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# Human metapneumovirus as a causative agent of acute bronchiolitis in infants

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**Abstract**

*Background:* Human Metapneumovirus (hMPV), has been recently isolated from children with acute respiratory tract infections (RTIs), including bronchiolitis, and classified in the Pneumovirinae subfamily within the Paramyxoviridae family. *Objectives:* Since most bronchiolitis studies fail to detect any viral pathogen in part of the samples, we sought for the presence of hMPV in a well characterized bronchiolitis cohort. *Study design:* Nasal washes were obtained from 56 children admitted to the hospital for acute bronchiolitis. RNA extraction and subsequent RT-PCR were used to detect hMPV, and correlated the presence of the virus with clinical characteristics of the disease. *Results and conclusions:* PCR revealed the presence of hMPV in 16% of bronchiolitis cases, whereas respiratory syncytial virus (RSV; 67.9%) was the most frequently encountered viral pathogen. hMPV was identified either as a unique viral pathogen or co-existed with RSV, with whom they shared a similar seasonal distribution. There were no differences in disease characteristics, either clinical or laboratory, between bronchiolitis cases where hMPV was present and those caused by RSV or other viral pathogens.

These findings suggest that hMPV is a common and important causative agent in infants with bronchiolitis, with clinical characteristics similar to that of RSV.
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*Keywords:* Human metapneumovirus; Bronchiolitis; Respiratory syncytial virus; Polymerase chain reaction; Diagnosis

## 1. Introduction

A new respiratory virus, human metapneumovirus (hMPV), has been recently isolated from nasopharyngeal aspirates of young children in the Netherlands (van den Hoogen et al., 2001). hMPV is the first human respiratory pathogen classified as a member of the Metapneumovirus genus, which together with Pneumoviruses constitute the Pneumovirinae subfamily within the Paramyxoviridae family (van den Hoogen et al., 2001; Boivin et al., 2002; Viazov et al., 2003). The clinical symptoms of the children from which the virus was isolated ranged from upper respiratory airway disease to severe bronchiolitis and pneumonia (van den Hoogen et al., 2001). Subsequent studies established the association of this virus with acute RTIs in all age groups (Maggi et al., 2003; Osterhaus and Fouchier, 2003; Boivin et al., 2003; Vicente et al., 2003; Stockton et al., 2002; Boivin et al., 2002; Peiris et al., 2003; Freymouth et al., 2003). However, several epidemiological as well as clinical aspects of hMPV infection are still to be firmly established and in this context the analysis of well defined respiratory disease cohorts is of particular importance (Jartti et al., 2002; Rawlinson et al., 2003).

We hypothesized that hMPV may be an important pathogen in the context of acute bronchiolitis of infancy. In addition to bronchiolitis cases being reported within the patients of published hMPV studies, this is further suggested by the observation that most bronchiolitis studies fail to identify a pathogen in a considerable proportion of cases.

We have recently reported the results of virological evaluation of a well-characterized cohort of infants admitted to

*Abbreviations:* NPW, nasopharyngeal wash; hMPV, human metapneumovirus; PCR, polymerase chain reaction; RSV, respiratory syncytial virus; RTI, respiratory tract infection; RV, human rhinovirus

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hospital with acute bronchiolitis, using reverse transcription polymerase chain reaction (PCR) for 11 respiratory pathogens (Papadopoulos et al., 2002). As expected, RSV was the predominant pathogen, while human rhinovirus (RV) was implicated in almost one-third of cases. Adenoviruses, influenza viruses, parainfluenza viruses and corona viruses, were involved to a smaller extent. Nevertheless, no pathogen was identified in almost one quarter of the samples (Papadopoulos et al., 2002).

Therefore, the aim of the present study was to assess the presence of hMPV in stored nasal secretions of this cohort and evaluate the relationship between the presence of the virus and the clinical and epidemiological characteristics of the disease.

## 2. Methods

### 2.1. Patients

The study was carried out in the Second Pediatric Clinic of the University of Athens, Greece, at the P&A Kyriakou Children's Hospital, and was approved by the hospital's Ethics Committee. Children were admitted in the hospital with the diagnosis of bronchiolitis, which was defined as an acute infection of the lower airway, characterized by increased respiratory effort (tachypnea of >50 respirations/min and/or use of accessory respiratory muscles), and expiratory wheezing and/or crackles. A detailed description of the cohort has been reported (Papadopoulos et al., 2002). Nasopharyngeal washes (NPW) have been obtained at admission, aliquoted and kept at −70 °C. A new aliquot was thawed and used for hMPV detection. Sufficient material for analysis was available in 56 cases.

