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Human metapneumovirus in paediatric patients

N. Principi, S. Bosis and S. Esposito

Institute of Pediatrics, University of Milan, Fondazione IRCCS 'Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena', Milan, Italy

ABSTRACT

Acute respiratory tract infections (ARTIs) are a leading cause of morbidity and mortality in children worldwide, but the aetiology of many ARTIs is still unknown. In 2001, researchers in The Netherlands reported the discovery of a previously unidentified pathogen called human metapneumovirus (hMPV). Since its initial description, hMPV has been associated with ARTI in Europe (Italy, France, Spain, the UK, Germany, Denmark, Finland and Norway), America (the USA, Canada, Argentina and Brazil), Asia (India, Japan, China and Singapore), Australia and South Africa in individuals of all ages. The incidence of infection varies from 1.5% to 25%, indicating that hMPV is a ubiquitous virus with a worldwide distribution. hMPV seems to play an important role as a cause of paediatric upper and lower respiratory tract infection, with similar, but not identical, epidemiological and clinical features to those of respiratory syncytial virus and influenza virus. Moreover, the socio-economic impact of hMPV-infected children on their families seems to be considerable, which suggests that, like influenza virus, hMPV infection may be a substantial public health problem for the community. It may be associated with significant morbidity and mortality in pre-term infants and children with underlying clinical conditions, although more adequately controlled studies are needed to confirm its importance in such patients. Many fundamental questions concerning the pathogenesis of hMPV disease and the host's specific immune response remain to be answered. Further studies are also required to properly define hMPV diagnosis, treatment and prevention strategies.

Keywords Emerging infections, epidemiology, human metapneumovirus, paediatric infections, respiratory tract infection, review

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INTRODUCTION

Acute respiratory tract infections (ARTIs) are a leading cause of morbidity and mortality in children worldwide [1,2]. A variety of viruses, including influenza viruses, respiratory syncytial virus (RSV), picornaviruses, coronaviruses, parainfluenza viruses and adenovirus, have been associated with different respiratory syndromes in all age groups [3,4]. However, the aetiology of a large number of ARTIs is still unknown. Although diagnostic methods may be inadequate, this suggests that other respiratory pathogens may remain to be identified.

During recent years, emerging virus infections that are probably associated with anthropogenic, social and environmental changes have been reported in humans and animals [3,5]. In 2001, researchers in The Netherlands reported the isolation of a previously unidentified RNA virus from children and adults with ARTI [6]. On the basis of morphological, biochemical and genetic analyses, the new virus seemed to be related closely to avian pneumovirus C, which at that time was the sole member of the *Metapneumovirus* genus, and which was the aetiological agent of an upper respiratory tract disease in turkeys and other birds [7–10]. The new virus was therefore classified as the first member of the *Metapneumovirus* genus of the Pneumovirinae sub-family of the Paramyxoviridae family that was capable of

Corresponding author and reprint requests: N. Principi, Institute of Pediatrics, University of Milan, Fondazione IRCCS 'Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena', Via Commenda 9, 20122 Milano, Italy
E-mail: Nicola.Principi@unimi.it

infecting humans, and was designated human metapneumovirus (hMPV) [7–10]. hMPV differs from the pneumoviruses (such as RSV), which also belong to the Pneumovirinae sub-family, in that it lacks two non-structural proteins and has a slightly different gene order [7–10]. Genotyping studies have determined that hMPV strains can be classified into two main lineages and four sub-lineages, named A1, A2, B1 and B2 [11]. Nucleotide sequence variation between hMPV types A and B is 11.8–47.7%, whereas that between the genotypes within each lineage is less [11].

