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A Review on Pathology of Human Metapneumovirus (HMPV)

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Abstract

Human metapneumovirus (HMPV) is one of the main pathogens responsible for acute respiratory infections in children up to 5 years of age, contributing substantially to health burden. Human Metapneumovirus (HMPV) frequently causes viral pneumonia which can become life-threatening if the virus spreads to the lungs and shows high morbidity and mortality, particularly in children and immunocompromised patients. Even though HMPV was only isolated in 2001, this negative-stranded RNA virus has probably been circulating in the human population for many decades. Interestingly, Almost all adults have serologic evidence of HMPV infection. Various methods for detecting HMPV have been developed and applied in clinical laboratories. When reviewing the literature, we found that polymerase chain reaction (PCR)-based assays have been Most frequently and consistently used to detect HMPV. This review will focus on the epidemiology, transmission, and clinical manifestationsin humans as well as the animal models of HMPV pathogenesis and host Immune response.

Keywords: Human metapneumovirus, polymerase chain reaction (PCR), respiratory tract infection, immune response, virus cultivation,

1. INTRODUCTION

Human metapneumovirus (HMPV) is a respiratory virus from the *Pneumoviridae family,* first described in 2001, when it was isolated from the respiratory tract of children from the Netherlands[1]. The start of the twenty-first century has seen the discovery of Several emerging or new respiratory pathogens causing human Disease, including severe acute respiratory syndrome coronavirus And human metapneumovirus (HMPV)[2]. Several studies have shown that upto 95% of children infected with HMPV were previously healthy, indicating that young age is one of the major factors influencing disease severity[3,4].ARTI that are confined to the upper respiratory tract typically result in mild respiratory symptoms. However, when the infection spreads to the lungs, this can lead to life-threatening pneumonia. Two members of the Pneumoviridae family, namely, human respiratory syncytial virus(HRSV) and human metapneumovirus (HMPV), frequently cause viral pneumonia in infants and children(<five years of age), the elderly (>65 years of age), and immune-compromised individuals[9,12,13].

HMPV is classified into two major genetic lineages, HMPV A and B, that are further subdivided into lineages A1, A2, B1, and B2[9,10,11]. Almost five years after the first description of the virus, several reports of animal models for HMPV have been published, the continuously increasing body of literature

makes it difficult even for experts in the field to follow recent developments. For this reason we have summarized the recent studies describing animal models for HMPV infections and discuss the advantages and disadvantages of these models. Several animal species have been determined to be permissive for HMPV infection[17, 18]. Because HMPV is a relevant respiratory virus, the need to understand how the immune system contributes to controlling this infectious agent can help develop new vaccines and therapies[19,20].

2. Genome Organization and structure:

Gene	Protein	Amino acid length	Function
N	Nucleoprotein	394	RNA genome encapsidation
P	Phosphoprotein	294	Polymerase co-factor
M	Matrix protein	254	Aids in viral assembly and budding
F	Fusion protein	539	Virus-cell binding and membrane fusion
M2	M2-1 protein	187	RNS transcription processivity factor
	M2-2 protein	71	Regulates RNA transcription/replication
SH	Small hydrophobic protein	177–183	Possible viroporin or innate immune inhibition
G	Attachment glycoprotein	229–236	Binds to cellular glycosaminoglycans
L	Large polymerase protein	2,005	Catalytic activity for viral replication

Figure 1. Summary of human metapneumovirus proteins and function.

