

Full Length Research Paper

Human metapneumovirus infections in adults associated with respiratory illness in Sao Paulo, Brazil

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The human metapneumovirus was associated with respiratory infections among children without others etiologic causes attributable. Researchers worldwide subsequently detected the virus in samples from all age groups. Our study was established on adults presented with acute respiratory infections from 2001 to 2003. Three groups of outpatients were enrolled: community patients, health care workers and kidney transplant recipients. Routine detection for seven different viruses was undertaken and negative results were set up for reverse transcription polymerase chain reaction (RT-PCR) with primers targeted to hMPV's F gene. Twenty four out of 185 patients (12.97%) were considered positive after amplification of a 347 bp fragment. The virus was detected from June to September in the 2001 to 2002 periods, peaks were higher than 2003 and circulation began earlier in that year. Patients older than 51 years were associated with the infection ($p = 0.006$). Community cases exhibited a higher incidence (18.92%, 11/59) than that of health care workers (10.75%, 10/92) and transplant patients (8.82%, 3/34). No characteristic symptoms could be associated with hMPV infection. Transplanted patients were less symptomatic than the other groups evaluated. Metapneumovirus infections should also be considered within the diagnostics possibilities of respiratory viruses in adult population.

Key words: Human metapneumovirus, epidemiology, reverse transcription polymerase chain reaction (RT-PCR).

INTRODUCTION

The human metapneumovirus (hMPV) was first described in 2001 by van den Hoogen (van den Hoogen and de Jong, 2001) as a paramyxovirus associated with a range of clinical syndromes including colds, bronchiolitis, exacerbations of asthma and obstructive airways disease, pneumonia, and occasionally severe infections in immunocompromised hosts. This virus has been associated with acute respiratory tract infections in young children worldwide (Jartti and van den Hoogen, 2002; Peret and Boivin, 2002; Williams and Martino, 2005; Kahn, 2006) and also in adults as well (Falsey, 2008; Johnstone and Majumdar, 2008).

Respiratory infections are responsible for mild illness in immunocompetent children and adults but morbidity and

mortality are higher in the very young and very old as well; most of the available data on the clinical manifestations of hMPV infection are from studies of children where the virus causes upper respiratory tract infections, bronchiolitis and pneumonia. Recipients of solid organ transplants also are at increased risk (Ison and Hayden, 2002; Kahn, 2006; van den Hoogen, 2007). Health Care Workers (HCW) which are exposed to respiratory infections daily has been shown by our previous study to be at greater risk to acquire viral infections (Bellei and Carraro, 2007a). Several studies have described the hMPV infections in children as common place within this age group but more studies are needed to better characterize these infections in Brazilian adults (Hamelin

Table 1. Characteristics of 185 patients selected for hMPV investigation at Hospital Sao Paulo.

Characteristics	Patients (%)	Mean	Median
Sex			
Male	63(34.05)		
Female	122(65.94)		
Age			
Time of symptoms onset		37.34	36
Exposure to children(≤5yrs)	61(34.07)	4.93	3.5
Origin			
Health Care Workers	92(49.72)		
Community	59(31.89)		
Kidney transplanted	34(18.37)		

and Boivin, 2005; Sumino and Agapov, 2005; Williams and Martino, 2005).

Therefore our report aims to describe clinical and laboratory data of infections caused by this virus from three distinct adult populations from 2001 to 2003 from Sao Paulo.

MATERIALS AND METHODS

The study period began in June 2001 and was concluded in September 2003. Subjects enrolled were adults referred by the attending physician at the emergency room, HCW and kidney transplant patients were enrolled from the outpatient office of the Nephrology Division at Sao Paulo Federal University.

Inclusion criteria

Adults (≥ 18 years) were considered eligible after evaluation by a physician, if presented with any acute respiratory infection (ARI) of possible viral etiology. Influenza-like illness (ILI) was defined when the patient reported fever with at least one respiratory symptom (cough, sore throat or nasal congestion) plus one constitutional symptom (headache, myalgia or chills).

