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# Human metapneumovirus: a new respiratory pathogen

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Human metapneumovirus is a recently recognized pathogen of acute respiratory tract infection (ARI) in children as well as elderly and immunocompromised adults. The virus belongs to the family *Paramyxoviridae*, sub family *Pneumovirinae* and genus *Metapneumovirus*. Through genetic analysis it has been characterized into two groups A and B which are further divided into four sub-lineages. The virus is difficult to grow in tissue culture and hence reverse transcriptase-polymerase chain reaction (RT-PCR) for N and L gene is the method of choice for diagnosis. The virus has been seen in all countries with seasonal distribution in winter months for temperate and spring/summer for tropical countries. F gene is the most conserved among different lineages and efforts are underway to design recombination vaccine using F gene.

[Broor S, Bharaj P and Chahar H S 2008 Human metapneumovirus: a new respiratory pathogen; *J. Biosci.* 33 483–493]

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## 1. Introduction

Acute respiratory tract infections (ARI) are a leading cause of morbidity and mortality worldwide (Denny and Loda 1986; Hijazi *et al* 1996). Of the 10 million deaths of children less than 5 years of age throughout the world 1.9 million children died from acute lower respiratory tract infections (ALRI) in the year 2000, 70% of them in sub-Sahara Africa and South Asia (Williams *et al* 2002). Although the incidence rates of ARI are almost similar in both developed and developing countries, the mortality is higher in developing countries (Shapiro 1998). The risk of pneumonia is 3-6 times higher in children from developing countries (10-20%) as compared to developed countries (3–4%) (Victoria *et al* 1999).

Approximately 0.5 million children die due to ALRI in India each year, accounting for one fourth of the 1.9 million global ALRI deaths (Reddaiah and Kapoor 1988; Ahmad *et al* 2000; Williams *et al* 2002).

All classes of micro organisms including viruses, bacteria and protozoa are capable of infecting the respiratory tract, but viruses and bacteria are the common pathogens. A variety

of viruses, including, respiratory syncytial virus (RSV), influenza viruses, parainfluenza viruses, adenoviruses, coronaviruses and picornaviruses, are associated with different respiratory syndromes in all age groups (Grondahl *et al* 1999; Weigl *et al* 2000). However, despite extensive diagnostic testing, a substantial portion of respiratory tract infections still cannot be attributed to any known pathogens. In about a third cases of ALRI (Liolios *et al* 2001) and a half of upper respiratory tract infections (URI) (Nokso-Koivisto *et al* 2002) no pathogen could be detected. These observations suggest that unknown pathogens may be responsible for a substantial proportion of respiratory tract disease.

Metapneumovirus is a recently discovered etiological agent of acute respiratory illness (ARI). It was first reported in 2001 from The Netherlands from nasopharyngeal aspirates (taken over a 20 year period) in 28 hospitalized children and infants with ARI having signs and symptoms similar to that of RSV infection (van den Hoogen *et al* 2001). The virus was found to be closely related to the avian pneumovirus, a member of the *Metapneumovirus* genus, and was called

**Keywords.** Acute respiratory infections; human metapneumovirus

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Abbreviations used: ALRI, acute lower respiratory tract infection; APV, avian pneumoviruses; ARI, acute respiratory tract infection; CPE, cytopathic effect; hMPV, human metapneumovirus; RSV, respiratory syncytial virus; RT-PCR, reverse transcriptase-polymerase chain reaction; SARS, severe acute respiratory syndrome; SH, small hydrophobic

human metapneumovirus (hMPV) (van den Hoogen *et al* 2002). Although the virus was not isolated until recently, antibodies to the virus have been shown to be present in all persons  $\geq 8$  years of age in serum samples collected since 1958 (van den Hoogen *et al* 2001). Thus the virus is not a newly emerging pathogen and did not recently "jump" to the human population from an animal reservoir.