### 2.2. Detection of hMPV by PCR

Viral RNA was extracted using a phenol–guanidine isothiocyanate method as previously described (Papadopoulos et al., 2002). Reverse transcription was performed at 37 °C in a 50 mM Tris–HCl, 75 mM KCl, 3 mM $MgCl_2$ buffer containing 10 mM DTT, 0.4 mM dNTPs, 0.5 μg random hexamer primers and 200 units of reverse transcriptase (Superscript™, Invitrogen). cDNA was stored at −20 °C until used. Primers (upper MPVNF: 5′ AGGCCCTCAGCACCAGACA3′; lower MPVNR: 5′TTGACCGGCCCCATAAGC3′) were designed against the N (nucleocapsid) region of the 00-1 isolate of hMPV (Genbank accession Nr. AF371337.2) to amplify a 318 bp fragment corresponding to nucleotides 505–822 of the published hMPV genome (van den Hoogen et al., 2001). PCR reaction was conducted in the presence of 2.5 mM $MgCl_2$, 400 μM dNTPs, 0.25 μM of each MPV primer and 2.5 units Taq polymerase (Invitrogen) for 40 cycles of 94 °C for 40 s, 55 °C for 40 s and 72 °C for 1 min. Subsequently, 20% of the PCR reaction volume was run in a 1.8% agarose gel and, when present, 318 bp amplicons were excised and further purified with the Concert Rapid Gel Extraction System kit (GibcoBRL), according to the manufacturers' instructions. Selected samples were vacuum dried and sequenced using the MWG AG Biotech (Ebersberg, Germany) sequencing facilities. Comparison of the sequenced fragments to the hMPV genome was done by submission to the Genbank using the standard nucleotide-nucleotide BLAST (blastn), whereas comparison between individual amplicons was conducted using blast2.

NPWs obtained from 10 healthy children, without symptoms or signs of upper respiratory infection were also analyzed as negative controls.

### 2.3. Statistical analysis

One-way analysis of variance was used for the comparison of continuous variables among subjects with hMPV, without hMPV and with RSV. Chi-square was performed for the analysis of ordinal or categorical data. Results of continuous data are expressed as mean ± standard error of mean. Probability values of <0.05 were considered significant.

## 3. Results

hMPV was present in 9/56 samples (16.1%). Sequence analysis of positive PCR products and comparison with the published sequences (van den Hoogen et al., 2002; Peret et al., 2002), confirmed the identity of the amplicons. RSV was the predominant pathogen in this cohort, found in 38/56 samples (67.9%), followed by human rhinovirus (eight cases: 14.3%), adenoviruses (four cases), coronavirus (three cases) and parainfluenza virus (one case). In 16 patients, two viruses were simultaneously present, while no pathogen was identified in nine cases. PCRs performed in NPWs of healthy control children obtained during the same period, were all negative for hMPV.

Among the hMPV positive samples, the virus was a unique pathogen in five cases (55.6%), while in four it was present together with RSV. No double positive cases of hMPV with other respiratory viruses were observed.

Most hMPV cases appeared in February and persisted, though gradually diminishing, until April (Fig. 1). The RSV season on that year was between December and March.

There were no differences in disease characteristics between bronchiolitis cases were hMPV was identified and those caused by RSV or other pathogens (Table 1). There was also no difference in disease severity between double positive RSV–hMPV cases and single positive hMPV (severity index: 8.3 ± 0.6 versus 8.4 ± 0.6, respectively). Similarly, no differences were found when components of the severity index (heart rate, respiratory rate, degree of wheezing, skin colour and oxygen saturation) were analyzed individually, or in laboratory findings (data not shown).

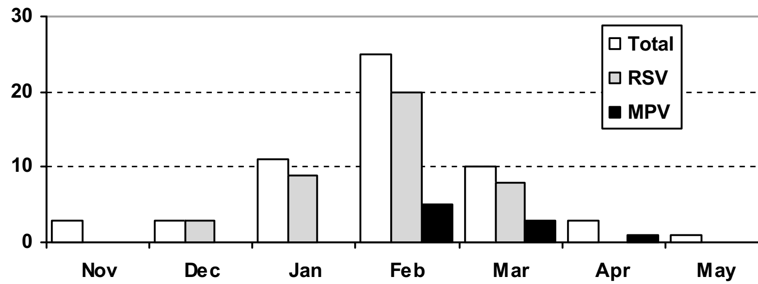


Fig. 1. Seasonal distribution of hMPV. The numbers of aspirates found to be infected by hMPV (closed bars) or RSV (dotted bars) during 1 year are depicted in comparison to the number of samples containing any respiratory virus.