The metapneumoviruses are enveloped, non-segmented, negative-sense, single-stranded RNA virus[2]. The HMPV genome harbors 8 genes that encode for 9 proteins. The order of these genes in the genome is N-P-M-F-M2-SH-G-L. The encoded proteins include the nucleoprotein (N protein), phosphoprotein (P protein), matrix protein (M protein), fusion glycoprotein (F protein), putative transcription factor (M2-1 protein), RNA synthesis regulatory factor (M2-2 protein), small hydrophobic glycoprotein (SH protein), attachment glycoprotein (G Protein), and viral polymerase (L protein)[5,6]. The F, M, and L genes as well as the N gene (the most conserved region), have been targeted to detect HMPV by reverse transcriptase-polymerase chain reaction (RT-PCR)[7,8]. Three transmembrane surface glycoproteins are embedded in the lipid envelope: F, G, and SH. The G protein is important for the attachment of the virion to the host cell. The F protein mediates fusion of the viral and host cell membrane. The exact function of the SH protein remains elusive. The F protein sequence is relatively well-conserved between different HMPV genotypes compared to G and SH which are more variable[11,14,15,16].

The putative G protein of hMPV is considerably smaller than the G protein of RSV (236 versus 299 amino acids). Unlike that in RSV, the region of the HMPV genome that encodes the putative G protein also carries an ORF immediately downstream of the G gene (in the same reading frame) and ORFs in the two other reading frames. It is unknown whether these accessory ORFs are expressed as separate proteins or are transcribed as part of the G protein through some RNA-editing event. The predicted amino acid sequences of the G gene of both HMPV and RSV indicate that these genes encode anchored type II glycoproteins[6,24]. HMPV exhibits a paramyxovirus-like morphology, ranging from 150 to 600 nm in size, enveloped with short protein spike projection[28].

3. Viral replication:

Replication of HMPV occurs in the nasal and lung tissues, and Airway epithelial cells are the primary target of HMPV. HMPV is thought to attach to the target cell via G protein interactions with heparan sulfate and other glycosaminoglycan[26,27]. Viral glycoproteins can be transported via the Golgi apparatus to the membrane and accumulate for the assembly of new viral particles. When the production of viral proteins reaches a threshold concentration, the RNA polymerase replicates the genome into positive-sense RNA. This positive-sense RNA will be used as the template for the new genomic negative-sense RNA, which will then be contained within the new viral particles[21].

