory insufficiency (4.5%). Among the hMPV positive children, one (4.5%) was premature and seven (31.8%) were admitted to intensive care units. The duration of hospitalization of all positive patients ranged from 4-50 days, with an average of  $15.0 \pm 16.9$  days.

Patients infected by hMPV and treated in the primary healthcare units had the following respiratory symptoms: rhinorrhea (82.8%), cough (79.3%), fever (75.9%), dyspnea (65.5%), headache (48.3%), myalgia (44.8%), pharyngitis (37.9%) and otalgia (20.7%). Antibiotic therapy was used in nine (31%) patients for an average of  $9.0 \pm 1.5$  days. Bronchodilator therapy was used in two (6.9%) patients for an average of  $4.0 \pm 1.4$  days. Corticoid therapy was not necessary. Only 11 (11/29, 37.9%) cases of hMPV infection related to a final diagnosis of respiratory condition were reported in the medical records and the most frequent ones were sinusitis (5/11, 45.5%), nasopharyngitis (4/11, 36.4%), bronchitis (1/11, 9.1%) and laryngopharyngitis (1/11, 9.1%).

Table II shows the clinical characteristics, treatment and diagnostic findings of the hMPV infected patients related to subtype. For both inpatients and those assisted in the primary healthcare service, there were no significant statistical differences among clinical characteristics, treatment and diagnostic findings for the different hMPV subtypes. One pediatric patient died due to complications associated with hMPV A2a infection. This patient was 5.1 months old, had Down syndrome, had been exposed to passive smoking and developed a secondary bacterial infection on the 23rd day of hospitalization, progressing to death from sepsis.

## DISCUSSION

hMPV has been frequently identified as a virus associated with respiratory tract diseases worldwide (Howe 2002, Bastien et al. 2003, Cuevas et al. 2003, Freymouth et al. 2003, Madhi et al. 2003, Peiris et al. 2003, Rao et al. 2004, Mirazo et al. 2005, Gray et al. 2006). We retrospectively assessed the presence and clinical importance of hMPV infection as a unique pathogen that was detected in 2.9% of ARIs in a population from Curitiba. RT-PCR was used to amplify a N gene that can detect both hMPV genotypes (Biacchesi et al. 2003). This is the first study to describe the circulation of all hMPV genotypes in Southern Brazil.

Depending on the population studied, the prevalence of hMPV as a cause of ARI ranges from 1.5-41% (Mahalingam et al. 2006). The present study included in and outpatients and the virus was present in 3% and 5.9% of the samples, respectively, which is similar to the results reported in other countries (Cuevas et al. 2003, Bouscambert-Duchamp et al. 2005, Chano et al. 2005).

Primary health care services had more cases of hMPV than HC-UFPR, particularly in 2007. Cuevas et al. (2003) also reported that hMPV was more prevalent in outpatients.

hMPV was responsible for ARIs in hospitalized children with a mean age of 7.6 months (Cuevas et al. 2003) and in patients treated in the primary healthcare service with a mean age of 19.7 years. According to the results of the present study, hMPV caused respiratory disease in all age groups (children, adults and elderly) (Rafiefard et al. 2008) and was more common in females (59%). In contrast, some reports have shown that hMPV infection is more frequent in males than in females (Gray et al. 2006, Mahalingam et al. 2006).

Most reports show that hMPV co-infections may occur with other respiratory viruses, mainly hMPV/RSV (Cuevas et al. 2003, Greensill et al. 2003), and studies in which only previously negative samples were tested might underestimate the percentage of hMPV-positive samples (van den Hoogen et al. 2004). This study has shown hMPV co-infection with RSV, PIV3, FLU B, FLU A and AdV. hMPV/RSV was the most frequent (43.7%) condition. Co-infections were more commonly found in children less than one year old (Aberle et al. 2005). Comparison between the children hospitalized for only hMPV infection and those with hMPV co-infections showed no significant differences in the clinical manifestations or in the final diagnosis (Xepapadaki et al. 2004).

Exact comparisons between the incidence of hMPV and other viruses in this study are difficult to make because hMPV was detected by a sensitive molecular technique, whereas other viruses were detected by IFA (Stempel et al. 2009). We believe that the detection of co-infections and incidence of others viruses may have been underestimated.