EPIDEMIOLOGY

Since its initial description in 2001, hMPV has been isolated from individuals of all ages with ARTI in Europe (Italy, France, Spain, the UK, Germany, Denmark, Finland and Norway), America (the USA, Canada, Argentina and Brazil), Asia (India, Japan, China and Singapore), Australia and South Africa [7,12–20]. The incidence of infection in different studies varies from 1.5% to 25%, thus indicating that hMPV is a ubiquitous virus with a worldwide distribution [12–20]. It is interesting to note that detection rates of hMPV have generally been higher in retrospective studies than in prospective studies, an observation consistent with a degree of selection bias. This indicates that large prospective studies are needed in order to clarify the role of hMPV in various clinical conditions.

The few seroprevalence surveys, from The Netherlands, Japan and Israel, have demonstrated that virtually all children are infected by the age of 5–10 years, which indicates that hMPV infection is acquired early in life [6,10]. However, as the range of antibody titres measured by immunofluorescence assays was higher in individuals aged >2 years than in children aged 6–24 months, it is possible that there is a booster effect as a consequence of re-infection with the same or a closely related virus. In addition, studies of samples collected previously have shown that hMPV is not a new pathogen, with serological evidence of human infection dating from 1958 in The Netherlands, and virus isolation during the last 10–20 years in Europe and Canada [7–10]. These findings suggest that hMPV has been in circulation for a long period, but has been recognised only recently because of the development of new diagnostic methods.

Surveys have indicated that hMPV has a seasonal distribution similar to that of RSV and influenza viruses, although the greatest number of hMPV infections are usually diagnosed at the end of winter or in early spring [12–22]. It has also been demonstrated that different hMPV genotypes may co-circulate in the population during a single year [20,23–25]. However, further studies over multiple years are needed to clarify the seasonal nature of hMPV infection and the quantitative importance of the different genotypes.

The similar seasonal distribution of several other respiratory virus infections may result in coinfection with hMPV and other respiratory viruses, but the role that hMPV plays as a co-pathogen is still not understood completely. Thus, it is not known whether dual infection by hMPV and RSV is associated with a more severe disease than that observed when a single virus is the aetiological agent. Greensill *et al.* [26] detected hMPV in 70% of infants with RSV bronchiolitis who required admission to a paediatric intensive care unit for ventilatory support, thus suggesting that hMPV may influence the severity of RSV disease. Similarly, Semple *et al.* [27] observed that dual infection with hMPV and RSV confers a ten-fold increase in the relative risk of admission to a paediatric intensive care unit for mechanical ventilation. Other recent data support this hypothesis, at least for children aged <3 years [28]. In contrast with these findings, but in agreement with other reports [14,29,30], there was no evidence of increased disease severity in a small group of children coinfected with hMPV and RSV or influenza viruses [17]. hMPV has also been identified in patients with SARS in Canada and Hong Kong [31,32], but probably did not increase the pathogenicity of the novel coronavirus identified as the cause of SARS. However, the possibility of coinfection may lead to an underestimation of the percentage of hMPV-positive samples identified in studies in which only samples negative for other respiratory viruses are tested. This means that all clinical samples (not just samples found to be negative for other viruses) must be tested in order to clarify further the epidemiology of hMPV infections.

The quantitative importance of hMPV in children with underlying disease also requires clarification. Van den Hoogen *et al.* [33] found that most hMPV-positive patients aged 5–65 years had an underlying disease such as cystic fibrosis, or

had received immunotherapy, and other studies found that 25–50% of hMPV-positive patients had an underlying disease [7,10,34]. In contrast, it has also been reported that hMPV is a frequent cause of ARTIs in otherwise healthy children, but has a marginal quantitative impact on patients with chronic underlying conditions [17]. Large-scale studies over a long period should reveal the true incidence of hMPV infections among patients with underlying diseases. Furthermore, as a case of life-threatening hMPV pneumonia requiring extracorporeal membrane oxygenation has been described in a pre-term infant [35], the importance of considering this newly discovered pathogen as a possible cause of pneumonia in neonates should be emphasised.