## 2. Virological characteristics

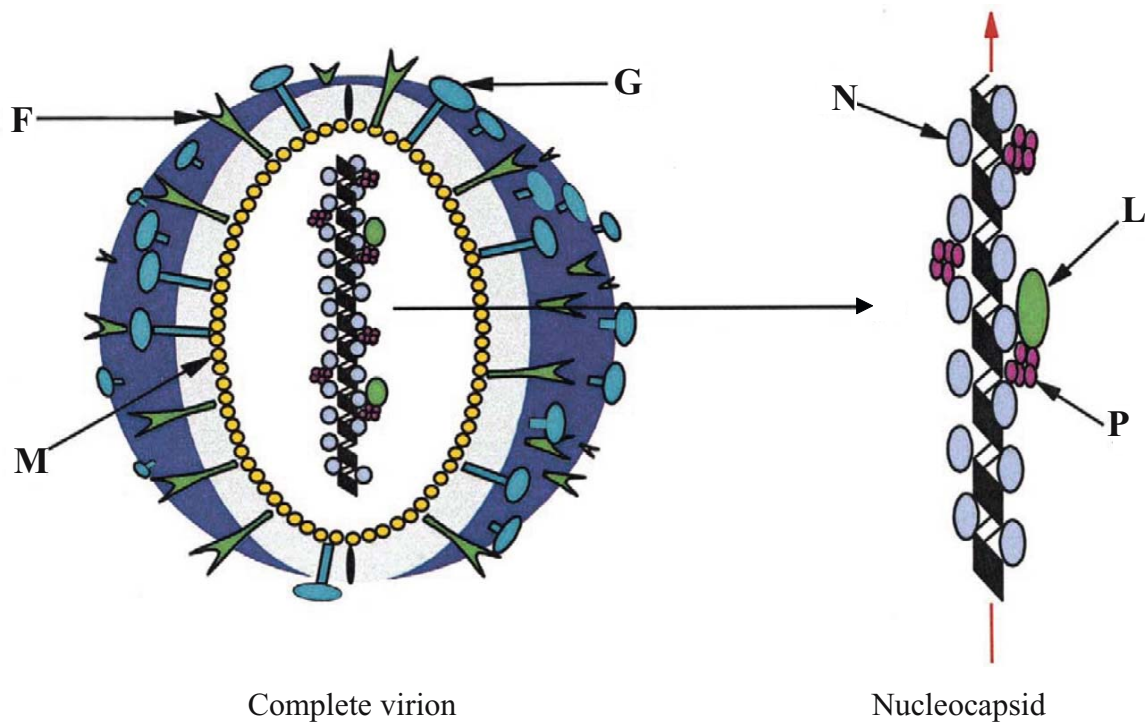
Genetic analysis of the virus showed that it is an RNA virus of the *Paramyxoviridae* family and *Pneumovirinae* subfamily along with RSV. The avian pneumoviruses (APV) (formerly turkey rhinotracheitis virus) is highly related to hMPV and these two viruses have been separated by taxonomists into a separate genus, *Metapneumovirus* (Lamb *et al* 2000).

Initial electron microscopic examination revealed that the viral isolate had morphology similar to paramyxoviruses. Spherical enveloped particles have a mean diameter of  $\sim 200$  nm. In addition, filamentous and pleomorphic particles are also present. The virion is surrounded by a lipid envelope derived from the plasma membrane of the host cell into which the three virus glycoproteins, the attachment (G), fusion (F), and small hydrophobic (SH) proteins, are inserted (Collins and Mottet 1993). The RNA genome associates with

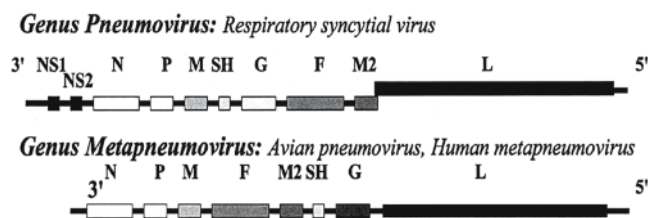
viral proteins to form the helical nucleocapsid (represented on the right and in the centre of the virion on the left in figure 1). The proteins consist of the nucleocapsid protein (N), the phosphoprotein (P), and the large polymerase (L) protein. The M2-1 transcriptional enhancer protein is also thought to be associated with the nucleocapsid. The nucleocapsid is surrounded by the matrix (M) protein, which forms a link between the nucleocapsid and the lipid membrane of the virus particle (Easton *et al* 2004).

## 3. Genome organization

Full length sequences of at least 4 hMPV genomes have been reported (van den Hoogen *et al* 2002; Biacchesi *et al* 2003; Herfst *et al* 2004). The negative sense non-segmented RNA genome of the virus is about 13 kb in length (Easton *et al* 2004). The hMPV genome is predicted to encode 9 proteins in the order 3-N-PM- F-M2-SH-G-L-5 (the M2 gene is predicted to encode 2 proteins, M2-1 and M2-2, using overlapping open reading frames, as in RSV) (van den Hoogen *et al* 2002) (figure 2). The genome also contains noncoding 3' leader which has the viral promoter, 5' trailer and intergenic regions, consistent with the organization of RSV (Herfst *et al* 2004). However there are some differences in the genome of APV/hMPV as compared to RSV, they lack the 2 nonstructural proteins NS1 and NS2



**Figure 1.** Schematic diagram of the pneumovirus particle (from Easton *et al* 2004).



**Figure 2.** Genomic organization of RSV and hMPV. (Modified from Easton *et al* 2004.)

located at the 3' end of RSV genomes. These proteins counteract host interferons; therefore the lack of these genes in the metapneumoviruses may have important implications for the relative pathogenicity of these viruses compared with RSV strains (Collins *et al* 2001). Sequence identity between APV and hMPV open reading frames is 56% to 88% (van den Hoogen *et al* 2002).