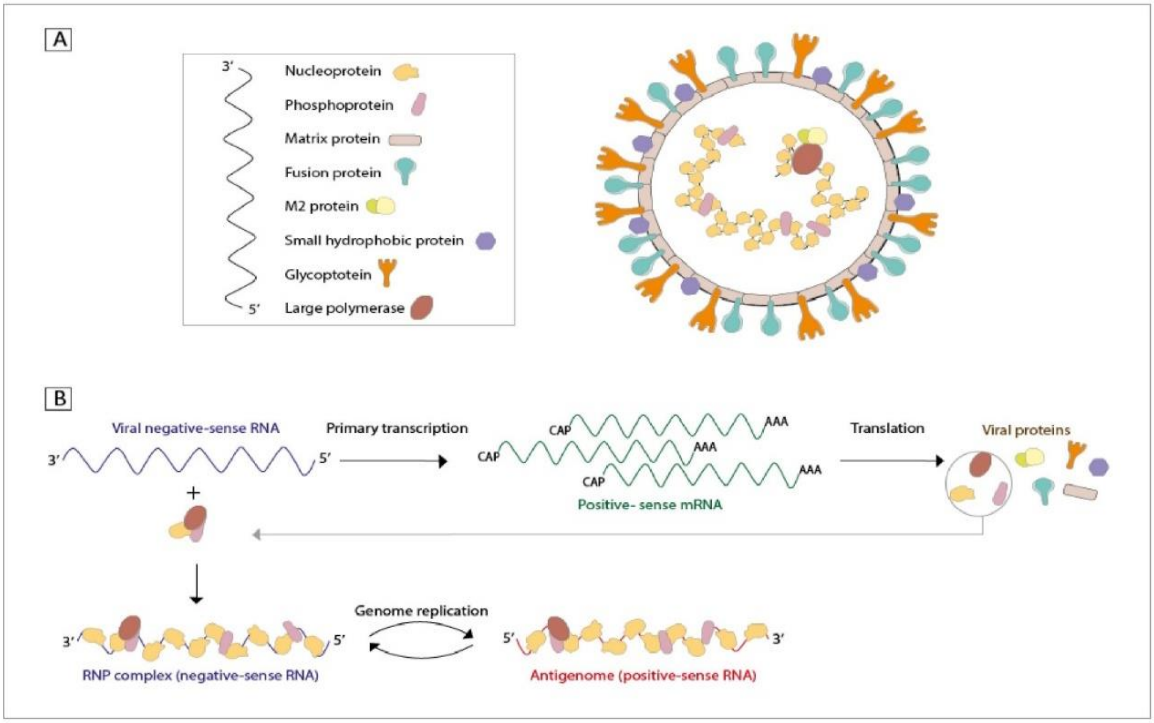

Figure 2. Human metapneumovirus (HMPV) virion structure with viral proteins and their function Schematic representation of the HMPV viral particle (A) and viral genome with encoded proteins (B):Nucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), matrix-2 proteins (M2-1 And M2-2), small hydrophobic (SH) protein, glycoprotein (G), and large (L) polymerase protein.

In the ribonucleoprotein complex, the viral RNA is entirely coated by the N protein, resulting in flexible helical nucleocapsids, which are also decorated with the P protein and can recruit the L and M2-1 proteins. In negative-stranded RNA viruses, the viral nucleocapsid serves to protect the viral RNA from degradation and as a template for viral replication and transcription[22]. This replication process takes place within cytoplasmic inclusion bodies, Created by the interaction between the N and P proteins[29]. As nascent viral proteins accumulate, the polymerase switches from transcribing monocistronic mRNA to replicating full-length positive-sense antigenome to serve as a template for progeny negative-sense genomes. Newly synthesized virions exit the host via budding from the plasma membrane, which is facilitated by the M protein[31,32].

4. Epidemiology

In 2001, human metapneumovirus was first identified in the Netherlands causing clinical symptoms in ch-

ildren, however serological studies demonstrated that this pathogen was already circulating in the Netherlands in 1958. Although infections with HMPV may be reported year-long, peak infection of HMPV in the northern hemisphere occurs in late winter and early spring, but infection can be found globally across all continents. The four different subgroups A1, A2, B1, B2 have not been known to cause varying levels of severity of infection compared to one another. In addition, there is not a predominance of one strain versus the others[23]. In 2004, a variant of HMPV, isolated from a 6 ½-year-old Girl with an acute exacerbation of asthma, was found to be genetically distinct from viruses of the four lineages of HMPV[30]. India has reported its first cases of HMPV, with at least nine infections confirmed across various regions, including Bengaluru, Ahmedabad, Chennai, etc. The affected individuals include infants and young children. Despite these cases, health experts and officials emphasise that there is no cause for alarm. The Indian Health Ministry has stated that the situation is under control and that the increase in respiratory illnesses during winter is expected. Based on the previous reports, HMPV infection appears to be seasonal, and co-infection with other respiratory pathogens is common[25].

5. Sign and symptoms:

Symptoms and Disease presentation of HMPV are similar to those of other respiratory viruses causing both upper and lower respiratory tract infections. Symptoms can include cough, rhinorrhea, sore throat, and fever as well as lower respiratory tract symptoms such as Wheezing, difficulty breathing, and hypoxia[33,34]. The clinical diagnoses most commonly associated with HMPV are bronchiolitis and pneumonia[35]. HMPV mainly infects and affects the lower respiratory tract (LRT), with the requirement for mechanical ventilation in the most severe cases[1,36]. Infection with HMPV has also been associated with the manifestation of neural-related Symptoms, such as encephalitis and febrile seizures[37,38]. The most severe symptoms associated with HMPV infections are often reported in infants between younger than one year old, but are highly prevalent during early childhood up to five years old[1,36].