Sample collection

Each patient was interviewed and had one nasal wash sample collected by our researcher. The samples were sent on ice to the Virology Laboratory for immediate storage. Direct fluorescence assay (DFA) was performed immediately and duplicate aliquots of each sample were frozen in -80°C for further analysis by PCR and for virus isolation.

Respiratory virus assays

After centrifugation, the cell pellet was fixed in two slides for the DFA, which screens for Influenza A and B, parainfluenza 1, 2 and 3, adenovirus and respiratory syncytial virus in a two-step procedure, according to the manufacturer's instructions (Light Diagnostics Simulfluor® Screen and Panel, Chemicon Int., Canada). Negative samples were tested by RT-PCR for Picornavirus and

Coronavirus OC43 and 229E according to the methods described previously. The 185 negative samples from 412 patients evaluated through those tests described were further analysed by another RT-PCR assay to detect hMPV viral gene. Negative samples were chosen for this study to evaluate the impact of this virus alone in the populations accessed and also to evaluate the clinical symptoms that could be associated with the infection, disregarding co-infections as a potential confounding parameter.

Reverse transcription polymerase chain reaction (RT-PCR)

Total viral RNA was extracted using QIAamp Viral RNA extraction kit (Qiagen, USA), according to the manufacturer's instructions. The hMPV's 347nt nucleoprotein fragment used flanking primers was designed by Falsey (2008) and the reaction was conducted as described below. Eight microliters of extracted RNA were added to a mix containing 100 U of M-MLV (Invitrogen, USA), 4 µl of 5× first strand buffer, 2 µl of DTT, 1 µl of dNTP mix (2 mM each), 2 µl of primer FR (25 µM) plus 15 U of RNAGuard (Amersham, USA) and molecular biology grade water to a final volume of 20 µl. The mixture was maintained on ice until the enzyme was added, then it was submitted to one hour at 37°C followed by an inactivation step of 70°C for 15 min in a PCT200 MJ Research thermocycler.

Two and a half microliters aliquots of each cDNA sample produced in the first step were used in the PCR. A mixture containing 2.5 µl of 10× buffer solution, 2.5 µl molecular grade glycerol 50% (v/v) (Invitrogen, USA), 2 µl of MgCl₂ (25 mM), 2 µl of a 25 µM primer FF, 0.5 µl of a dNTP mix (20 mM each) plus 2.5 U of Taq polymerase (Promega) was added to each reaction tube containing molecular biology grade water to a final volume of 25 µl. The reaction was conducted at 95°C for 5 min followed by 40 cycles of 94°C for 30 s, 53.2°C for 30 s and 68°C for one min. A final extension step of 10 min at 72°C and then maintained at 4°C until a electrophoresis on a 2% agarose gel was performed.

Statistical analysis

To correlate positive with negative cases distributions between the groups a χ^2 (chi-square) partition test for numerical data alongside with Fishers exact test and Students T test, epidemiological and clinical variables were analyzed. Level of significance was established at less than 0.05 ($\alpha \leq 5\%$). Calculations were done with Microsoft Excel version 2010-SP1.

RESULTS

From June 2001 to September 2003, one hundred and eighty five patients previously tested negative for other viruses were enrolled for the study. Subjects mean age was 37.3 years (median of 35 years), 66% of those patients were female and 34% had contact with children under five years old. Risk group distribution and symptoms duration are shown in Table 1.

The RT-PCR for hMPV was positive in 24 (12.97%) patient's nasal washes. Figure 1 represents hMPV infections in a monthly distribution during the study period. All hMPV positive samples were identified within winter months and no gaps were found during its season, but the variations that were found were in accordance with the risk groups studied. The age distribution of hMPV infections had a high probability of association ($p = 0.006$) when the group of patients with the elderly (51+

Table 2. Distribution of positive and negative cases according different risk group of patients during the study period.

