

# Human metapneumovirus in Pediatric Patients with Acute Respiratory Tract Infections in the Aseer Region of Saudi Arabia

Ali Mohammed Alsuheel, Abdelwahid Saeed Ali<sup>1</sup>, Ahmed Musa Al-Hakami<sup>1</sup>, Ayed Abdullah Shati, Harish C. Chandramoorthy<sup>1</sup>, Saleh Mohammed Al-Qahtani

Department of Child Health, College of Medicine, King Khalid University, <sup>1</sup>Department of Microbiology and Clinical Parasitology, College of Medicine, King Khalid University, Abha, Saudi Arabia

## Abstract

**Background:** Human metapneumovirus (hMPV) is a *Paramyxovirus* known to cause acute respiratory tract infections in children and young adults. To date, there is no study from the Aseer region of Saudi Arabia determining the proportion and severity of hMPV infection among pediatric hospitalized patients with respiratory infections.

**Objectives:** The objective of this study is to determine the presence of hMPV antigens in the nasopharyngeal secretions of pediatric patients hospitalized with respiratory tract infections in the Aseer region of Saudi Arabia.

**Materials and Methods:** This prospective, serological hospital-based study included all pediatric patients who were admitted to Aseer Central Hospital, Abha, Saudi Arabia, from July 2016 to November 2017 with upper and/or lower respiratory tract infections. Basic demographics of patients and their clinical data on and after admission were recorded. Direct fluorescent antibody assay was used to detect the presence of hMPV antigens in the obtained nasopharyngeal secretion specimens.

**Results:** During the study, 91 pediatric patients were hospitalized due to upper and/or lower respiratory tract infections, of which 9.9% were positive for hMPV. These patients were aged 9 months to 16 years, were from Abha city or its surrounding localities and were mostly (77.8%) hospitalized during autumn or winter. The most common diagnosis on admission was bronchopneumonia (55.5%) and aspiration pneumonia (22.2%), and some patients also had underlying chronic conditions such as chronic heart disease (22.2%) and bronchial asthma (11.1%).

**Conclusions:** The results obtained indicated that hMPV is a potential etiologic factor for the commonly occurring acute respiratory infections in hospitalized children from the Aseer region of Saudi Arabia. hMPV infection was also found to be associated with complicated respiratory conditions such as bronchopneumonia, chronic heart disease and bronchial asthma.

**Keywords:** Bronchiolitis, *Human metapneumovirus*, pediatric, pneumonia, respiratory infection, Saudi Arabia

**Address for correspondence:** Prof. Abdelwahid Saeed Ali, Department of Microbiology and Clinical Parasitology, College of Medicine, King Khalid University, Abha, Saudi Arabia.  
E-mail: abumalaz2002@gmail.com

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## INTRODUCTION

Human metapneumovirus (hMPV) is a single-stranded RNA-enveloped virus, recently classified in the order *Mononegavirales*, family *Pneumoviridae*, genus *Metapneumovirus* and species hMPV. It was first isolated in the Netherlands by van den Hoogen *et al.*,<sup>[1,2]</sup> and is now a known causative agent of upper and lower respiratory tract infections in children and adults. hMPV infections have been reported in Australia,<sup>[3]</sup> Canada,<sup>[4]</sup> the United States,<sup>[5]</sup> the United Kingdom,<sup>[6]</sup> Hong Kong,<sup>[7]</sup> South Africa,<sup>[8]</sup> Mexico,<sup>[9]</sup> Spain<sup>[10]</sup> and Peru.<sup>[11]</sup> However, in the Middle East, there have only been few reports of hMPV infections, mainly as sporadic infections in Egypt,<sup>[12]</sup> Jordan,<sup>[13]</sup> Kuwait<sup>[14]</sup> and Saudi Arabia.<sup>[15]</sup>