6. Clinical Manifestations of HMPV infection

6.1 Lower Respiratory Tract Disease

Evidence from many studies has demonstrated that HMPV is responsible for a substantial proportion of LRTI in infants and Young children and is second only to RSV as a cause of bronchiolitis in early childhood. These children were followed as part of the Vanderbilt University Vaccine Clinic center in Nashville. In 20% of cases of LRTI (characterized by wheezing, rales, tachypnea, and dyspnea) which could not be attributed to a culturable respiratory Virus, hMPV was detected. The incidence of HMPV-associated LRTI in young children varies with geographical location and time of year. The incidence estimates range from 5 to 15% in most studies [36,39,40,41,42,43]. The clinical manifestations of HMPV infection in young children are indistinguishable from the clinical manifestations of RSV infection. Features of HMPV infection included tachypnea, fever, cough, hypoxia, and changes on chest radiographs such as infiltrates, hyperinflation, and peribronchial cuffing [36,39, 43,44,45]. The most common diagnoses in hospitalized children who test positive for HMPV are bronchiolitis, pneumonia, and bronchitis. There are no manifestations of infection that are specific for HMPV-associated disease. In the limited number of population-based studies performed, children 2 years old appear to be the most likely to be hospitalized due to HMPV-associated LRTI . HMPV has been associated with severe LRTI requiring intensive care[46].

6.2 Upper Respiratory Tract Infection

Like other common human respiratory viruses, HMPV is also associated with URI. While the definition of URI varies among studies, HMPV may be responsible for 5 to 15% of cases URI in children. In a recent study, Williams et al.Screened respiratory specimens collected over a 20-year period from children 5 years old with URI, characterized by coryza, Conjunctivitis, pharyngitis, otit media (OM), or stomatitis.These children did not have a prior virological diagnosis. Of the 2000 specimens screened by real-time RT-PCR, the percentage of URIs attributed to HMPV was 1 to 5%. This varied from year to year. Overall, the percentage of URIs associated with HMPV was lower than that observed with influenza virus, Parainfluenza viruses, adenovirus, and RSV. In one study, 50% of the children with HMPV associated URI were diagnosed with otitis media [47]. In another study, one-third of children with HMPV-associate LRTI were diagnosed with concomitant acute OM [48].

6.3 Wheezing and Asthma

A causative role of respiratory viruses in the initiation and **p**rogression of asthma remains a controversial issue. However, It is fairly well established that common respiratory viruses, Such as rhinoviruses, can cause wheezing in some children and That infection with these viruses may induce exacerbations of Reactive airways disease or asthma. Investigation of the Inflammatory mediators and cytokines induced by HMPV during infection should shed light on the mechanisms of HMPV-Induced wheezing. Whether these potential mechanisms of wheezing are specific for HMPV or a more general response to a wide variety of respiratory pathogens remains to be determined[49].

6.4 HMPV in the Adult Population

HMPV has been associated with ILI, bronchitis, pneumonia, and exacerbations of both asthma and chronic obstructive pulmonary disease (COPD) in adults[50]. The major risk factor for HMPV-associated respiratory tract disease in adults without COPD is advanced age and underlying cardiopulmonary disease[51]. The role of HMPV in exacerbations of COPD is less clear. HMPV has been detected in individuals with acute exacerbations of COPD. However, the duration of viral shedding in individuals with COPD is not established, and therefore a causal role may be difficult to define. Further investigations are needed to define the role of HMPV in COPD[52,53].

6.5 Infection in the Immunocompromised Host

In one study of lung transplant recipients, 9 of 25 Individuals who were screened during a 1-year period tested Positive for HMPV at least one time. Several fatalities were Reported. It is difficult to determine the clinical features of HMPV disease in these individuals, as many of them had concurrent bacterial or fungal infections. HMPV was reported in an immunocompromised child, who had two HMPV infections with two different strains of the virus during a 10-month period . HMPV has been detected in children with human immunodeficiency virus(HIV) infection, though because of the small numbers of children in the study, it is difficult to determine whether HMPV-associated disease was more or less severe in HIV-infected children than in non-HIV-infected children[54].

