Research Article
Future Virology

The role of human metapneumovirus in pediatric respiratory tract infection in Qatar

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Aim: The human metapneumovirus (hMPV) has been recently discovered as an etiological agent of acute respiratory infections in infants and children, with similar clinical symptoms to those caused by respiratory syncytial virus. The aim of this study was to determine the prevalence of hMPV and its potential role as causative agent of respiratory tract infections in children in Qatar. **Methods:** In the present study, we examined 84 nasopharyngeal aspirates from children with respiratory tract infections, presenting at Al-Saad Pediatric Emergency Center in Doha as outpatients, for the presence of respiratory viruses. **Results:** A total of 56 out of 84 (66.7%) cases were positive for the presence of respiratory viruses. Out of the 56 positive cases 54 (96%) contained hMPV; whereas 12 out of 56 (21.4%) contained human parainfluenza virus. A total of 14 out of 56 of the positive patients were infected with more than one virus. hMPV was in samples infected with one or more respiratory tract infection viruses and was the most frequently isolated virus from infants less than 6 months of age. **Conclusion:** This is the first report demonstrating the prevalence of hMPV in children suffering from respiratory tract infections in Qatar. Detection of this virus may have significant clinical implications in this patient population in Qatar.

Respiratory tract infections are ranked as the second leading cause of death in children younger than 5 years of age, regardless of geographical area [1]. In the pediatric population, respiratory syncytial virus (RSV), parainfluenza viruses and influenza virus are the major causes of bronchiolitis and lower respiratory tract infections (LRTI). However, a viral agent can only be identified in 40% of LRTIs, even with use of state-of-the-art genomic amplification methods [2]. Furthermore, an infectious agent cannot be identified in nearly half of upper respiratory tract infections (URTI) occurring in children [3]. These observations suggest that previously unknown pathogens may be responsible for a substantial proportion of respiratory tract diseases.

Human metapneumovirus (hMPV) is a negative-sense, ssRNA virus that was first described in 2001 as a novel paramyxovirus isolated from the respiratory tract of children in The Netherlands [4]. hMPV has been identified worldwide [5–9] and appears to have a seasonal distribution (winter and spring) [10]. Since its initial description, hMPV has been identified as a leading cause of LRTIs in previously healthy infant and child outpatients [11]. The incidence of hMPV-associated LRTI in young children varies with geographical location and time of year, and can range from 5 to 15% [12–18]. However, higher rates have also been reported [19,20]. hMPV infections are associated with hospitalization of children [15,16,21–23]. hMPV has also been associated with URTIs and may be responsible for 5–15% of cases of URTIs in children [24]. Information on the biology, prevalence and clinical significance of hMPV is scarce.

The pneumovirus, RSV, is the most closely related human pathogen to hMPV. In hospitalized children, hMPV infection has been shown to be as serious as RSV infection and therefore deserves the same attention [25]. Since the circulation of hMPV may overlap with that of RSV, simultaneous infection with both RSV and hMPV may contribute to severe disease.

Virologic diagnosis from respiratory secretions is important because clinical, laboratory, and radiological signs cannot sufficiently discriminate between viral and bacterial respiratory tract infection in infants and children [22]. In this study, we sought to determine the frequency of hMPV infection in children with respiratory infections from Qatar. To the best of our knowledge, this is the first study of its kind conducted in Qatar.

Materials & methods

Patients

A total of 84 pediatric outpatients, aged 1 month to 5 years, who presented at Al-Saad Pediatric Emergency Department between

Keywords
■ bronchiolitis ■ children
■ human metapneumovirus
■ Qatar

February 2007 and March 2008 were recruited for this study. The patients' symptoms consisted of fever, runny nose, coughing, sneezing, sore throat, pneumonia, broncholitis and other relevant signs and symptoms of URTIs and LRTIs.

The majority of the individuals (57%) were non-Qatari while 43% belonged to the Qatari population. Males accounted for 68% of the study population.

Sample collection

Nasopharyngeal aspirate was collected by adding two drops of normal saline to each nostril. A feeding tube (infant size) was connected via a mucus trap to a hand vacuum pump with tubing. The end of the catheter was inserted into each nostril to the posterior pharynx along the floor of the nasopharynx.

The volume of the samples varied from approximately 0.5–1.5 ml. A total of 2 ml of RSV buffer (containing distilled water and phosphate buffer saline with pH 7.4 + 0.2), was added directly to each sample immediately after collection. Within 24 h of collection, the samples were stored in an ice box (4°C) and transported to the research laboratory at Qatar University, where they were aliquoted into smaller tubes of 150–500 µl, which were stored at -86°C to prevent RNA degradation. Samples were centrifuged at 500–1000 Xg for 3 min at 4°C to remove cellular or particulate debris prior to analysis.