PATHOLOGY

In experimental animals, hMPV infection is associated with airway epithelial cell changes and increased inflammatory cell infiltrates, predominantly mononuclear cells, in the lung interstitium [36–39]. In addition, hMPV infection causes increased myofibroblast thickening adjacent to the airway epithelium and staining of cellular debris [36]. Moreover, like RSV, hMPV can persist for several weeks in the lungs, despite an established immune response, suggesting that this virus uses specific strategies to overcome host defences [36].

Data regarding the pathology of hMPV in humans are scarce, and have mostly been collected for individuals with underlying lung disease [10]. Specimens obtained by bronchoalveolar lavage within 4 days of the identification of hMPV in the nasal secretions of children with an acute episode of respiratory infection have demonstrated that hMPV affects primarily airway epithelium [40]. Infection of the airway epithelial cells results in cell degeneration and/or necrosis, with ciliacytophthoria and round, red cytoplasmic inclusions on a background of haemosiderin-laden macrophages, abundant neutrophils and prominent mucus [40]. These findings, especially the red cytoplasmic inclusions, are similar to those seen in infections by RSV, parainfluenza viruses and measles virus. Lung biopsies, performed at least 1 month after a positive hMPV nasal assay, have shown that later stages of the disease caused by hMPV include expansion of peribronchiolar lymphoid tissue, squamous metaplasia, haemosiderin and accumulation of intra-alveolar foamy macrophages [40]. These features indicate chronic/healing airway inflammation, with a degree of concomitant airway obstruction and impairment of the mucociliary escalator, and correlate well with the bronchiolitis and wheezing noted clinically in patients with hMPV infection.

CLINICAL MANIFESTATIONS

hMPV seems to be an important respiratory pathogen that causes both upper and lower respiratory tract infections in children [7–18], who, unlike adults, are rarely asymptomatic, although most reports are biased toward descriptions of the most severe symptoms in hospitalised subjects. The fact that most severe cases are found in paediatric patients suggests that naturally acquired infection induces partial protection against the disease. However, it should be emphasised that there is no cross-protection among the different virus strains. A recent report has described a child who suffered from two episodes of hMPV infection during a 1-month period, each caused by a different strain [41]. Moreover, the degree of severity seems to be related not only to age, but also to the strain causing the infection, with a possible link between the A2 strain, which is the most frequent, and severe disease [10].

Diagnosis of hMPV infection may be made on the basis of signs and symptoms, ranging from rhinopharyngitis to bronchitis and pneumonia, and some patients may be admitted to intensive care units [7–10,21,42]. Table 1 summarises the

Table 1. Clinical characteristics and outcomes among children seen for acute respiratory infection in an emergency department, grouped by virus RNA detection

Characteristics	hMPV-positive (n = 41)	RSV-positive (n = 117)	Influenza-positive (n = 209)
Clinical presentation			
Common cold, no. (%)	3 (7.3)	20 (17.1)	43 (20.6)
Pharyngitis, no. (%)	11 (26.8)	20 (17.1)	73 (34.9)
Acute otitis media, no. (%)	5 (12.2)	10 (8.5)	34 (16.3)
Croup, no. (%)	3 (7.3)	4 (3.4)	7 (3.3)
Acute bronchitis, no. (%)	4 (9.8)	15 (12.8)	20 (9.6)
Wheezing, no. (%)	10 (24.4)[a]	30 (25.7)[a]	14 (6.7)
Pneumonia, no. (%)	5 (12.2)	18 (15.4)	18 (8.6)
Clinical outcome			
Hospitalisation, no. (%)	2 (4.8)	16 (13.7)	11 (5.3)
School absence, median days (range)	10 (3–15)	10 (3–12)	12 (5–15)

[a]$p < 0.0001$ vs. influenza-positive children; no other statistically significant differences.
hMPV, human metapneumovirus; RSV, respiratory syncytial virus.
Adapted from Principi *et al.* [16].

clinical data for a group of children seen in an emergency department for acute respiratory infection, grouped according to virus diagnosis. In this population, hMPV caused signs and symptoms that sometimes resembled those of RSV infection (bronchiolitis, asthma exacerbation and pneumonia) and sometimes those of influenza (fever and upper respiratory tract infection) [16]. Boivin *et al.* [34] also reported that the clinical presentation of hMPV is similar to that of other common winter virus infections. hMPV has also been associated with febrile seizures, rash, diarrhoea, an enlarged liver, and altered liver function test results (Table 2) [13].