Year	2001		2002		2003	
	Positive	Negative	Positive	Negative	Positive	Negative
Population						
Community	1	20	0	13	10	15
Health care workers	1	15	2	51	7	16
Kidney transplanted	0	0	2	20	1	11
Total	2	35	4	84	18	42

Table 3. Clinical symptoms frequencies among hMPV positive and negative cases different risk group of patients investigated during the study period.

Symptom	Health care workers (%)		Community (%)		Renal transplant (%)	
	Positive (n=10)	Negative (n=83)	Positive (n=11)	Negative (n=47)	Positive (n=3)	Negative (n=31)
Fever	40	64	81	74	0	29
Cough	70	78	91	79	67	77
Coryza	90	84	91	72	67	81
Sore Throat	50	67	73	68	33	39
Headache	90	73	73	70	33	55
Myalgia	90	78	55	66	67	48
Chills	50	53	55	49	0	26

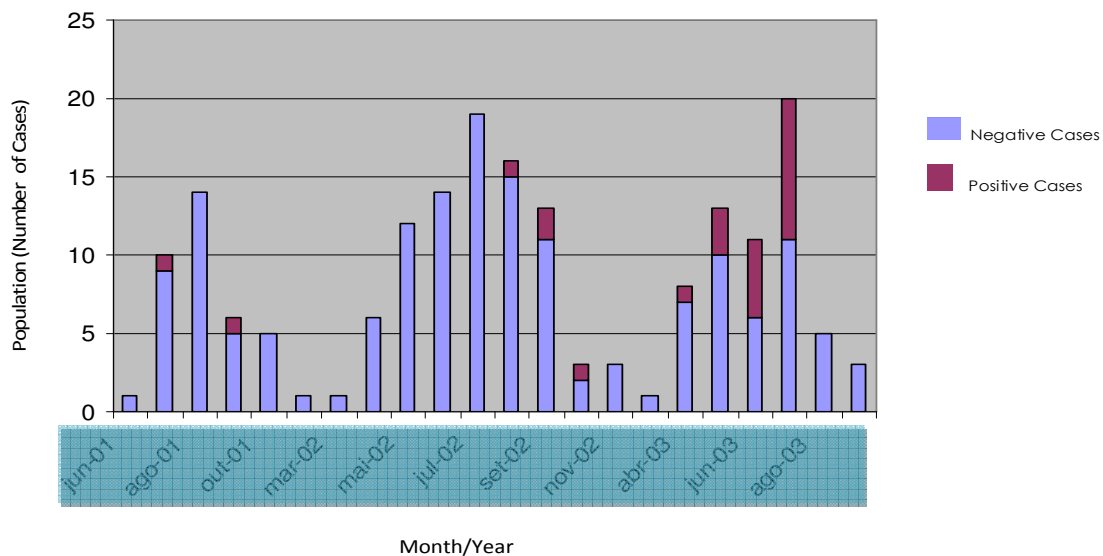


Figure 1. Laboratory confirmed hMPV infection cases distribution among adult population presenting with acute respiratory infection from 2001 to 2003.

y.o.) was compared to the group with younger patients from all groups.

The variable “contact with patient” was not associated with higher risk of hMPV infection for the HCW population, clinical data shown in Table 1. The history of household or professional contact with a child with less than 5 years old was also not associated with higher risk for infection. Cases from the community had higher positivity results (18.92%, 11/59; symptoms associated with this group are shown in Table 2), particularly in 2003. Health care workers and kidney transplant patients had 10.9%

(10/92) and 18.6% (11/34) positivity, respectively.

All positive samples were collected from patients that had symptoms in 5.1 days on average (mean 4.9 days). ILI case definition was reported by 45.8% of positive cases except for transplant patients, this group did not present with ILI at that moment. In all, the transplant positive cases were less symptomatic than the other groups evaluated together. The clinical data revealed that hMPV positive cases presented mainly coryza (87.5%), cough (79.1%) and headaches (75%) but this data was not statistically significantly different from negative cases

(Tables 1 to 3).