Along with respiratory syncytial virus (RSV) infections, hMPV is now recognized as a primary etiologic agent for acute upper and lower respiratory tract infections in pediatrics.<sup>[16,17]</sup> In a study from Mexico, it was found that the number of hMPV infections increased in children aged 24–36 months as compared with those in younger age groups, whereas RSV infections were inversely proportional to increase in age.<sup>[9]</sup> Co-infection with both viruses can also occur, resulting in a more complicated and serious clinical disease.<sup>[3,18]</sup> In addition to the pediatric population, studies have also found hMPV to infect adults<sup>[19]</sup> and elderly people.<sup>[20]</sup> In terms of transmission, hMPV spreads through contact with contaminated secretions, i.e., droplet, aerosol or fomites. Hospital-acquired hMPV infections have also been reported.<sup>[5]</sup>

In young children, infections are usually not asymptomatic, and bronchiolitis, with or without pneumonia, is the most common associated presentation.<sup>[18,20,21]</sup> Other reported conditions include bronchial asthma exacerbation, otitis media, pneumonitis, flu-like illness and community-acquired pneumonia.<sup>[9]</sup> In adults, hMPV has been associated with bronchitis, pneumonia and exacerbations of both bronchial asthma and chronic obstructive pulmonary disease.<sup>[22,23]</sup>

In terms of detection, reverse transcriptase–polymerase chain reaction (RT-PCR) is a sensitive and commonly used method to detect hMPV.<sup>[5]</sup> Real-time RT-PCR is also commonly used for detecting hMPV in clinical specimens with many genomic target sequences.<sup>[24,25]</sup> A touch-down genomic amplification protocol for the diagnosis of acute viral respiratory tract infections has also been used previously.<sup>[26]</sup> The enzyme-linked immunosorbent assay for hMPV diagnosis is a simple and specific serological test for anti-hMPV antibodies detection.<sup>[27]</sup> Immunofluorescence using specific antibodies is routinely used for detecting

hMPV antigens, particularly in epidemiological studies.<sup>[28]</sup> However, cell culturing techniques have a low sensitivity in detecting hMPV from respiratory tract secretions, as the virus exhibits extremely limited types of cell tropism.<sup>[2,10,29,30]</sup>

In Saudi Arabia, there is a paucity of data regarding the occurrence of hMPV and its role in complicated clinical cases of commonly reported respiratory infections. Therefore, the current study aimed to determine the role of hMPV in the respiratory tract infections' severity and complications among hospitalized children in the Aseer region, where no such study has previously been conducted.

## MATERIALS AND METHODS

The study was conducted after obtaining approval from the Ethical Committee of College of Medicine, King Khalid University, Saudi Arabia (KKU Research Ethics Committee Meeting No. REC # 2014-01-08; dated January 5, 2014)

### Study design and samples

This prospective, serological study included pediatric patients who were admitted to Aseer Central Hospital, Abha, Kingdom of Saudi Arabia, from July 2016 to November 2017 with upper and/or lower respiratory tract infections. Aseer Central Hospital is the largest tertiary care referral hospital in the Aseer region, and thus its sample is representative of the area.

Data such as age, gender, clinical presentation and current medications were collected using an objectively prepared questionnaire. Informed consent was obtained from the parents/guardians of all patients before sample collection.

### Nasopharyngeal sampling

Nasopharyngeal secretions were collected from all hospitalized patients included in this study using the standard collection method. Briefly, physicians collected the nasopharyngeal secretions with the help of a sterile feeding tube connected to a vacuum pump. Following the vacuum application, the tip of the tube was cut and placed into a sterile container labelled with the patient's name and identification number. The container was then transported to the Virology Laboratory at the Department of Microbiology and Clinical Parasitology, College of Medicine, King Khalid University, Abha, Saudi Arabia, and either processed on the same day or stored at  $-70^{\circ}\text{C}$ .