7. Diagnosis

The standard method for HMPV diagnosis has been nucleic acid Amplification tests, such as RT-PCR[55,56,57] Early diagnosis of HMPV infection will help to develop effective measures against the disease, such as limiting the outbreak and providing timely care for the patients. Since the highly conserved F protein amino acid sequences of HMPV and RSV, limited serological technologies were developed for detecting HMPV specifc antibodies. Thus a variety of molecular diagnosis methods, probing viral nucleic

acids, have been invented for HMPV molecular detection, which mainly include the reverse transcription polymerase chain reaction (RT-PCR), real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR),and reverse transcription loop-mediated isothermal amplifcation (RT-LAMP)[58,59,60].

Several animal species have been determined to be permissive for HMPV infection. These Models, which are described in more detail Below, include small animals such as mice,Cotton rats, hamsters, guinea pigs, ferets, and primates, including chimpanzees, Rhesus macaques and African green monkeys. In most of the susceptible animals HMPV replicates to high titers and induces high levels of virus-neutralizing antibodies in the serum. Although HMPV Infection in most of these models does not mimic the signs of human disease, experimental animal infections appear to be Extremely useful for investigation of many Characteristics of HMPV infection, including pathogenesis and antiviral immunity. Thereby the establishment of animal models of HMPV infection will also facilitate Studies of both innate and adaptive immune Responses, the characteristics of which are not very well understood[61,62,63].

7.1 RT-PCR

RT-PCR is commonly used to detect HMPV. This technique involves the reverse transcription of RNA into complementary DNA (cDNA) and the amplification of specific DNA targets using PCR. Simultaneous performance of RT-PCR and real-time PCR, which enables combined nucleic acid amplification and detection in a single step, is routinely used to detect viral RNA[64]. Nucleic acid extraction and purification are basic steps used for most RT-PCR systems. The sample concentration can be measured to improve the clinical sensitivity. These procedures are automated in numerous laboratories for high-quality and reproducible results.. Including control material is necessary to validate the extraction and purification steps. Primers and probes enabling amplification and detection of viral targets have been reported[65,66,67]. Primers against conserved regions have been preferred for reliable amplification or for identifying sequence variants. However, these primers can cause decreased amplification efficiency when homologous primers incorporate into products. Several HMPV genes such as P, M, F, or N have served as targets for RT-PCR. Among them, the N gene is most conserved and has shown high sensitivity and reliability for all four genotypes; thus, it has been widely targeted in RT-PCR assay[68,69,70]. The PCR thermal cycling programs consist of three steps (denaturation, annealing, and elongation) and are slightly varied, according to previous reports for HMPV[71]. Quantification can be performed by RT-PCR as either a one-step or a two-step reaction. One of the main differences between these two procedures is the number of used tubes. For one-step RT-PCR, most processes going from the reverse transcriptase reaction to PCR amplification are conducted in a single tube. However, cDNA synthesis and PCR amplification occur in separate tubes in two-step reactions. The advantage of using a one-step protocol is that it minimizes experimental variation. However, using RNA (which is prone to degradation) as the starting templates for one-step reactions makes it difficult to repeatedly assay the same samples over a period of time. In addition, one-step protocols are reported to be less sensitive than two-step protocols. Two-step reaction show high reproducibility and it is possible to perform several different PCR assays after diluting a single cDNA sample .Although two-step protocols are vulnerable to DNA contamination, they have been the preferred methods when using DNA-binding dyes such as SYBR Green. Primer dimers can be easily eliminated by manipulating the melting temperatures[72,73,74].

7.2 Animal models

Although early studies demonstrated that HMPV does not Replicate or cause disease in birds, small animal models such as Mice, cotton rats, and hamsters as well as non-human primates are semi-permissive40.

