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AN OVERVIEW OF THE HUMAN METAPNEUMOVIRUS AS A SIGNIFICANT RESPIRATORY PATHOGEN

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ABSTRACT

A serious respiratory infection, human metapneumovirus (hMPV) was discovered for the first time in the Netherlands in 2001. The genus Metapneumovirus, subfamily Pneumovirinae, and family Paramyxoviridae are its members. In all age groups, but especially in small children, the elderly, and people with impaired immune systems, hMPV is a major contributor to respiratory tract infections. It shares a strong relationship with the respiratory syncytial virus (RSV). hMPV is found all over the world and causes a significant number of respiratory diseases. Usually occurring in late winter or early spring, seasonal outbreaks coexist with other respiratory viruses such as influenza and RSV. By the age of five, practically all children have been exposed to hMPV, and reinfections are frequent throughout life. From minor upper respiratory tract infections to serious lower respiratory tract diseases, hMPV can cause a wide range of respiratory ailments. The respiratory tract's epithelial cells are the main target of hMPV infection. It triggers an inflammatory reaction marked by the production of cytokines and the infiltration of immune cells. Similar to RSV, it can cause mucus formation and epithelium damage, which can clog airways. The virus is a target for vaccine development because it uses the fusion (F) protein for viral entrance and cell-to-cell dissemination while lacking hemagglutinin and neuraminidase. A major respiratory pathogen that causes acute respiratory infections all throughout the world is the human metapneumovirus. Despite having many

clinical and epidemiological similarities to RSV, more study is necessary to create efficient therapies and vaccines that will lessen the disease's impact on public health.

KEYWORDS: Human metapneumovirus, Respiratory diseases, Viral pneumonia, Clinical features of hMPV, hMPV Diagnosis.

INTRODUCTION

Childhood mortality is primarily caused by acute respiratory infections (ARI). Setting health care priorities requires estimates of the number of children who die from ARI globally. In order to demonstrate a correlation between ARI-related deaths and all-cause deaths in children under five, we show that the percentage of deaths directly related to ARI decreases from 23% to 18% and then 15% (95% CIs range from ±2% to ±3%) as the annual mortality rate for children under five drops from 50 to 20 and then to 10/1000. There is evidence that the use of verbal autopsy contributes significantly to the variation in estimates of ARI in children. According to this data, ARI killed 1·9 million (95% CI 1·6–2·2 million) children worldwide in 2000, with 70% of those deaths occurring in Africa and Southeast Asia.[1,2] Children in underdeveloped nations are more likely to get pneumonia (10–20% versus 3–4% in developed nations).[3,4] Despite the fact that upper respiratory tract infections are typically less severe, they nevertheless have a substantial negative impact on society due to missed work, missed school days, and increased medical expenses. Determining the etiological agents of these infections is crucial because of this. We have established the significance of recognized viral infections such as the coronavirus, rhinovirus, influenza virus, parainfluenza virus, and human respiratory syncytial virus (hRSV) via decades of study and epidemiological investigations. Nevertheless, a significant percentage of respiratory tract infections remain unattributable to any recognized pathogen in spite of these investigations.[1,5] Although hMPV mainly affects children, it can also infect adults and others with weakened immune systems. The sickness brought on by a hMPV infection might manifest clinically as anything from a minor upper respiratory tract infection to potentially fatal acute pneumonia and bronchiolitis. The Paramyxoviridae family, which is divided into the subfamilies Paramyxovirinae and Pneumovirinae, is a member of the order Mononegavirales. The two genera Pneumovirus and Metapneumovirus comprise the subfamily Pneumovirinae. hMPV belongs to the genus Metapneumovirus, whereas hRSV is classified under the genus Pneumovirus. Two genotypes of hMPV, A and B, have been identified by whole genome analysis. The attachment (G) and fusion (F) surface glycoprotein

sequence variability further subdivides these two genotypes into subgroups A1, A2, B1, and B2. A2a and A2b are the new divisions of subgroup A2.[6,7] There may be a new subgroup developing inside the A major subgroup since one study described a strain that is under main subgroup A but does not belong to subgroups A1 or A2.[8] Nevertheless, the majority of retrospective studies had drawbacks, such as being small, not include patients who tested positive for other viruses, providing only a cursory description of the disease's clinical characteristics, and lacking information on the severity of the illness and death rates. Furthermore, because these investigations lacked a control group, they were unable to distinguish between HMPV as a pathogenic or colonizing virus. More recently, Stockton et al. found HMPV RNA in 2.2% of 405 specimens from influenza-like illness patients who saw general practitioners in England, despite the fact that few swabs were taken from children under the age of five.[9] The development of reverse genetics platforms marked a significant advancement in the study of the molecular biology of hMPV, however a trustworthy vaccine to prevent hMPV infection is still lacking. Here, recent discoveries in hMPV molecular virology, diagnosis, and control methods are examined.