DISCUSSION

The hMPV incidence among the adult population from different risk groups from Sao Paulo, Brazil was accessed by this work. Negative cases from previously immunofluorescence screened cases, from three distinct groups of patients (community population, health care workers and kidney transplant) over three years, were analyzed by RT-PCR reactions and their clinical data correlated with the results. All hMPV positive samples were identified within winter months. This winter pattern concur with previous studies regarding the circulation period for this virus (Falsey and Erdman, 2003; Falsey and Criddle, 2006).

Our study detected this virus in 12.97% of the negative samples tested. This reflects that hMPV is present in approximately one in each ten adults presented with respiratory symptoms in our population, without any other confirmed viral infection. Considering that there are few case reports available for hMPV infections in adults and/or in the elderly from Brazil, limited conclusions can be drawn and more epidemiological studies are needed to confirm the data. In Brazil, Cuevas et al. (2003) investigated 120 children for hMPV infections and found 15.2% of positive cases among them, a incidence that is much higher than the one found by our study, since this group is at more risk of infection and at greater risk of coming into contact with other children, higher positivity rates were expected; but in comparison to our study groups, the assays exhibited an overall comparable result, a more detailed and prospective study must be conducted to identify variables attributable to this infection within these groups in order to better understand some of the symptoms observed in the patients studied here.

Previous studies found that positive cases of hMPV infections in adults presenting with ARI had incidences ranging from 2.2% (Stockton and Stephenson, 2002) to 3.4% (Falsey and Erdman, 2003). Kaye et al. (2006) found 5.4% of hMPV infections in adults from the community, a result that is similar to the incidences observed in 2001 and 2002 of our study. In 2003, there was a raise in positivity up to 30%, which in turn could be explained by different subtype prevalence as observed and hypothesized by Gerna et al. (2005), whose similar results were further confirmed to be due to different subtype circulation. Since our study was primarily concerned with establishing ARI etiology within the three samples studied, no such claim can be made to whether viral subtype is responsible for the differences in cases observed here.

The association between advanced age and infections by hMPV suggested by other authors (Boivin and Abed, 2002; Falsey and Erdman, 2003; Honda and Iwahashi, 2006) was also found by our study when comparing the all samples together ($p = 0.006$). Patients older than 51

years had more infections by hMPV than the other four age groups. Such an association was also described by Kaye et al. (2006). The twenty positive samples for hMPV, out of the 373 obtained, had 50% of those positive cases belonging to that age group (< 75 y.o.) in that report.

No previous detection of hMPV in kidney transplanted patients was reported here in Brazil. Our results for this population showed fewer positive cases than those published by Larcher et al. (2005) whose group identified the virus in 25% of lung transplant recipients. It is also important to notice that we recruited patients from our ambulatory and most hMPV studies to date were based on hospitalized patients, which might overestimate its incidence. The transplanted patients evaluated here were less symptomatic than the other two groups. The positive cases had even less symptoms, with an exception made for myalgia; without statistical significance (67 versus 48%; $p > 0.05$). These findings may reflect that the treatment protocols that the transplant recipients undertake, such as corticosteroids plus immunosuppressive therapy, may be a reason for masking flu-like symptoms (Bellei and Carraro, 2007b; Vu and Bridevaux, 2011).

All three population's clinical aspects did not reveal typical symptoms for predicting hMPV infections (Tables 1 to 3). Falsey et al. (2003) previously reported no evidence for an association between fever and hMPV infections, however, the same group in another report (Falsey and Criddle, 2006) show that 80% of hMPV cases studied had fever, against 48% from control cases. This suggests that this factor may still be under unidentified conditions.

Our results point to a significant percentage of hMPV infections in adults from Hospital Sao Paulo. Community acquired hMPV infections affects nearly one out of five patients without a diagnosed etiology presented with influenza-like illness during winter months according to our results. Our study was not designed to detect hMPV co-infections and from this reason, the clinical data described here were directly associated with hMPV. This study suggests that hMPV is a frequent pathogen among adults in Brazil. Metapneumovirus infections should also be considered among the most common respiratory viruses by the attending physician in periods of respiratory viruses with known circulation.

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