Table 1
Disease characteristics of patients in which hMPV was or was not (w/o) detected in comparison to those recorded in the presence of RSV

| Variable | MPV ($n = 9$) | w/o MPV ($n = 47$) | RSV ($n = 38$) |
|---|---|---|---|
| Gender (%) (male) | 67 | 70 | 71 |
| Age (months) | 5.3 ± 1.1 | 5.4 ± 0.5 | 5.0 ± 0.5 |
| Birth weight (g) | 3118 ± 150 | 3150 ± 71 | 3103 ± 86 |
| Smoking (%) | 44 | 74 | 65 |
| Family history of atopy (%) | 33 | 24 | 19 |
| Duration of hospitalization (days) | 5.3 ± 0.9 | 4.8 ± 0.4 | 5.1 ± 0.5 |
| Fever (%) | 44 | 35 | 34 |
| Severity score[a] | 8.3 ± 0.4 | 8.3 ± 0.2 | 8.3 ± 0.2 |

Unless otherwise indicated data are presented as number of positive/number of reported (%).

[a] Severity score was calculated by adding individual scores (1–3) for cardiac rate (<120, 120–160, >160), respiratory rate (<40, 40–60, >60), wheezing (expiratory, expiratory + inspiratory, audible without auscultation), skin color/feeding (normal, mild cyanosis/difficult feeding, cyanosis/feeding not possible) and $SaO_2$ (>98%, 94–98%, <94%). The severity score ranges from 3–15.

## 4. Discussion

Although RSV is indisputably the most common cause of acute bronchiolitis, virological analyses have failed to reveal a pathogen in a considerable fraction of cases in most studies. In this context, the recently isolated hMPV (van den Hoogen et al., 2001), could partially account for previously undiagnosed cases.

The present study provides evidence of frequent involvement of hMPV in acute bronchiolitis in infants, using PCR-based detection. hMPV was present in 16% of bronchiolitis cases, a percentage only second to RSV, comparable to that of human rhinovirus (Papadopoulos et al., 2002), and clearly higher than that of the other respiratory viruses tested. Similarly, Viazov et al. (2003) detected hMPV in 17.5% (and RSV in 23.8%) of young children with respiratory tract illness, with most the cases diagnosed as bronchiolitis (Viazov et al., 2003). In another study, the percentage of hMPV within the bronchiolitis subgroup was 21% (Maggi et al., 2003).

hMPV was either a unique pathogen, or co-existed with RSV. Greensill et al. (2003), analyzed bronchoalveolar lavage fluids collected from infants with RSV bronchiolitis and found that 70% of them were co-infected with hMPV (Greensill et al., 2003). In contrast, Vicente et al. (2003) found no hMPV co-infections with any other virus in a pediatric population with acute RTI (Vicente et al., 2003). The simultaneous presence of hMPV with RSV may reflect the considerably overlapping seasonal distribution of the two viruses. hMPV seasonal distribution observed in this study resembles those recently reported in Finland and Canada (Jartti et al., 2002; Boivin et al., 2003). Finally, it should be noted that this is the first report of hMPV infection in Greece and the East Mediterranean area, further confirming the worldwide distribution of this virus (van den Hoogen et al., 2001; Stockton et al., 2002; Peret et al., 2002; Howe, 2002; Jartti et al., 2002; Freymouth et al., 2003; Viazov et al., 2003; Peiris et al., 2003; Vicente et al., 2003; Maggi et al., 2003).

No differences were found between cases where hMPV was identified and those caused by RSV or other viral pathogens. We also found no difference in disease severity, or laboratory tests, between double positive RSV–hMPV cases and single positive hMPV cases. All the above support the notion that hMPV is able to induce bronchiolitis which is clinically identical to that caused by RSV.

Current evidence suggests that hMPV may account for a fraction of respiratory infection cases whose causative agent remained unidentified in previous cohorts (Osterhaus and Fouchier, 2003). Additional studies should be undertaken in order to characterize the role of hMPV in different human respiratory tract diseases and provide important information for future development of specific antiviral therapies and vaccines.

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