

# Human Metapneumovirus (hMPV) in Acute Respiratory Infection: A Clinic-Based Study in Egypt

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## Abstract

**Objective** To estimate the prevalence of Human metapneumovirus (hMPV), its epidemiological and clinical features in infants and children with respiratory infections, attending outpatients' clinic of Mansoura University Children Hospital (MUCH).

**Methods** After taking history, clinical examination and appropriate investigations, nasopharyngeal aspirates were collected from 600 infants and children with symptoms and signs of respiratory infections. Samples were examined by RT-PCR for hMPV.

**Results** The overall prevalence of hMPV infection among studied patients was 8% (95% CI=6.1–10.4). The rate was significantly higher among children aged 2–24 mo compared to other age groups (11.9% vs. 3.7% and 4.0% for 2–24, 25–60, 61–108 mo respectively). Also it was significantly higher among females than males (12.6% vs. 6.6%). Cough, wheezing, rhinorrhea, fever and chest wall retraction were the most frequent presentations (81.2%, 68.8%, 66.7%, 64.6% and 56.3%; respectively). Antibiotics, bronchodilators and oxygen administration were the most

common treatments offered (60.4%, 31.2% and 27.1%; respectively).

**Conclusions** hMPV is an emerging cause of acute respiratory infection in Mansoura University Children Hospital (MUCH), and may have a significant clinical impact on infants and children and thus, must be considered in etiological diagnosis.

**Keywords** Human · Metapneumovirus · Respiratory infections · Children

## Introduction

Respiratory tract infections are a leading cause of morbidity and mortality worldwide. All classes of microorganisms including viruses, bacteria and protozoa are capable of infecting the respiratory tract [1]. A variety of viruses, including respiratory syncytial virus (RSV), influenza virus, parainfluenza virus, adenovirus, coronavirus and picornavirus, are associated with different respiratory syndromes in all age groups [2].

In half of the upper respiratory tract infections (URTI) in children, an infectious cause cannot be determined [3]. Also the etiology of a majority of lower respiratory tract infections (LRTI) is thought to be viral [4], yet in only 40% of cases a viral agent can be identified [5]. These observations suggest that unknown pathogens may be responsible for a substantial proportion of respiratory tract diseases [5].

Recently, an increasing number of studies demonstrated that the human metapneumovirus (hMPV) causes mild to severe respiratory infections in both sexes of all ages in different countries of the world and may be a frequent but somewhat undervalued pathogen [6]. hMPV induces clinical

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symptoms ranging from upper to lower respiratory illness such as bronchiolitis, bronchitis and pneumonia [7].

hMPV grows poorly in cell culture. The replication of hMPV in vitro is restricted to a limited number of cell lines. A more important defect is the long incubation cycle [7]. These properties may explain why the virus was not identified until recently [7]. hMPV-specific antibodies have been developed for immunofluorescence assays, though this method may not be as sensitive as RT-PCR for the detection of hMPV [8]. As infections with hMPV are universal, serologic testing for diagnosis can only help if four fold increases in antibody titers or seroconversion is demonstrated [9].

Currently, RT-PCR is the most common method used to detect hMPV. Several genes of hMPV have been primer targets for genomic amplification. Cote et al. [10], reported that primers that bind regions of the N and L genes are highly sensitive for the detection of hMPV strains of both genotypes.

A previous study [11] done in Egypt found a prevalence of 13.6% of human metapneumovirus in adult patients with lower respiratory tract infections. To the best of the authors' knowledge, hMPV has not been studied previously among children in Egypt. Hence, this study is planned to determine the frequency, epidemiology and clinical characteristics of this virus in children with respiratory tract infection manifestations using a rapid fairly accurate test, RT-PCR.

## Material and Methods

This study was carried out in outpatients' clinic of Mansoura University Children Hospital (MUCH), Egypt during the calendar year of 2010. Mansoura is the largest city in the northeast of Egypt. Outpatients' clinic offers daily free services to all sick children. According to the hospital statistics, a total of 35,268 children attended the outpatients' clinic during the study period. Acute respiratory infection (ARI) accounted for about one-third of diagnoses. Six hundred children were included in the study after the consent of their parents. Depending on the logistics and parents' agreement, 600 child were included (600/11,756 =5.1% of children with ARIs). Data and specimen (nasopharyngeal aspirate) collection were carried out 3 d per wk. Children with ARIs diagnosed during the day were enlisted and a systematic random sample of them was selected (one case per fixed number of cases) for specimen collection. The number specimens collected varied from 3 to 5 per day according to the number of children with ARIs.