Although the function of each of the gene products has not been formally tested, it is likely that the function of the proteins can be predicted by comparison with other paramyxoviruses. The F (fusion), G (glycosylated) and SH (short hydrophobic) proteins are integral membrane proteins on the surface of infected cells and virion particles. The F protein appears to be a classic viral fusion protein, with a predicted nonfurin F1/F2 cleavage site near a hydrophobic fusion peptide and 2 heptad repeats in the extra cellular domain that facilitate membrane fusion. The predicted G protein of hMPV exhibits the basic features of a glycosylated type II mucin-like protein but interestingly lacks the cluster of conserved cysteines sometimes termed the "cysteine noose" that is found in the RSV and APV G proteins. G gene of hMPV tends to be highly variable like RSV G gene which may be due to host immune selection pressure (Crowe 2004). The function of the SH proteins of both the viruses remains unknown. The N (nucleoprotein), P (phosphoprotein) and L (large, polymerase) proteins are replication proteins in the nucleocapsid, the M2-1 and M2-2 proteins are regulatory proteins and the M (matrix) protein may coordinate viral assembly of viral nucleocapsids with envelope proteins (Crowe 2004).

#### 4. Genetic and antigenic diversity

The classification and naming of serotypes/serogroups, genotypes, subgroups, strains, variants and isolates of hMPV is still evolving. Based on genomic sequence and phylogenetic analysis, there are two major genotypes of hMPV, designated A and B (Biacchesi *et al* 2003; van den Hoogen *et al* 2003). These analyses are based on sequencing of the N, M, F, G, or L gene, and the genotype groupings are concordant regardless of which gene is studied (Bastein *et al* 2003; Biacchesi *et al* 2003; Boivin *et al* 2004; Peret