### Specimen processing

Specimens were processed according to the manufacturer's instructions for the direct fluorescent antibody (DFA) kit (Oxoid Ltd., Cambridge, UK) with minor modifications. Samples were transferred to an Eppendorf tube containing

1 mL of phosphate-buffered saline (PBS; pH 7.5). The specimens were gently vortexed for 30 s to reduce the viscosity and dilute the mucus. Samples were then centrifuged at 3000 rpm for 5 min to separate the cells from the mucus. The supernatant was removed, and the cells in the pellets were used for DFA staining. The authors chose to use DFA because it has been found to be a useful technique for wider hMPV epidemiological studies.<sup>[31]</sup>

### Preparation of cells

The cell suspension was washed several times with PBS and the final cell deposit was resuspended in 2 mL PBS (pH 7.5). The cells were then gently agitated by pipetting up and down until the cellular material was released from the mucus. Additional PBS was added until a smooth suspension was obtained, and any visible flecks of mucus were removed. After the cell separation process was completed, the obtained cell suspension was centrifuged at room temperature (15°C–30°C) for 10 min at 3500 rpm and the supernatant was discarded. The final cell deposit was resuspended in PBS to dilute any remaining mucus and maintain high cell density.

### Preparation of slides and direct fluorescent antibody staining of cells

A volume of 25 µL of the resuspended cell deposit was placed in slides with 6-mm-diameter wells. The specimens were then allowed to air dry thoroughly and fixed with fresh acetone at room temperature (15°C–30°C) for 10 min. The slide was air-dried after fixation. A volume of 25 µL of IMAGEN™ hMPV reagent (Oxoid Ltd., Cambridge, UK), which contains monoclonal antibodies against hMPV conjugated to fluorescein isothiocyanate, was added to the fixed cell preparation on the slide to cover the wells. The same amount was also added to the positive control slide. The slides were then incubated with the reagent in a moist chamber for 15 min at 37°C. Following incubation, excess reagent was washed off with PBS, and the slide was gently washed in an agitating bath containing PBS for 5 min. The PBS was drained off, and slide was allowed to air dry at room temperature (15°C–30°C). One drop of IMAGEN™ hMPV mounting fluid was added to the center of each well, and cover-slip was placed over the mounting fluid and specimen to ensure that there are no trapped air bubbles. The stained slides were immediately examined under epifluorescence microscope at ×400 and then ×1000. Apple-green fluorescence was observed in the cells infected with hMPV, whereas non-infected cells appeared as red color because they were stained with the Evans blue counterstain. Images of these cells were captured using a microscopic camera (Nikon-DS-Fi1, Nikon Corp., Tokyo, Japan) and archived.

## RESULTS

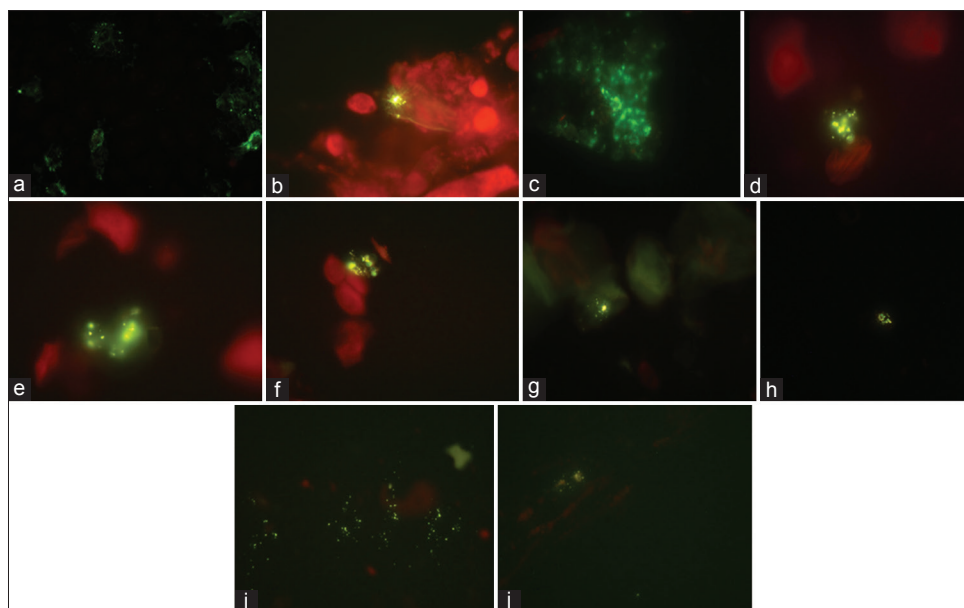
During the study, a total of 91 pediatric patients were hospitalized based on upper and/or lower respiratory tract infections. Of these 9 (9.9%) patients tested positive for hMPV antigens, as demonstrated by DFA from the nasopharyngeal secretions [Figure 1]. Table 1 provides the demographic and clinical presentation data of all hMPV-positive patients. The age of these patients ranged from 9 months to 16 years and all were Saudi nationals except one infant, who was a Jordanian by nationality but was born and raised in Saudi Arabia.