The research protocol was approved by the research ethics committee of Faculty of Medicine, Mansoura University and by the Administration of MUCH.

Each child was subjected to history taking, clinical examination, and appropriate laboratory and radiological examination and then nasopharyngeal aspirate was collected. Treatment was offered and final diagnosis was recorded. Nasopharyngeal aspirates were collected by syringe with 3 or 4 ml of buffered saline which was sequestered into the nose and aspirated again, then immediately installed into viral transport media. The samples were placed immediately in cold packs or refrigerator (ice box with ice), transported immediately to MDICU where they were stored at 2°C–8°C.

RNA was extracted using TriFast™ reagent, according to the manufacturer's instructions. First strand cDNA synthesis from extracted RNA was done using The revertAid™ Minus first strand cDNA synthesis kit (Fermentas), according to the manufacturer's instructions. Then PCR was performed with primers for amplification of M gene, hMPV- MF1 (AAGT GAATGCATCAGCCCAAG) and hMPV- MR1 (CACA GACTGTGAGTTTGTCAA), which amplified the region between nucleotides 212 and 331, were used to give an amplicon of 120 bp. Each PCR reaction mixture contained 10 µl of cDNA, 2.5 U Taq DNA polymerase (Fermentas), 2 mmol/L MgCl<sub>2</sub>, 4 µmol each primer, 300 µmol/L each dNTP, 50 mmol/L KCl and 10 mmol/L Tris Cl (pH 8.5). PCR cycling conditions were 95°C for 10 min followed by 45 cycles of 95°C for 1 min, 58°C for 1 min, 72°C for 1 min and a final extension step for 10 min in a master cycler instrument Perkin Elmer cetus (Norwalk, Conn). Positive samples were confirmed by PCR amplification of a longer product of M gene. The longer M product used primers for hMPV- MR1 and a new primer, hMPV MF2 (ATGGAGTCCTATCTAGTAGTAC) which amplified the region between nucleotides 1 and 331 of the M gene, yielded an amplicon of 331 bp product. The cycling conditions were same for the first PCR but with annealing temperature of 62°C. Amplicons were analyzed on agarose gels (1%) after ethidium bromide staining. As negative control distilled water was used, positive controls were not available for use.

Statistical analysis of the results was performed with SPSS (Statistical Package for Social Sciences) program version 16. Mean, standard deviation, and percentage were used as descriptive statistics. Categorical variables were analyzed by chi-square test. Difference at *P* value of ≤0.05 was considered significant.

## Results

Table 1 shows that the overall prevalence of hMPV infection among studied patients was 8% (95%CI=6.1–10.4). The prevalence rate was significantly higher among children aged 2–24 mo compared to other age groups (11.9% vs. 3.7% and 4.0% for 2–24, 25–60, 61–108 mo respectively). Also it was significantly higher among females than males (12.6% vs.

**Table 1** Prevalence of hMPV and its variation according to some factors

	Total	h-MPV Positive N (%)	Significance test
Overall	600	48(8.0)	
Age (mo)			
2–24	311	37(11.9)	$\chi^2=13.3, P=0.0013$
25–60	190	7(3.7)	
61–108	99	4(4.0)	
Sex			
Male	457	30(6.6)	$\chi^2=5.37, P=0.021$
Female	143	18(12.6)	
Season			
Winter	364	30(8.2)	$\chi^2=1.91, P=0.59$
Spring	176	11(6.3)	
Summer	19	2(10.5)	
Fall	41	5(12.2)	
Diagnosis			
Pneumonia	378	27(7.1)	$\chi^2=1.99, P=0.57$
Bronchial asthma	103	8(0.8)	
Bronchiolitis	88	9(10.2)	
Croup	31	4(12.9)	

6.6%). However, the prevalence does not show any variation with diagnosis and season of infection.

Clinical presentations and treatment were enlisted in Table 2. Cough, wheezing, rhinorrhea, fever and chest wall retraction were the most frequent presentations (81.2%, 68.8%, 66.7%, 64.6% and 56.3%; respectively). Antibiotics, bronchodilators and oxygen administration were the most treatments offered (60.4%, 31.2% and 27.1%; respectively).