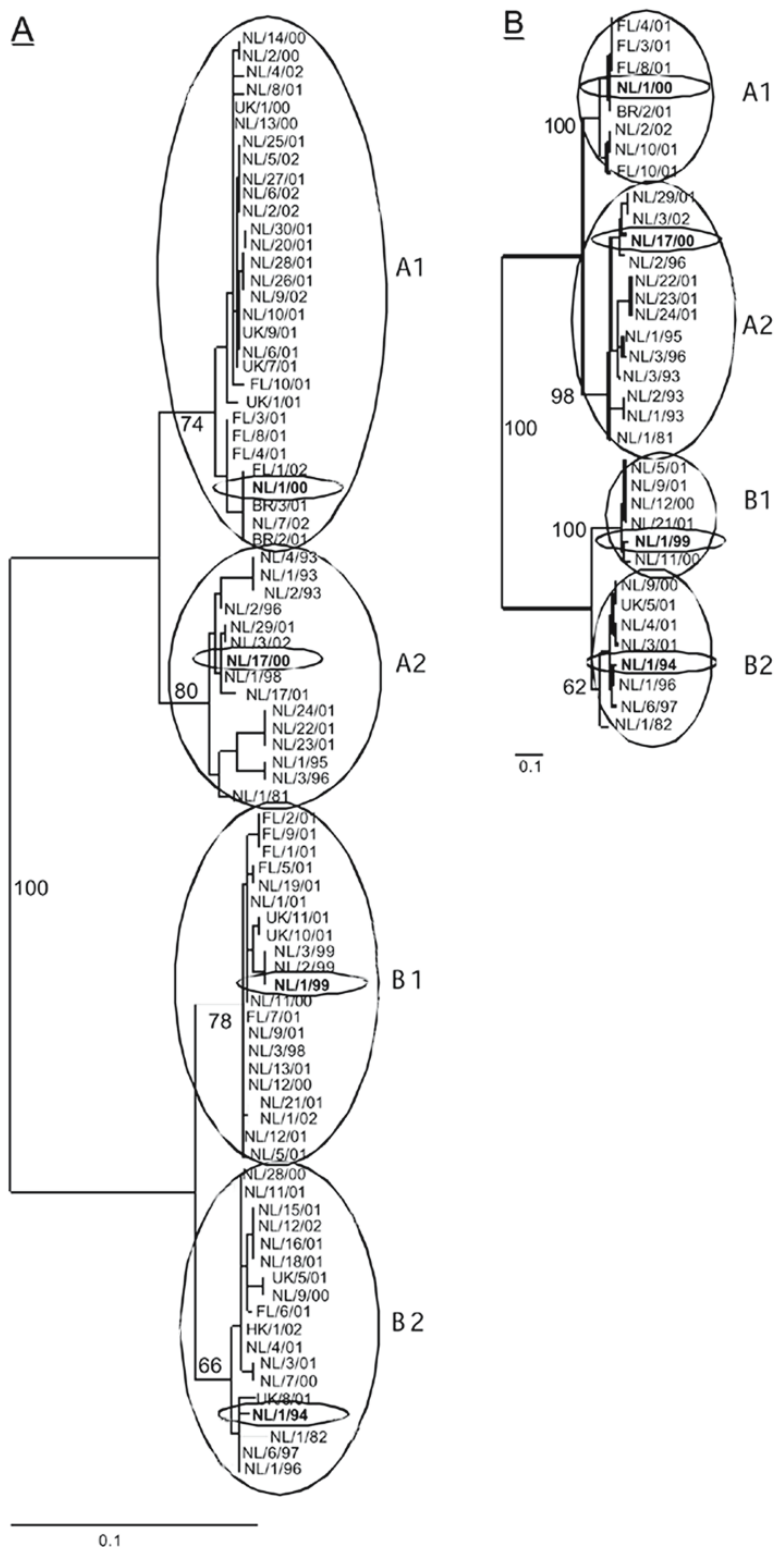
*et al* 2004). Full length sequences of genomes from viruses representing the 2 major subgroups show that the diversity between hMPV subgroup A and B sequences is greatest for the SH and G proteins (59 and 37% identity, respectively), (van den Hoogen *et al* 2002; Biacchesi *et al* 2003; Herfst *et al* 2004) and is more than RSV subgroup A and B. hMPV F protein, which is predicted to be the principal target of protective antibodies, is more conserved in hMPV strains than RSV. The overall level of genome nucleotide sequence identity and aggregate proteome amino acid sequence identity between the two hMPV subgroups were 80 and 90%, respectively, similar to the respective values for RSV A and B groups (Hamelin *et al* 2004).

Based on the sequence analysis of two surface glycoprotein encoding genes namely F and G genes each of the major lineages are further subdivided into two genetic sub-lineages known as A1, A2 and B1, B2, the significance of this diversity is unclear at this time (Boivin *et al* 2004; van den Hoogen *et al* 2004) (figure 3). In a recent report Huck and colleagues have reported a new sub lineage within the A lineage. Previously reported A2 lineage is found to consist of two clusters namely, A2a and A2b. Among all the sub-lineages of hMPV the A2 sub-lineage shows the greatest diversity (Huck *et al* 2006).

The genetic diversity in hMPV strains also affects antigenic diversity, but to what extent has not been resolved. Studies with experimental infection of animals have suggested that the two lineages exhibited 48% antigenic relatedness based on reciprocal cross-neutralization assay with post-infection hamster sera (Skiadopoulos *et al* 2004). However cross protection studies in hamsters and nonhuman primates have shown that each strain provided a high level of resistance to reinfection with the homologous or heterologous strain, supporting the conclusion that the two hMPV genetic lineages are highly related antigenically and are not distinct antigenic subtypes or subgroups (Herfst *et al* 2004; Skiadopoulos *et al* 2004; van den Hoogen *et al* 2004). hMPV F protein is a major antigenic determinant that mediates extensive cross-lineage neutralization and protection (MacPhail *et al* 2004; Skiadopoulos *et al* 2004). The extent of cross-protection in rodents cannot be extrapolated directly to the human situation because the animals are only semi permissive hosts for hMPV replication. Although difficult to assess, the extent of cross-protection is important to estimate because vaccine developers will choose to develop either a monovalent or a bivalent vaccine formulation based on this factor.

#### 5. Clinical features of infection

hMPV has been associated with ARI in all age groups, with more severe diseases occurring in young children, elderly individuals and immunocompromised hosts.



**Figure 3.** Phylogenetic trees constructed based on the (A) partial F gene (Open reading frame [ORF] position 780–1,221) or (B) the complete G coding region (start G ORF to start L ORF). Trees were generated by maximum likelihood analysis using 100 bootstrap values. The four prototype viruses are shown in boldface, with ovals drawn around them (from van den Hoogan et al 2004).