Several studies have indicated that, like RSV, hMPV may induce airway alterations and may be related to the onset and exacerbation of childhood asthma. Two successive studies by Jartti *et al.* [15,43] detected hMPV in 10/132 (8%) and 12/293 (4%) children hospitalised for acute expiratory wheezing, which suggests that hMPV may trigger this disease [15,43]. Our own study found that wheezing was diagnosed in 25.7% of children with hMPV, and in 23.4% of those with RSV infection [17]. However, the fact that hMPV-positive children are older than children who are RSV-positive may explain why, when wheezing is diagnosed, asthma exacerbation is more common in the former and bronchiolitis in the latter, and why hMPV-positive children have less severe disease, as demonstrated by the lower frequency of hospitalisation. Nevertheless, preliminary results concerning the association between asthma and hMPV infections warrant further research, not least because asthma is a difficult clinical diagnosis to make in children aged <2 years.

Table 2. Characteristics of 32 children admitted with human metapneumovirus (hMPV) infection compared to age-matched controls with respiratory syncytial virus (RSV) or influenza A infection

Characteristics	hMPV No. positive/total (%)	RSV No. positive/total (%)	Influenza A No. positive/total (%)
Influenza-like illness in family contact	10/19 (52.6)	7/29 (24.1)	19/24 (79.1)
Febrile seizure	5/32 (15.6)	1/32 (3.1)	3/32 (9.4)
Congested pharynx	12/32 (37.5)	11/32 (34.4)	11/32 (34.4)
Rash	4/32 (12.5)	1/32 (3.1)	4/32 (12.5)
Enlarged liver	2/32 (6.3)	0/32 (0.0)	4/32 (12.5)
Otitis media	4/32 (12.5)	0/32 (0.0)	0/32 (0.0)
Diarrhoea	2/32 (6.3)	1/32 (3.1)	3/32 (9.4)
Crepitations	18/32 (56.3)[a]	14/32 (43.8)[a]	3/32 (9.4)
Wheezing	9/32 (28.1)[a]	12/32 (37.5)[a]	2/32 (6.3)
Asthma exacerbation	6/32 (18.8)	2/32 (6.3)	2/32 (6.3)
Acute bronchiolitis	3/32 (9.4)	10/32 (31.3)	0/32 (0.0)
Pneumonia	12/32 (37.5)[a]	5/32 (15.6)[a]	1/32 (3.1)
Abnormal chest X-ray	17/25 (68.0)[a]	11/18 (61.1)[a]	1/17 (5.9)
Lymphopenia ($\leq 1.5 \times 10^9$/L)	9/31 (29)	2/27 (7.4)	12/29 (41.4)
Neutropenia (ANC $< 1 \times 10^9$/L)	2/31 (6.5)	0/27 (0.0)	4/29 (13.8)
Elevated transaminase	2/15 (13.3)	0/5 (0.0)	3/11 (27.3)

[a]$p < 0.05$ vs. influenza A.
ANC, absolute neutrophils count.
Adapted from Peiris *et al.* [13].