From the patient's demographics, it was observed that hMPV antigens were detected not only in patients from Abha but also among those from its bordering areas, namely, Alghama, Bilahmar, Ahud Rufida, Sarat Abeedah, Khamis Mushait and Bilasmer. Three of the nine positive cases were found from Abha (33.3%), and one positive case from each of the previously mentioned six cities was reported (11.1%). Of the nine hMPV-positive patients, seven (77.8%) were hospitalized during the autumn and winter of 2016–2017. In the hMPV-positive patients, the symptoms included fever (77.8%), cough (77.8%), shortness of breath (66.7%), nasal congestion (11.1%), cyanosis (11.1%) and stridor (11.1%). These patients also had underlying chronic illnesses such as chronic heart disease (22.2%) and bronchial asthma (11.1%), and most had tachypnea (88.8%). On physical examinations, bilateral crepitation and wheezing were found to be the major findings along with bronchopneumonia (55.5%) and aspiration pneumonia (22.2%).

## DISCUSSION

In Saudi Arabia, although few studies have reported the incidence, epidemiological elements and genetic diversity of hMPV in some regions,<sup>[15,32,33]</sup> there are no reports on the association between hMPV infections and the clinical presentations of respiratory tract infections among pediatrics. The current study found that in the Aseer region of Saudi Arabia, about 10% of hospitalization among pediatrics with respiratory tract infections between July 2016 and November 2017 was due to hMPV infections. In addition, this study also found that hMPV infections were associated with presentation of acute respiratory symptoms, thereby highlighting the role of the infection in complicating the course of the disease.

The results of the present study concur with several other studies demonstrating an association between hMPV infection and acute respiratory conditions such as bronchopneumonia and pneumonia.<sup>[3,5]</sup> A previous study



**Figure 1:** (a) Positive control; (b-j) Positive cases for *Human metapneumovirus* as detected by direct fluorescent antibody test in nasopharyngeal secretions of hospitalized children