The virus causes a variety of clinical syndromes in children that are typical of the paramyxoviruses, including upper and lower respiratory tract illnesses. The clinical characteristics of hMPV infections are not distinctive, thus, differentiating it from other respiratory viruses on clinical grounds is not possible (Stockton *et al* 2002). A study conducted at Vanderbilt University Medical Center on the association of the virus in a cohort of 2000 subjects of age 0–5 years, followed during a 25-year period revealed that hMPV was associated with common cold (complicated by otitis media in one third), bronchiolitis, pneumonia, croup and exacerbation of reactive airway disease (Williams *et al* 2004). The clinical profile of illness caused by hMPV was similar to that caused by RSV. In outpatients with ALRI, hMPV was detected in 12% of cases, which was second only to RSV (Williams *et al* 2004). Hoarseness has also been observed more frequently in hMPV infection as compared to RSV (Falsey *et al* 2003). Further compared with RSV infections, the children who develop hMPV infection are somewhat older, and the severity of disease is usually somewhat less than RSV (Boivin *et al* 2003; Peiris *et al* 2003; van den Hoogen *et al* 2003; Viazov *et al* 2003).

Human MPV infection is not restricted to the very young children but also occurs in adults and elderly subjects. In adults it usually causes flu like illness and colds (Boivin *et al* 2002). In fragile elderly hMPV causes more severe disease than in healthy elderly or young adults (Falsey *et al* 2003). In a study among 10 elderly of > 65 years of age who had underlying illness, 4 developed pneumonitis and 2 died due to hMPV infection (Boivin *et al* 2002).

Immune status of infected subjects is also important in determining the severity of illness. hMPV infection has been reported in immunocompromised individuals. In 3 patients with acute lymphoblastic leukemia who had ALRI, hMPV was the sole pathogen detected and one of them died (Pelletier *et al* 2002). hMPV infection has also been reported in HIV infected children but it is not clear if the severity of illness is more in this group (Madhi *et al* 2003).

Respiratory virus infections are often associated with recurrent wheezing in children and exacerbations of asthma in older patients. Acute wheezing and asthma exacerbations have been associated with hMPV infection in some studies (Jartti *et al* 2002; Peiris *et al* 2003) but all studies have not shown this association (Rawlinson *et al* 2003). In some studies in children with asthma hMPV was found more frequently than RSV (Peiris *et al* 2003; van den Hoogen *et al* 2003).

A study by Vicente *et al* (2006) suggested that hMPV genotype A might be more pathogenic than genotype B, causing greater clinical severity in children whereas no differences in disease severity associated with either genotype (Agapov *et al* 2006) was observed in another study.

## 6. Co-infections

Because the seasonal distributions of hMPV and RSV overlap, the potential for dual infection exists. Several studies have found hMPV –RSV co-infection rate of approximately 5-10% (Esper *et al* 2004; Viazov *et al* 2003; Williams *et al* 2004; Xepapadaki *et al* 2004). However, Greensill *et al* (2003) reported that 70% of RSV-infected children who required intensive care in Liverpool, United Kingdom, were co-infected with hMPV, suggesting that dual infection with RSV and hMPV may predispose for severe infection particularly in otherwise healthy children. In another study from the United Kingdom, hMPV and RSV co-infection conferred an increased risk of admission to the pediatric intensive care unit (Semple *et al* 2005). However, such synergistic association has not been found in other, population-based and case-control studies of hospitalized children (Boivin *et al* 2003; Esper *et al* 2003; Maggi *et al* 2003). The possible synergistic interaction between hMPV and the severe acute respiratory syndrome (SARS) coronavirus has been recently postulated during the 2003 SARS outbreak in Canada and Hong Kong as many individuals were found to have co-infection with hMPV (Chan and Li 2003; Poutanen *et al* 2003). On the other hand, such synergy between hMPV and SARS was not confirmed in experimental macaque models (Fouchier *et al* 2003). In one case report, in an infant who had SARS, fatal encephalitis was correlated with hMPV infection as hMPV RNA was detected post-mortem in brain and lung tissue (Schildgen *et al* 2005).

## 7. Re-infections

Cases of severe hMPV infections in adults (Boivin *et al* 2002) and reinfection in immunocompromised subjects (Pelletier *et al* 2002) suggest that, despite the universal infection in childhood, new infections can occur throughout life due to incompletely protective immune responses and/or acquisition of new genotype. Since severe disease is seen mainly in pediatric patients, it suggests that naturally acquired infection induces partial protection against the disease. However, it should be emphasized that there is no cross – protection among the virus strains. A recent report has described a child who suffered from two episodes of hMPV infection during a one month period, each caused by a different strain (Vargas *et al* 2004).