Table 1. Distribution of demographic and clinical characteristics of 84 children participating in the study.

Demographic and clinical characteristics of hospitalized patients	n/84 (%) (Total = 84)†
Nationality	
Qatari	36 (43)
Non-Qatari	48 (57)
Gender	
Male	57 (68)
Female	27 (32)
Sample	
Positive	56 (66.7)
Negative	28 (33.3)
Clinical features	
Broncholitis	53 (63.1)
Pneumonia	11 (13.1)
URTI	4 (4.8)
Bronchial asthma and URTI	10 (11.9)
Broncholitis and pneumonia	1 (1.2)
Broncholitis, bronchial asthma and URTI	1 (1.2)
URTI and broncholitis	4 (4.8)
Virus frequency‡	
hMPV‡	54 (64.3)
hPIV (1,2 and 3)‡	12 (14)
RSV‡	4 (4.8)
IV (A and B)‡	2 (2.4)

†Age range: 1 month to 5 years
‡In Positive cases only (N = 56) (taking into account overlaps due to dual and multiple infections). hPIV: Human parainfluenza virus; IV: Influenza virus; RSV: Respiratory syncytial virus; URTI: Upper respiratory tract infection.

Methods

Laboratory testing

A multiplex quantitative reverse transcription (RT)-PCR enzyme hybridization assay (Hexaplex; Prodesse, Inc., Milwaukee, WI, USA), which combines primers originating from highly conserved regions of seven respiratory viruses (RSV subtypes A and B; parainfluenza virus types A and B, RSV and hMPV) with probes for the detection of PCR products using enzyme hybridization assay was applied on the 84 specimens. The assay is based on nucleic acid extraction RT to generate cDNA from target viral and internal control RNA, PCR amplification of cDNA, enzyme hybridization and colorimetric detection of DNA amplicon. The assay provided rapid simultaneous detection, identification, and quantitation of these viruses in nasal wash specimens in a single test. The primer and the probes utilized were evaluated using multiple virus isolates from each group and resulted in specific PCR products from all tissue culture-positive specimens without cross-reactivity among these seven viruses or with other common human respiratory viruses.

RNA extraction

Viral RNA was extracted from nasopharyngeal aspirate specimens with Roche High Pure™ Viral Nucleic Acid Kit (Roche) as per the manufacturer's instructions.

hMPV RT-PCR

Complementary cDNA was synthesized through reverse transcription of purified RNA using an RT Master Mix containing murine leukemia virus RT (Applied Biosystems). The PCR assay was carried out according to the instructions of the manufacturer. Each RNA sample was run with a housekeeping gene to verify RNA integrity.

Statistical methods

Frequency distribution was calculated for all the clinical characteristics in the study. To see association between clinical characteristics

and hMPV, chi square tests were performed. A p value of 0.05 (two tailed) was considered as statistically significant. SPSS 14.0 statistical package was used for the analysis.

Results

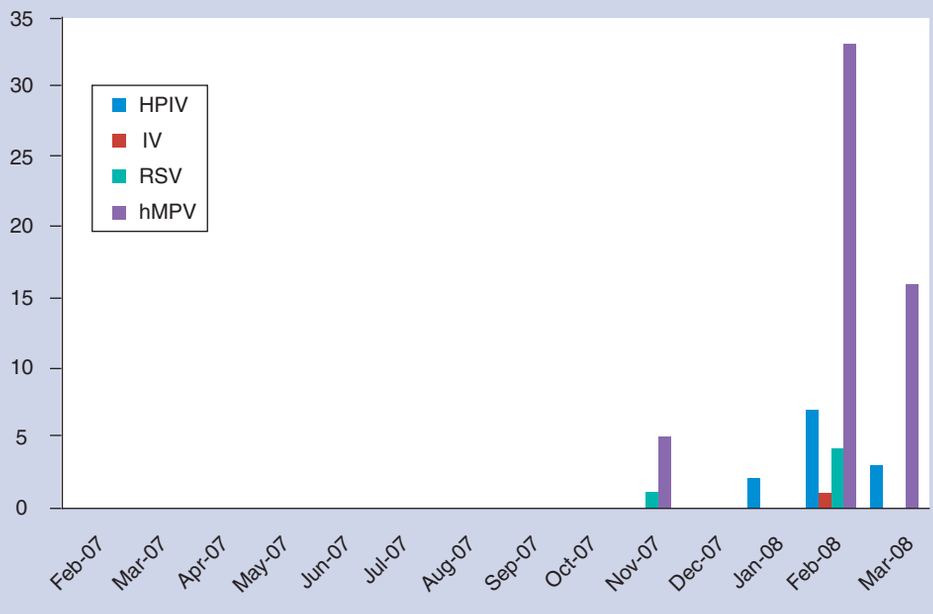
The most common diagnosis in patients was broncholitis (n = 53/84; 63.1%), followed by pneumonia (n = 11/84; 13.1%) and then bronchial asthma-associated URTI (n = 10/84; 11.9%). Most of the children presented with a cough, nasal discharge, shortness of breath and fever. Respiratory viruses were detected in 56 (66.7%) of the 84 children included in the study (Table 1).