**Table 1: Clinical and laboratory parameters of hMPV-positive patients**

Specimen ID	Age (years + months)	Gender	Underlying illness	Temperature (°C)	Respiratory rate (BPM)	Pulse rate (beats/min)	BP (mmHg)	O <sub>2</sub> saturation (room air) (%)	CBC	Duration of stay (days)	Diagnosis
3	1 + 4	Female	None	39	76/min (T)	150	60/38	80	WBC: 4.21 Hb: 8.6 Plt: 465	30 (14 in PICU)	BPN
9	1 + 0	Female	Nonketotic hyperglycemia	38	50/min (T)	109	81/40	86	WBC: 18 Hb: 13.2 Plt: 367	7	BPN
16	11 + 0	Male	Subglottic stenosis	37	32/min (T)	93	105/70	93	WBC: 7.4 Hb 12.5 Plt 160	2	Subglottic stenosis
24	3 + 0	Male	Down syndrome, CHD, asthma	37	56/min (T)	140	103/59	75	WBC: 15.5 Hb: 10.9 Plt: 377	12 (4 in PICU)	BPN
29	15 + 0	Male	Severe scoliosis, Cerebral palsy	38	28/min (T)	81	128/80	82	WBC: 5.6 Hb: 14 Plt: 279	6	Aspiration pneumonia
35	16 + 0	Female	CHD, Cerebral palsy	36.5	22/min	74	137/74	95	ND	4	Pharyngitis, tonsillitis
57	14 + 0	Male	None	36.8	30/min (T)	83	130/80	90	WBC: 7.6 Hb: 9 Plt: 346	9	BPN
65	0 + 9	Female	Lissencephaly epilepsy	37	55/min (T)	120	103/54	75	WBC: 6.1 Hb: 9.7 Plt : 406	14	BPN
87	5 + 0	Male	None	38	52/min (T)	130	103/70	70	WBC: 11 Hb: 9.6 Plt: 379	12	Aspiration pneumonia

WBC – White blood cells per µl; HB – Hemoglobin g/dL; Plt – Platelets per µl; BP – Blood pressure; CBC – Complete blood count; PICU – Pediatric intensive care unit; BPN – Bronchopneumonia; CHD – Chronic heart disease; T – Tachypneic patient; ND - Not done

implicated hMPV as a causative agent for severe and acute respiratory infections among pediatric patients,<sup>[34]</sup> which is similar to the findings of the current study. Another study found that children with hMPV infection are likely to have immunodeficiency; however, the current study was not able

to substantiate these findings as none of the patients were found to be immunocompromised.<sup>[13]</sup>

The prevalence of hMPV infection of the current study (about 10%) was similar to that reported in studies from



Saudi Arabia and Kuwait.<sup>[14,15]</sup> However, other similar studies that used direct immunofluorescence assays for the detection of hMPV antibodies in sera of patients revealed much higher prevalence rates.<sup>[35]</sup> This suggests that different results can be obtained with different diagnostic techniques for hMPV infection. In the current study, we used DFA with monoclonal antibody, which has previously been shown to have 100% specificity, thereby indicating the reliability of our results.<sup>[31]</sup> The current study did not find any gender predilection in terms of the infections; these findings are in accordance with that of Bastien *et al.*<sup>[4]</sup> and Kahn.<sup>[29]</sup>

In this study, all nine hMPV-positive children were from Abha or its surrounding areas. These areas are known for their high altitude, low temperature and low oxygen tension. It was well known that the prevalence of viral respiratory infections is high in cold and dry areas.<sup>[36]</sup> In addition, almost 78% of the hMPV-positive cases were in children who had been admitted during the autumn and winter seasons. It is also known that the majority of the viruses responsible for bronchiolitis and bronchopneumonia have their peak infectivity during winter and late autumn, with only sporadic cases through other seasons.<sup>[37,38]</sup>

The hMPV-positive patients in the current study had fever, cough, nasal congestion, cyanosis, stridor and shortness of breath, while some also had underlying chronic illnesses such as chronic heart disease and bronchial asthma; similar hMPV-associated illnesses were observed in another study.<sup>[39]</sup> Physical examination revealed wheezing and bilateral crepitation and clinically, the most common presentations were bronchopneumonia and aspiration pneumonia. These findings are in line with those of Williams *et al.*,<sup>[40]</sup> who found significant association between hMPV and wheezing exacerbations and/or bronchiolitis in infants and young children.

## CONCLUSIONS

In the Aseer region of Saudi Arabia, hMPV was found to be responsible for about one-tenth of hospitalizations in children with acute respiratory tract infections. This study also confirmed that hMPV infection is associated with presentation of acute respiratory symptoms.

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## Conflicts of interest

There are no conflicts of interest.

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