## 8. Pathogenesis

The conventional model of attachment for the members of subfamily *Pneumovirinae* including genus *Pneumovirus* and *Metapneumovirus* involves the interaction of viral

G protein with a molecule or molecules on the host cell surface (Levine *et al* 1987). While the precise nature of the cellular receptor and mechanism of entry has not been identified, current evidence suggests that one or more cellular glycosaminoglycans or heparin-like molecules are involved in virus attachment and entry (Feldman *et al* 2000). Following G-protein-mediated attachment, the F glycoprotein promotes pH-independent fusion between the cell membrane and the virus envelope. For the F protein to become functional, it has to be cleaved by cellular proteases, and the hydrophobic amino terminal region of the F1 component then promotes the fusion process, which introduces the internal components of the virion into the cytoplasm of the host cell, where the remainder of the infectious cycle takes place. Recent experimental work using primates (chimpanzees, cynomolgus and rhesus macaques, African green monkeys) and small animals (hamsters, cotton rats, mice and ferrets) has been performed to characterize the pathogenesis associated with this viral infection; hMPV replicates to a various extent in the upper and lower respiratory tracts of these experimental animals, although clinical symptoms after intranasal challenge have only been observed in chimpanzees, *Cynomolgus macaques* and BALB/c mice so far (van den Hoogen *et al* 2001; Alvarez *et al* 2004; Kuiken *et al* 2004; Skiadopoulos *et al* 2004; Hamelin *et al* 2005). In BALB/c mice and cotton rats, time course studies have indicated that peak viral titers are found around day 4–5 after infection and decrease thereafter Alvarez *et al* (2004a) and Hamelin *et al* (2005), have demonstrated that hMPV may present an initial biphasic replication pattern in lungs of BALB/c mice, with hMPV RNA still detectable more than 180 days after infection. Such persistence could be explained by an aberrant T helper cell type 2-like immune response, with impaired virus clearance after primary hMPV infection (Alvarez and Tripp 2005).

Significant pulmonary inflammatory changes have been found in BALB/c mice and cotton rats (Alvarez *et al* 2004; Hamelin *et al* 2005). Interestingly significant inflammatory changes were still present in the cotton rat animal model more than 21 days after viral challenge (Hamelin *et al* 2005). Increase in many cytokines and chemokines such as interleukin IL-2, IL-8, IL-4, INF- $\gamma$ , macrophage inflammatory protein 1 $\alpha$  and monocyte chemotactic protein has been observed in the lungs or bronchoalveolar lavage of both mice and cotton rats in response to hMPV challenge (Alvarez *et al* 2004, Hamelin *et al* 2005). In humans, hMPV infection has also been associated with an increase of IL-8 in upper respiratory tract secretions and with chronic inflammatory changes of the airways, with presence of, intra alveolar foamy and hemosiderin-laden macrophages (Laham *et al* 2004, Vargas *et al* 2004). When compared with RSV, hMPV infections in humans seem to induce lower levels of inflammatory cytokines such as IL-

12, tumor necrosis factor  $\alpha$ , IL-6 and IL-1 $\beta$  (Laham *et al* 2004). Thus far, no experimental human volunteer studies have been reported.

## 9. Epidemiology

Since its initial description in 2001, hMPV has been isolated from individuals of all ages with ARI, and has been identified in every continent. hMPV infections have been documented in Europe [United Kingdom ( Stockton *et al* 2002 ), Finland (Jartti *et al* 2002 ), Italy (Maggi *et al* 2003), France (Freymuth *et al* 2003), Germany ( Viazov *et al* 2003), Spain (Vicente *et al* 2003), Norway (Dollner *et al* 2004)]; America, Canada (Boivin *et al* 2002), United States (Esper *et al* 2003, Falsey *et al* 2003), Brazil (Cuevas *et al* 2003) Australia (Nissen *et al* 2002), Asia [HongKong (Peiris *et al* 2003), Japan (Ebihara *et al* 2004), Korea (Kim and Lee 2005) Thailand (Samransamruajkit *et al* 2005)] and Africa [Yemen, (Al-Sonboli *et al* 2005), South Africa (Ludewick *et al* 2005)]. In a preliminary study from Pune in India hMPV was found in 5 of 26 children with ARI (Rao *et al* 2004). In a study of children seen in a large referral hospital in Delhi hMPV was detected in 12% of ARI cases by reverse transcriptase-polymerase chain reaction (RT-PCR) (Banerjee *et al* 2007).