In Curitiba, during the coldest months of the year, an increased number of NPAs were collected (Tsuchiya et al. 2005). hMPV is related with winter months (Jartti et al. 2002, van den Hoogen et al. 2004, Bouscambert-Duchamp et al. 2005) and we report that this virus had two peaks in the year, one in the autumn and another in the late winter and spring, that correlated with lower temperature months in the region. In other Brazilian Regions, the presence of the hMPV correlates with rainy seasons, as reported by Cuevas et al. (2003), and with temperature decreases.

In May and June 2007, a higher incidence of hMPV (51.6%) was reported, although the hMPV B2 circulated in May and the hMPV B1 subtype circulated in June. All genotypes (A and B) of hMPV were detected during the study period. Unlike Carneiro et al. (2009), we found that different genotypes can co-circulate during the same year, with A1 and B2 hMPV subtypes being detected in August, September and October 2006. Matsuzaki et al. (2008) and Oliveira et al. (2009) also reported the circulation of the A and B genotypes in 2006. Although the study was only conducted over three consecutive years, it was observed that at least two hMPV subtypes circulated in the same year and occurred in both 2006 and 2007. However, in 2008, 90% of the hMPV detected were subtyped and only subtype A2a was identified. It was observed that the hMPV genotypes changed after a period of circulation (subtypes A1 and B2 in 2006; subtypes B2 and B1 in 2007 and subtype A2a in 2008) and that subtype A2a did not circulate in the previous years of this study, occur-

TABLE II

Clinical characteristics, treatment and diagnosis from patients with different human metapneumovirus (hMPV) subtype infection

	Inpatients (n = 26 <sup>a</sup> )				Outpatients (n = 25 <sup>a</sup> )			
	A1 (n = 3)	A2a (n = 6)	B1 (n = 6)	B2 (n = 11)	A1 (n = 2)	A2a (n = 3)	B1 (n = 7)	B2 (n = 13)
Subtype infection								
Female n (%)	3 (100)	4 (66.7)	4 (66.7)	6 (54.5)	1 (50)	1 (33.3)	6 (85.7)	7 (53.8)
Male n (%)	0 (0)	2 (33.3)	2 (33.3)	5 (45.4)	1 (50)	2 (66.7)	1 (14.3)	6 (46.2)
Age (median ± SD)	2.1 ± 0.1 months	2.8 ± 3.6 months	14.0 ± 10.7 months	3.4 ± 3.9 months	41.9 ± 47.4 years	26.6 ± 21.0 years	34.9 ± 20.9 years	11.7 ± 14.6 years
Time of hospitalization (median days ± SD)	13.0 ± 13.0	12.0 ± 30.3	3.5 ± 7.3	15.0 ± 8.9	-	-	-	-
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
<b>Symptoms</b>								
Dyspnea	1 (33.3)	6 (100)	5 (83.3)	9 (81.8)	1 (50)	3 (100)	3 (42.8)	9 (69.2)
Wheeze	1 (33.3)	6 (100)	1 (16.7)	3 (27.3)	0	0	0	2 (15.4)
Cough	3 (100)	5 (83.3)	5 (83.3)	10 (90.9)	2 (100)	3 (100)	2 (28.6)	12 (92.3)
Fever	1 (33.3)	4 (66.7)	2 (33.3)	7 (63.6)	1 (50)	2 (66.7)	3 (42.8)	12 (92.3)
Rhinorrhoea	2 (66.7)	2 (33.3)	0	3 (27.3)	2 (100)	3 (100)	3 (42.8)	12 (92.3)
Apnea	0	2 (33.3)	1 (16.7)	2 (18.2)	0	0	0	0
Cyanosis	1 (33.3)	4 (66.7)	1 (16.7)	5 (45.4)	0	0	0	0
Vomiting	2 (66.7)	3 (50)	2 (33.3)	4 (36.4)	0	0	0	1 (7.7)
Tachypnea	2 (66.7)	6 (100)	3 (50)	6 (54.5)	0	0	0	0
Headache <sup>b</sup>	-	-	-	-	1 (50)	3 (100)	3 (42.8)	4 (30.8)
Myalgia <sup>b</sup>	-	-	-	-	0	3 (100)	3 (42.8)	4 (30.8)
Pharyngitis <sup>b</sup>	-	-	-	-	1 (50)	2 (66.7)	1 (14.3)	5 (38.5)
Otalgy <sup>b</sup>	-	-	-	-	0	1 (33.3)	0	4 (30.8)
<b>Treatment</b>								
Admitted to ICU	1 (33.3)	3 (50)	1 (16.7)	3 (27.3)	-	-	-	-
Oxygen therapies	2 (66.7)	4 (66.7)	5 (83.3)	9 (81.8)	-	-	-	-
Mechanical ventilatory support	1 (33.3)	2 (33.3)	0	3 (27.3)	-	-	-	-
Antibiotics use	2 (66.7)	4 (66.7)	2 (33.3)	6 (54.5)	0	0	0	5 (38.5)
Inhaler therapies	2 (66.7)	5 (83.3)	4 (66.7)	7 (63.6)	0	0	0	3 (23.1)
Bronchodilators use	1 (33.3)	3 (50)	5 (83.3)	8 (72.7)	0	0	0	2 (15.4)
Corticoid use	1 (33.3)	2 (33.3)	2 (33.3)	4 (36.4)	0	0	0	0
<b>Diagnosis</b>								
Acute respiratory insufficiency	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Bronchiolitis	0 (0)	4 (66.7)	2 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Pneumonia	0 (0)	1 (16.7)	1 (16.7)	1 (9.1)	0 (0)	0 (0)	0 (0)	0 (0)
Laryngotracheobronchitis	1 (33.3)	0 (0)	0 (0)	1 (9.1)	0 (0)	0 (0)	0 (0)	0 (0)
Bronchopneumonia	0 (0)	0 (0)	0 (0)	5 (45.4)	0 (0)	0 (0)	0 (0)	0 (0)
Trachibronchitis	0 (0)	0 (0)	2 (33.3)	2 (18.2)	0 (0)	0 (0)	0 (0)	0 (0)
Nasopharyngitis	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	1 (33.3)	1 (14.3)	1 (7.7)
Laryngopharyngitis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (7.7)
Bronchitis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (7.7)
Sinusitis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (28.6)	3 (23.1)
None diagnosis	2 (66.7)	0 (0)	1 (16.7)	2 (18.2)	1 (50)	2 (66.7)	6 (85.7)	7 (53.8)