Several studies show that cotton rats are the most permissive small animal model and that peak virus titers occurred at day four post infection[75]. Viral lung replication and disease vary between different inbred mouse strains; most work has been published in the BALB/c model, which exhibits substantial disease symptoms. BALB/c and C57BL/6 mice may exhibit clinical symptoms such as difficulty breathing, weight loss, and ruffled fur, partly depending on the virus strain and inoculum. Histological scoring revealed that lung pathology is most severe between days 5 and 7 but is significantly decreased by day 14. Viral replication occurs for up to 10–14 days in mice, and peak viral load is at day 5. Similar to older humans, aged mice have increased disease severity, higher viral titers, and diminished immune response compared with younger mice. However, unlike humans, in whom re-infection occurs throughout life, immunocompetent mice cannot be productively re-infected with HMPV. Of note, most work has been published in the BALB/c inbred strain, based on the extensive body of RSV research, but some investigators have focused on the C57BL/6 model[76,77]. In contrast, cotton rats, hamsters, and ferrets infected with HMPV do not manifest observable clinical symptoms. In hamsters and ferrets, there is high viral replication in the respiratory tract compared with mice. Both African green monkeys and rhesus macaques are permissive for HMPV infection, but neither exhibits clinical symptoms. HMPV replication and neutralizing antibody production are higher in African green monkeys compared with rhesus macaque[78].

8. Pharmaceutical Treatment:

8.1 Antivirals

Treatment consists of supportive care as there are no licensed Antivirals against HMPV. Two potential treatments that have been investigated are ribavirin and immunoglobulin. Ribavarin is a nucleoside with activity against RNA viruses and exhibits In vitro activity against HMPV and exhibited some efficacy in Mice. Commercial intravenous immunoglobulin (IVIG) contains neutralizing activity against HMPV and as noted above, Antibodies alone exhibit efficacy both prophylactically and Therapeutically in mice. There are anecdotal reports of human use of ribavirin and IVIG121 but no controlled trials and No guidelines to recommend the use of these measures[79,80,81]. Other compounds, such as NMSO3, a sulfated sialyl lipid that has been shown have potent antiviral activity against RSV in tissue culture cells, have been shown to have anti-HMPV activity in vitro. 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[82].

8.2 Vaccine development

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 [83].

There have been several promising live-attenuated vaccines. A Cold-adapted, live-attenuated HMPV vaccine provided complete Protection in hamsters. While antibody levels were increased After immunization in cynomolgus macaques, immunization did not provide complete protection from viral replication after Challenge. Recombinant HMPV (rHMPV) viruses lacking the G, M2-1, M2-2, or SH protein have exhibited an attenuated and Immunogenic phenotype in animal models. Mutations in The

methyl transferase domains of the polymerase or the integrin-Binding RGD motif of the F protein were attenuated, immunogenic, and protective in cotton rat[84]. Vectored vaccine approaches that have been effective in animal models include chimeric rHMPV containing the avian metapneumovirus P protein1, alphavirus-vectored HMPV F bovine PIV3 vectored F, or Sendai virus vectored . The establishment of a human challenge model and a successful test of a live-attenuated candidate in seropositive adults provides a platform for future clinical trials[85]. Another method of vaccination is with heat-killed or formalin-inactivated virus, but a major concern for non-replicating HMPV vaccines is the experience in the 1960s with formalin-inactivated RSV (FI-RSV) vaccines. FI-RSV induced an aberrant immune response that failed to protect and led to enhanced respiratory disease in vaccines upon natural RSV infection. Animal studies replicated the results of the FI-RSV clinical trials. Similar to FI-RSV, FI-HMPV and heat-inactivated HMPV vaccines in mice, cotton rats, and macaques led to enhanced disease following viral infection and to high mortality and increased levels of cytokines and lung inflammation[86,87,88].

9. Conclusion

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. 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. In clinical laboratories, The main methods used for HMPV detection are PCR Based assays including conventional RT-PCR, multiplex RT-PCR, and microarray-based approaches. Robust animal models have been established, and candidate vaccines and antibodies have been developed. However, there is still much in the field regarding pathogenesis, immunity, antivirals, and vaccines that is yet to be discovered. The human respiratory system is a playground for HMPV. Ultimately, it may take a combination a directly acting antiviral agent and a host response-modulating drug to control severe disease caused by HMPV.

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