Role of hMPV in ARI has been evaluated in many studies mostly using molecular methods. In young hospitalized children hMPV has been detected in 5-10% of ARI cases. (Peret *et al* 2002; Bastein *et al* 2003; Bovin *et al* 2003; Esper *et al* 2003, 2004; McIntosh and McAdam 2004). However in one study from Italy the rates of hospitalizations due to hMPV infection varied from 7-43% (Maggi *et al* 2003). The rates of detection of hMPV in adults are usually lower than children with rates of about 3% in general community (Stockton *et al* 2002).

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 (Hamelin *et al* 2004).

Most hMPV infections occur in < 5 years of age, with children <2 years of age being most at risk for serious hMPV infections. In one study the peak was seen at 4–6 months of age (van den Hoogen *et al* 2004). However, hMPV infections tend to occur in slightly older children compared to RSV (Peiris *et al* 2003; van den Hoogen *et al* 2003).

The activity of hMPV in temperate climates peaks between December and February (Maggi *et al* 2003; van den Hoogen *et al* 2003) where as in subtropics it peaks in spring-summer (Peiris *et al* 2003). The peak of activity of hMPV at any given location often coincides with or follows the peak of RSV activity (Boivin *et al* 2003; Esper *et al* 2004).

Outbreaks of hMPV are to be a local phenomenon unlike influenza virus, where two or three strains spread across the globe each year. Strains of hMPV differ from community to community, and strains identified in one location may be quite similar to strains identified in other locations in different years. For example, the prototype strain identified in The Netherlands is genetically similar to strains identified in Australia; New Haven, Connecticut and Quebec, Canada in different years (Esper *et al* 2004). We in Delhi also found that 2 lineages of hMPV circulated during the same season i.e. in 2005-06 A2b and B1 lineages were seen with A2b being the predominant genotype where as in 2006-07 B1 and B2 were found to co-circulate with predominance of B1 (Broor S, *et al*, unpublished data).

Although formal transmissions studied have not been carried out, transmission most likely is through large particle respiratory secretions and fomites as is true for RSV (Human Metapneumovirus 2006). Nosocomial transmission has been reported in hospital setting (Pieris *et al* 2003).

### 9.1 Seroepidemiology

Presence of hMPV antibodies in serum samples obtained in 1958 showed that the virus has been circulating for at least for the past 50 years in The Netherlands (van den Hoogen *et al* 2001). By the age of 5 years, >90% of individuals screened have evidence of hMPV infection. The seroprevalence of hMPV specific antibody in adults is nearly 100% (van den Hoogen *et al* 2001, Leung *et al* 2005). The hMPV-specific antibodies in infants <3 months of age has been detected in >90% of the cases tested, indicating that maternally derived antibodies are present in young children (Leung *et al* 2005). Whether these hMPV specific antibodies protects against infection or lessens the severity of illness is not known.

## 10. Diagnosis

### 10.1 Virus isolation

hMPV replicates poorly in conventional cell culture and is relatively difficult to isolate. Many strains show reliable cytopathic effect (CPE) in tertiary monkey kidney cell line/LLCMK-2 cells (Peret *et al* 2002). The CPE is quite variable, some strains show syncytia formation and other only produce rounding (Boivin *et al* 2002). CPE is usually observed after 10-21 days (mean 17 days). Confirmation of CPE is done by RT-PCR or indirect immunofluorescence staining using hMPV specific antibodies.

Recent reports have shown that hMPV can replicate efficiently in Hep-2 cells but does not produce good CPE. van den Hoogen *et al* (2004) reported that Vero cell clone

118 is permissible for expression of virus from all four lineages and CPE is easier to observe. Many laboratories are now using this cell line for routine virus isolation (van den Hoogen *et al* 2004). In addition shell vial culture for rapid isolation of hMPV has also been described (Reina *et al* 2007). They showed that shell vial culture using commercial LLCMK-2 cells should be a method for isolating hMPV from respiratory samples in pediatric population.

### 10.2 Direct immunofluorescence assay

Direct immunofluorescence assay (DFA) using virus specific antibodies is a rapid method to detect respiratory viruses and is commonly used in diagnostic laboratories. Commercially available hMPV specific antibodies have been developed for direct immunofluorescence assays, though this method may not be as sensitive as RT-PCR for the detection of hMPV (Ebihara *et al* 2005, Landry *et al* 2005, Percivalle *et al* 2005).