a: considered only the cases hMPV subtyped; b: these symptoms are difficult to analyze in children less than one year old; ICU: intensive care unit; SD: standard deviation. Data are number of cases (%), unless otherwise indicated.

ring only in 2008. A periodical change in the predominant hMPV genotype/subtype has been previously reported, suggesting that antigenic shift may play a role as a mechanism of immune evasion (Larcher et al. 2008). Matsuzaki et al. (2008) explain that the predominant genotype and the most affected age group may be closely related to genotype-specific immune status within a community.

Boivin et al. (2004) have reported that in children less than three years, the hMPV genotype A is three times more common than genotype B (Boivin et al. 2004). In addition, group B strains occurred more frequently in adults. Rafiefard et al. (2008) reported that genotype A was dominant in both age groups. In the present study, genotype B was prevalent in both age groups. The different results obtained by the referred studies can be explained by the dynamic fluctuations of the two genotypes in different countries/continents (Rafiefard et al. 2008). During the study period, genotype B was the most detected (73%).

Symptoms of hMPV infection were similar to previous reports (Cuevas et al. 2003, Boivin et al. 2004, Chano et al. 2005). The final diagnosis showed that this virus was associated with lower respiratory tract disease (bronchiolitis, laryngotracheobronchitis and bronchopneumonia) that may cause an influenza-like illness in pediatric patients and in the adult population (Falsey et al. 2003, Xepapadaki et al. 2004, Bouscambert-Duchamp et al. 2005, Rafiefard et al. 2008). Among the hMPV infected patients submitted to HSCT proceedings, their underlying conditions may have contributed to their hospitalization and, in some cases, to the progression to a lower respiratory tract infection (Debur et al. 2010).

Previous reports that described the relationship between hMPV genotype and disease severity found no difference in clinical progression (Vicente et al. 2006, Agapov et al. 2006, Matsuzaki et al. 2008). Similarly, no correlation was observed between viral subtype and disease severity in the analyzed samples. However, a lower number of each hMPV subtype was detected in this study. Further studies comparing more cases are needed to clarify whether the hMPV subtype can be related to the severity of the infection or not. We suggest that hMPV infection should be routinely investigated in inpatients of all age groups to determine the risk of progressing to complications due to the occurrence of a lower respiratory tract infection.

In conclusion, hMPV has been reported as a respiratory pathogen in all age groups. Both hMPV genotypes can circulate in a single season and the predominant subtype switches in successive seasons. The disease severity did not correlate with hMPV subtype in the analyzed samples. Further studies, including analyzing additional consecutive years, are needed to determine the rule of hMPV genotypes/subtypes and to determine their pathogenicity.

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