The pathogenesis of hMPV disease also requires further clarification, since the results of different studies are contradictory. In children who were positive for hMPV, Jartti *et al.* [43] found two chemokines that have been linked to RSV disease, namely interleukin-8, a chemotactic factor mainly for neutrophils, and RANTES (regulated upon activation, normal T-cell expressed and secreted), a chemotactic factor for eosinophils. In comparison to children with RSV infection, the hMPV-positive children had lower concentrations of RANTES and higher concentrations of interleukin-8 in their respiratory secretions [43]. In contrast, Laham *et al.* [44] found that although both hMPV and RSV were associated with rhinorrhoea, cough and wheezing, hMPV elicited significantly lower levels of respiratory inflammatory cytokines than did RSV. It is, therefore, not known whether hMPV and RSV share a common pathogenic mechanism or cause disease via different mechanisms.

Although the available data suggest that hMPV mostly causes mild disease in otherwise healthy children, the question of whether hMPV may cause severe problems in children with underlying conditions requires further clarification. hMPV was the only pathogen detected in two patients with acute lymphoblastic leukaemia and ARTI who subsequently died (aged 7 months and <5 years, respectively) [45,46]. Although there was no pathological examination in these cases, that fact that no other pathogens were identified suggests that hMPV infection was the cause of death. In agreement with these observations, Van den Hoogen *et al.* [33] found that hMPV-positive patients aged >5 years had underlying diseases and presented with more severe clinical signs than those generally observed in younger patients. However, a separate study showed that a child aged 5 years with a diagnosis of acute lymphoblastic leukaemia who was infected with hMPV recovered uneventfully [13]. Although studies of immunocompromised individuals have so far been relatively small, they seem to show that the impact of hMPV is similar to that of RSV and influenza virus infections.

Table 3. Clinical and socio-economic impact of different virus infections among the household contacts of the children in whom a single infectious agent was demonstrated

Characteristics	Households of hMPV-positive children (n = 128)	Households of RSV-positive children (n = 507)	Households of influenza-positive children (n = 806)
Disease similar to that of the infected child, no. (%)	16 (12.5)[a]	24 (4.7)	78 (9.7)[a]
Additional medical visits, no. (%)	16 (12.5)[b]	16 (3.2)	78 (9.7)[b]
Anti-pyretic prescriptions, no. (%)	14 (10.9)[a]	18 (3.6)	104 (12.9)[b]
Antibiotic prescriptions, no. (%)	6 (4.7)	11 (2.2)	36 (4.5)
Hospitalisation, no. (%)	0	0	3 (0.4)
Lost working days, median (range)	4 (2–10)[a]	2.5 (2–7)	4 (1–10)[a]
Lost school days, median (range)	4 (3–15)[a]	2 (2–4)	5 (1–15)[a]

[a]$p < 0.05$ and [b]$p < 0.0001$ vs. households of RSV-positive children; no other statistically significant differences.
hMPV, human metapneumovirus; RSV, respiratory syncytial virus.
Adapted from Bosis *et al.* [17].

Few data are available concerning the socio-economic impact of hMPV infection on children and their households. It was reported that the household contacts of hMPV-positive children, as well as those of influenza-positive children, fell ill significantly more frequently, required more medical visits, received more anti-pyretic prescriptions, and were also absent more frequently from work or school than those of RSV-positive children (Table 3) [17]. These findings show that hMPV infection in children may considerably affect their families.

The clinical features described for hMPV disease are based on studies of small numbers of patients. Collectively, the available data indicate that the clinical presentation of hMPV is similar to that of RSV and influenza. Socio-economically, the impact of childhood hMPV infection on children and their families seems to be similar to that of influenza viruses, and significantly greater than that of RSV. Further clinical studies are needed to elucidate the quantitative and qualitative characteristics of hMPV infection, and the groups at risk for severe complications.

DIAGNOSIS

Because hMPV replicates poorly in the conventional cell cultures used for the diagnosis of respiratory viruses, it is relatively difficult to isolate and has probably circulated unreported for some considerable time. Most studies have only detected reliable cytopathic effects in tertiary monkey kidney (tMK) and LLC-MK2 cells [6–10]. The cytopathic effect varies, with some strains inducing RSV-like syncytia formation, and others causing focal rounding and cell destruction. More cell lines have been explored following the original isolation of hMPV, and some laboratories now use Vero or human laryngeal carcinoma (HEp-2) cells successfully [7–10]. In the absence of commercially available antibodies, the cytopathic effect of hMPV can be confirmed by using RT-PCR to test infected supernatants.