### 10.3 Molecular diagnostics

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. Cote *et al* (2003) reported that primers that bind regions of the N and L genes are highly sensitive for the detection of hMPV strains of both genotypes. However, it is reported that the P gene of hMPV is an ideal target for the molecular detection and genotyping as P gene provides the conserved region for designing RT-PCR primers and adequate variability to permit the accurate genotyping of the virus into 2 main lineages and 4 sub-lineages (Mackay *et al* 2004).

Real time PCR has also been described for hMPV which allows amplification and quantitation of this pathogen in clinical samples. Real-time RT-PCR is a powerful method to detect hMPV and other respiratory viruses. The assay has been developed to detect the N gene of all known hMPV lineages (Maertzdorf *et al* 2004) and real time RT-PCR targeting the N and / or L gene is more sensitive, specific, and rapid method to detect this virus in clinical samples than conventional RT-PCR (Mackay *et al* 2003). Nucleic acid sequence based amplification assay (NASBA) targeting the M gene has also been developed for detection of hMPV infection in respiratory specimens (Dare *et al* 2007).

### 10.4 Serology

Enzyme-linked immunosorbent assay (ELISA) can also be used for hMPV serological testing using the hMPV-infected

cells (Falsey *et al* 2003, Hamelin *et al* 2005). Recently, ELISA methods using viral N or F protein expressed in prokaryotic (Hamelin and Boivin 2005) or recombinant vesicular stomatitis virus (VSV) (Leung *et al* 2005) and recombinant baculovirus system (Liu *et al* 2007) have been developed to detect antibodies against hMPV. Use of these assays in sero-epidemiologic studies might be helpful for detection of antibody response against hMPV infections thus increasing the understanding of the human immune responses to hMPV and permitting better understanding of the epidemiology of this virus. However, since infections with hMPV are universal, serologic testing for diagnosis can only help if four fold increases in antibody titers or seroconversion is demonstrated (Prins and Wolthers 2004).

### 11. Vaccine candidates and animal models

The development of a safe and effective vaccine to protect against hMPV is a reasonable goal. Several promising vaccine candidates have been tested in animal models. A live recombinant human parainfluenza virus that contains the hMPV F gene has been shown to induce hMPV-specific antibodies and to protect experimental animals from hMPV challenge (Skiadopoulos *et al* 2004).

A chimeric bovine/human parainfluenza virus 3 expressing the hMPV F elicits neutralizing antibodies against both Parainfluenza virus and hMPV (Tang *et al* 2005). However, the results of these animal challenge studies should be interpreted cautiously. There are many limitations of the small-animal model for testing potential hMPV vaccines, not the least of which is that the pathogen is highly host restricted. The safety and efficacy of a recombinant hMPV vaccine may be difficult to predict. Chimeric hMPV/avian pneumovirus recombinant viruses in which the N or P gene of hMPV was replaced with the corresponding gene from avian pneumovirus are attenuated in African green monkeys (the P gene chimera is 10- to 1,000-fold more attenuated than the N chimera), though both are as immunogenic as wild-type hMPV (Pham *et al* 2005). This represents another strategy for the development of recombinant attenuated live hMPV vaccines.

### 12. Therapy

Other than influenza virus, antiviral therapy for respiratory viruses has not shown tremendous potential. It is unknown what role the host's inflammatory response plays during hMPV disease. Nonetheless, antiviral compounds have been tested with hMPV.

The antiviral activity of ribavirin to inhibit the replication of hMPV is equivalent to that observed with RSV (Wyde *et al* 2003). Other compounds, such as NMSO<sub>3</sub>, a sulphated

sialyl lipid that has been shown to have potent antiviral activity against RSV in tissue culture cells, have been shown to have anti-hMPV activity *in vitro* (Wyde *et al* 2004). It is likely that an hMPV-neutralizing monoclonal antibody for prophylaxis of high-risk infants (similar to the anti-RSV F humanized monoclonal antibody currently used for prevention of severe RSV disease) will be developed and tested. The progress towards an effective antiviral strategy for hMPV is currently limited by the scant data on pathogenesis of the virus in the natural host.

### 13. Conclusions

Since the discovery of hMPV in 2001, the virus has been identified worldwide. hMPV is a common respiratory pathogen, particularly in infants and young children. The virus is associated with both upper and lower respiratory tract infections and may be a trigger for asthma. There are two major genotypes of hMPV, whether these genotypes represent distinct serotypes re-mains controversial. The major challenges faced by the medical and scientific communities are: the understanding of the pathogenesis of hMPV disease and the development of a safe and effective vaccine to protect against infection and disease caused by this newly recognized respiratory virus.

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