Peak incidence of hMPV was in late winter – mainly February followed by March – while other viruses were higher only in February (Figure 1). No infections were presented at the hospital in the remaining months of the year. No samples were collected in December. Most cases were associated with recurrent bronchiolitis. hMPV was present at a higher frequency among children below 6 months of age and the infection rates decreased with increasing age.

In 42 out of 56 (75%) patients positive for respiratory viruses, the infection was caused by a single virus. Dual and multiple infections were detected in 14 out of 56 of these patients (11 with dual infection and three with multiple infections). hMPV was detected in all dual and multiple infections and accounted for 54 out of 56 cases of infected patients (40 single, 11 dual and three multiple infections with hMPV). There were no significant differences in clinical symptoms between hMPV-positive and hMPV-negative patients (Table 2). RSV was found in five out of 56 respiratory virus-positive patients (9%). Human parainfluenza viruses 1–3 were detected in 12 out of 56 positive samples (21.4%), and influenza virus was detected in one sample. There were no significant clinical differences between co-infections and single infections.

Discussion

This study aimed to analyze the frequency of hMPV infections in a nonpreselected group of young children with symptoms of respiratory tract diseases in Qatar. This is the first pediatric study investigating the role of hMPV in causing respiratory tract infection in Qatar.



	Feb-07	Mar-07	Apr-07	May-07	Jun-07	Jul-07	Aug-07	Sep-07	Oct-07	Nov-07	Dec-07	Jan-08	Feb-08	Mar-08
HPIV	0	0	0	0	0	0	0	0	0	0	n/a	2	7	3
IV	0	0	0	0	0	0	0	0	0	0	n/a	0	1	0
RSV	0	0	0	0	0	0	0	0	0	1	n/a	0	4	0
hMPV	0	0	0	0	0	0	0	0	0	5	n/a	0	33	16

Figure 1. Positive detection of human metapneumovirus in different age groups.
hMPV: Human metapneumovirus; hPIV: Human parainfluenza virus; IV: Influenza virus; RSV: Respiratory syncytial virus.

Table 2. Clinical characteristics of the study population, associated with human parainfluenza virus infection.

Characteristics	hMPV negative n/84 (%)	hMPV positive n/84 (%)	Significance
Nationality			
Qatari	14 (16.7)	22 (26.2)	0.599
Non-Qatari	16 (19)	32 (38)	
Age			
≤6 month	14 (16.7)	30 (35.7)	0.81
6 months–1 year	6 (7.1)	8 (9.5)	
1–2 years	4 (4.8)	6 (7.1)	
2–3 years	2 (2.4)	7 (8.3)	
3–4 years	2 (2.4)	2 (2.4)	
4 years and above	0 (0)	1 (1.2)	
Sex			
Male	19 (22.6)	38 (45.2)	0.508
Female	11 (13.1)	16 (19)	
Presenting symptoms/signs			
Coughing	30 (35.7)	54 (64.3)	–
Wheezing	22 (26.2)	38 (45.2)	0.773
Shortness of breath	26 (31)	48 (57.1)	0.763
Cyanosis	2 (2.4)	6 (7.1)	0.506
Apnea	0 (0)	1 (1.2)	0.453
Nasal discharge	30 (39)	47 (61)	0.039
Eye discharge	16 (19)	21 (25)	0.201
Fever	25 (29.8)	46 (54.8)	0.822
Feeding difficulties	15 (17.9)	34 (40.5)	0.248
Choking during feeding	0 (0)	1 (1.2)	0.453

hMPV: Human metapneumovirus.

The most common diagnoses identified within our cohort were broncholitis, followed by pneumonia and then bronchial asthma associated with URTI. Coughing and nasal discharge were the most common signs and symptoms in this study population. Bronchiolitis was the most common diagnosis associated with hMPV infections in this study, suggesting an important role for this virus in clinical worsening of RTIs. All hMPV positive cases were associated with coughing.