**Table 2** Clinical presentation and treatment of cases with hMPV infection

	N (%)
<b>Clinical presentations<sup>a</sup>:</b>	
Cough	39(81.2)
Wheezing	33(68.8)
Rhinorrhea	32(66.7)
Fever	31(64.6)
Chest wall retraction	27(56.3)
Sore throat	20(41.7)
Hyperventilation	20(41.7)
Hypoxemia	19(39.6)
Tachycardia	18(37.5)
Feeding difficulties	18(33.3)
<b>Treatment<sup>a</sup>:</b>	
Antibiotics	29(60.4)
Bronchodilators	15(31.2)
Oxygen administration	13(27.1)
Corticosteroids	8(16.7)
Artificial respiration	4(8.3)

<sup>a</sup>Categories are not mutually exclusive

## Discussion

Human metapneumovirus (hMPV) is an emerging virus associated with a significant proportion of both upper and lower acute respiratory infection in infants and young children [12, 13]. Because of the unavailability of rapid antigenic detection assays in the past and slow growth in tissue culture, molecular methods have become the method of choice for the diagnosis of hMPV infection [10].

In the present study, the incidence of hMPV was 8%. This result is more or less similar to previous studies in other countries with incidence ranging from 5 to 10% in children with ARI cases [14–20]. Higher detection rates were reported from some countries [21, 22] while lower levels observed in some studies [23, 24]. This variation could be attributed to different localities, age group, genetic susceptibility, sampling technique and detection method.

Epidemiological findings suggest that hMPV may circulate worldwide and may have a seasonal distribution in winter months for temperate and spring/summer for tropical countries [13, 25]. In this study incidence of hMPV is insignificantly higher during fall and summer seasons, a situation that shows a mix of the temperate and tropical countries.

Caracciolo et al. [12], in Italy, observed a high incidence of hMPV infection (25.3%) during winter-spring season. A study in northern Taiwan concluded that hMPV circulates in children during spring and early summer [24]. In Netherlands, hMPV was found primarily in winter months and rarely detected in summer months [15]. In Korea, hMPV infections peak in winter and spring [20].

In the present study hMPV infection was highest in children less than 2 y. Most hMPV infections occur in children less than 5 y of age, with children <2 y of age being most at risk for serious hMPV infections [8, 13, 15, 26]. In Japan, hMPV was significantly higher among children with ARI aged 3–5 y (20.9%) compared to those aged 1–3 y (16.5%) and 5–10 y(16.0%) [14].

The authors found that the incidence of hMPV is significantly higher among female than male children. However, Ebihara et al. [14] and Hara et al. [19], reported no sex differences in detection of hMPV.

The virus causes a variety of clinical symptoms in children that are typical of the paramyxoviruses, including upper and lower respiratory tract illnesses [13]. The virus was significantly more frequent among children diagnosed with croup (12.9%), bronchiolitis (10.2%) and pneumonia (7.1%), compared to 0.8% among asthmatic children. Previous studies concluded that hMPV has likely been a major factor in pneumonia and bronchiolitis [7, 19, 23, 27]. The diagnoses were bronchiolitis, bronchopneumonia, infantile asthma or upper respiratory tract infection in China [28]. In Italy, the diagnoses were bronchiolitis, pneumonia and upper respiratory tract illness [12].

The clinical characteristics of hMPV infections are not distinctive. Thus, differentiating it from other respiratory viruses on clinical grounds is not possible [29]. In the current study 81.2% of hMPV infections were associated with cough, 60.6% were associated with fever, 66.7% with rhinorrhoea and 68.8% with wheezing. The same symptoms were reported with different percentages in different studies from different localities [18, 27, 28, 30].

Antibiotics were by far the most frequently prescribed treatment (about 60%) followed by bronchodilators (31%), oxygen administration (27.1%), corticosteroids (16.7%) and artificial respiration (8.3%).

In a Netherlands' study the most frequent treatments were antibiotics (60%), bronchodilators and oxygen administration (36%, each), corticosteroids (20%) and artificial respiration (12%) [18].

Because at the time of consultation 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 and to control wheeze. This indicates that testing for hMPV in patients with RTI may reduce unnecessary use of antibiotics and corticosteroids.

A weakness of this study is the fact that only outpatients' infants and children attending a single hospital with infections severe enough to seek medical advice were included. Therefore, the prevalence of hMPV in these children may underestimate its prevalence in the community. A previous study [11] in Egypt found a prevalence of 13.6% of human metapneumovirus in adult patients with lower respiratory tract infections. The present results cannot be extrapolated to the entire population of Egyptian children. Despite this limitation, this study confirms the etiologic role of hMPV in respiratory infections among children in Egypt. A wide-scale community-based study is recommended for further characterization of the viral genotype, its epidemiology, co-infections and re-infections.

## Conclusions

hMPV infection is frequent in infants and children presented with different clinical manifestations of respiratory tract infections in MUCH and thus must be considered in etiological diagnosis though no treatment is yet known.

**Conflict of Interest** None.

**Role of Funding Source** None.

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