RT-PCR has become the method of choice for the diagnosis of acute hMPV infection, because of the unavailability of rapid antigen detection tests, and the slow and restrictive growth of the virus [7–10]. Most PCR protocols published to date rely on amplification of the L (major polymerase subunit), N (nucleoprotein), or F (fusion protein) gene, using primer sequences derived mainly from the prototype strain 001 from The Netherlands (GenBank accession number AF371337) [47–49]. Because of the existence of two hMPV lineages showing significant genetic variability, hMPV detection may be underestimated when inappropriate primers are used. New rapid and sensitive hMPV assays, based on real-time PCR, allow amplification and detection of hMPV in ≤2 h [47–49]. In comparison with conventional RT-PCR, real-time RT-PCR is more sensitive, faster and more cost-effective, and may thus be the best means of detecting hMPV routinely.

Serological tests only permit a retrospective diagnosis and, because infection is almost universal in childhood, seroconversion or a ≥4-fold increase in antibody titres must be demonstrated to confirm recent infection [7–10,48]. A recent serological survey of hMPV was based on use of a novel ELISA using hMPV-fusion protein expressed in recombinant vescicular stomatitis virus [49]. Large serological surveys using this and other simple ELISAs are needed to permit a better understanding of the worldwide epidemiology of hMPV infection. Detection of hMPV antigens in nasopharyngeal secretions by an immunofluorescent antibody test has also provided interesting results [50,51].

TREATMENT AND PREVENTION

No treatment is registered currently and no specific prevention procedures are recommended

for the management of hMPV infection. Ribavirin and a polyclonal intravenous immunoglobulin preparation have been found to have similar in-vitro antiviral activity against both hMPV and RSV [52], but clinical studies are required to confirm these observations. However, given the well-known limitations of these medications (i.e., severe adverse events, difficult administration and high costs), they should be used with caution and probably considered only for treating immunocompromised patients with severe hMPV disease, as in the case of RSV infection. Furthermore, high-titred intravenous immunoglobulin preparations active against hMPV could be used in patients with severe disease [9].

Studies on the development of a specific vaccine are currently in progress in experimental animals. Biacchesi *et al.* [25] were able to generate recombinant hMPVs lacking some genes that were at least 40-fold and 600-fold, respectively, restricted in replication in the lower and upper respiratory tract compared with wild-type recombinant hMPV [25]. However, many more studies are required before there is the possibility of an effective and safe vaccine for humans.

CONCLUSIONS

The recent identification of a presumably old virus pathogen is an exciting development in the field of respiratory viruses. Considering the available studies as a whole, hMPV appears to play an important role as a cause of paediatric upper and lower respiratory tract infection. In general, many of the epidemiological and clinical features of hMPV infection seem to be similar to those of RSV and influenza, although some differences have been noted [7–20]. Moreover, the socio-economic impact of hMPV-infected children on their families seems to be significant, suggesting that, like influenza, hMPV infection may be a substantial public health problem for the community [16,17]. hMPV can cause morbidity and mortality in pre-term infants [35] and children with underlying clinical conditions, including immunocompromised patients [33,34,45,46], although further adequately controlled studies are needed to confirm this.

Many fundamental questions concerning the pathogenesis of hMPV disease and the host's specific immune response remain to be answered. Further surveillance studies are necessary to define the full spectrum of childhood hMPV infection completely, as well as the risk-factors associated with severe hMPV disease. At least two circulating serotypes of hMPV have been identified [8,10], and this must be taken into account when developing diagnostic tests or measures for prevention and treatment of infection.

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