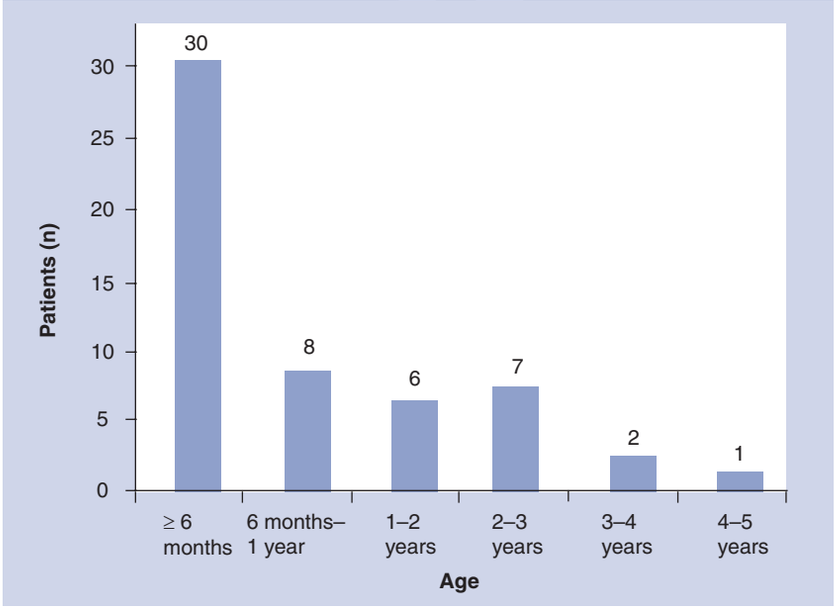


Figure 2. Human metapneumovirus positive detection among children of different age groups.

Respiratory viruses were detected in 66.7% out of the 84 children included in this study. The hMPV virus was identified in 54 of 83 patients recruited (64.3%). hMPV virus was the most commonly detected virus in the patients, accounting for 74% of all respiratory infections followed by 18% of cases with human parainfluenza virus. This is in contrast to another Spanish paediatric study where RSV was the most common causative agent for respiratory diseases (76.1%) followed by hMPV infection (14%) [26]. The frequency of hMPV (74%) infection in the current study was comparable to that of RSV infection in the Spanish study (76.1%).

No correlation was demonstrated between Qatari and non-Qatari nationals for susceptibility to a specific respiratory virus. However, males were more likely than females to be infected with hMPV (68%), though not significantly so. We were not able to obtain more detailed data for non-Qatari individuals (e.g., ethnic origin) and this will be a subject of further investigation in future studies.

Human metapneumovirus infections were mainly detected in late winter, between February and March, while other viruses were detected at a higher rate in February. These findings are consistent with other studies, which have demonstrated a higher prevalence of hMPV infections in spring and late winter [12,16,21,27,28]. Interestingly, in the French [8], Dutch [11] and Norwegian studies [19], hMPV was found at a higher rate in December and January. Owing to an inability to collect samples during December in the current study, the incidence of infection compared with the other respiratory viruses detected elsewhere, could not be completely assessed. However, we believe that there were generally more cases in February and March than November and December, though this would have to be studied further in a larger and longer-term study in the future.

The rate of dual infection in our study population was similar to that observed in the Spanish study (26%) [29]. While data from other reports suggest that dual infection with hMPV and other respiratory viruses is rare (68–71) [6,29,30], both this study and the Spanish study [29] have demonstrated relatively high co-infection rates. Taking

into account our observations on the occurrence of dual infections caused by both hMPV and other viruses, one might presume that a preselection of patients negative for other respiratory viruses could lead to an underestimation of the true incidence of hMPV infection. The molecular technique used in this study could detect multiple viruses simultaneously, therefore eliminating the need to detect only negative samples.

Little information on the clinical presentation and the impact of hMPV infection exists. In the current study, no significant differences in diagnosis or severity of infection were detected between co-infections and single infections. This suggests that the presence of hMPV in the setting of another viral respiratory infection does not affect severity of the condition.

The rate of hMPV infection decreased with increasing age of patients. This suggests a possible concordance with a previous study speculating that by the age of 5 months almost all of the children had encountered hMPV [31]. The results from our study suggest that for young children, hMPV could be a pathogen as important as RSV.

Conclusion

This is the first report on the incidence of hMPV-related infections in children in Qatar, though studies have demonstrated the presence of hMPV in the other Middle Eastern countries [32–34]. Our data demonstrate a high incidence of the virus in children 6 months to 5 years of age with respiratory tract infections. A major limitation of this study is the lack of data in the month of December and further studies will need to be performed in order to obtain data for this month. Future studies are planned in Qatar with larger patient samples, covering all 12 months of the year, with possible longer-term extensions.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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