Molecular Virology

The size of the hMPV virion ranges from 150 nm to 600 nm, and it is pleomorphic. Similar to other Paramyxoviridae family members, hMPV has a similar genomic orientation. The structure of the avian pneumovirus (aMPV), especially type C, and hMPV are fairly similar. The genomes of hMPV and hRSV are very similar, with the exception of a few variations in the gene order and the hMPV genome's lack of non-structural genes. Both the NS1 and NS2 nonstructural proteins have been found to be strong multifunctional antagonists of the interferon (IFN) signaling pathways for hRSV.[10] T he difference in the degree of host innate immune response seen during hRSV and hMPV infections may be due to the absence of these proteins.[10] The hMPV genome has eight genes that code for nine proteins and is made up of negative-sense single-stranded RNA. From 30 until the end of the genome, the genes are arranged as follows: N–P–M–F–M2–SH–G–L. These proteins include the viral polymerase (L protein), the attached glycoprotein (G protein), the small hydrophobic glycoprotein (SH protein), the phosphoprotein (P protein), the matrix protein (M protein), the fusion glycoprotein (F protein), the putative transcription factor (M2-1 protein), the RNA synthesis regulatory factor (M2-2 protein), and the nucleoprotein (N protein).[12] The lipid envelope envelops the RNA core, which is encircled by the M protein. The F, SH, and G surface glycoproteins are present in this envelope as spikes that range in size from 13 to 17 nm. A

nucleocapsid with a diameter of 17 nm is formed by the core nucleic acids, which are linked to the P, N, L, M2-1, and M2-2 proteins. Heparan sulphate receptors on the cell surface are where hMPV binds and fuses with the aid of the G and F proteins. Following the fusion process, the viral nucleocapsid replicates by entering the host cell's cytoplasm. The freshly created viral genome travels toward the host cell membrane after joining the viral P, N, L, and M2 proteins. The F, SH, and G proteins are now visible on the membrane's outer side when the virion sprouts out of the cell.[13,14] During virus replication, the P protein stabilizes the L protein, enabling the creation of the virus ribonucleoprotein (RNP) complex. Through its interactions with the RNP complex, the M protein is essential for virus assembly and budding. The viral genome is encapsulated by the N protein, which shields it from nuclease activity. By lowering the host's innate immunity, the M2-2 protein contributes significantly to virulence in addition to controlling viral transcription and replication.[15,16] Similar to other Paramyxoviridae family members, hMPV uses particular ways to impede the host's innate immune system. By controlling pattern recognition receptors, including toll-like receptors, retinoic acid-inducible gene-like receptors, and other signaling molecules, the virus counteracts cellular reactions. Infection decreases antigen-specific T cell activation and disrupts dendritic cell function. As a result, virus removal is still not complete, and the likelihood of re-infection rises.[17,18] 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 were shown[19,20] (Figure:1).

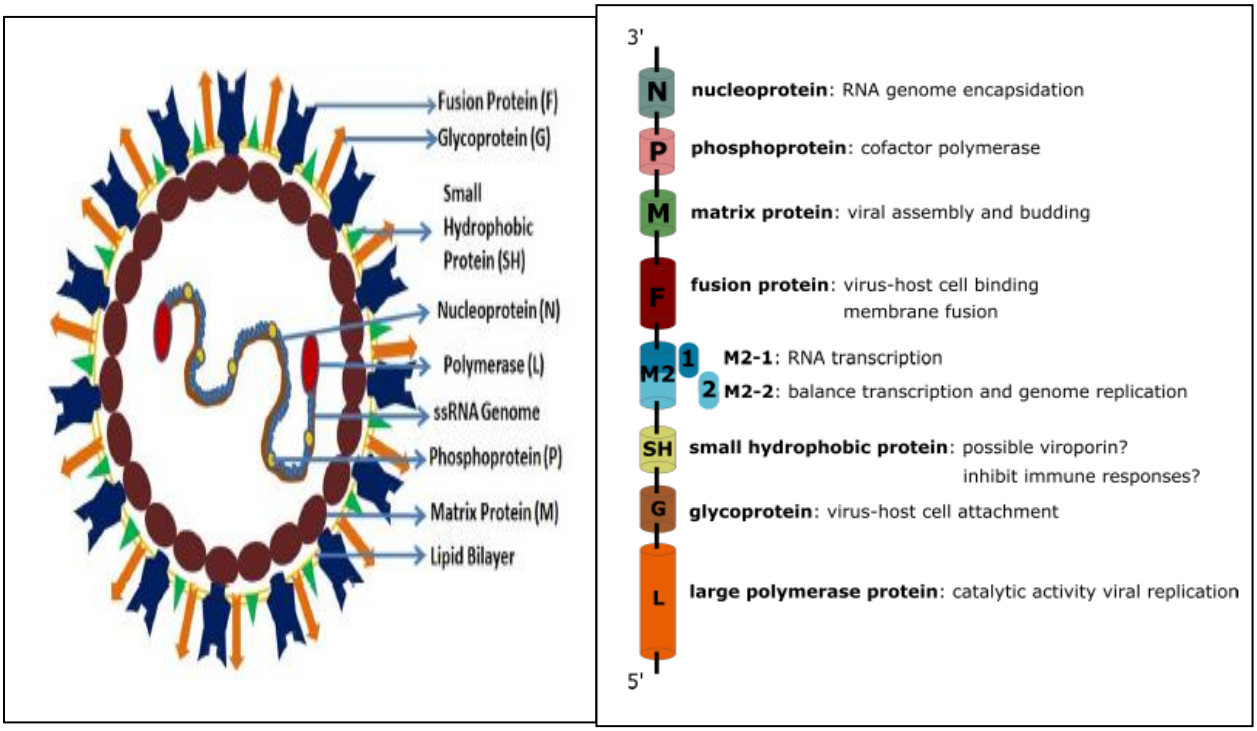

Figure 1: Schematic representation of the hMPV viral particle (A) and viral genome with encoded proteins (B):nucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), matrix-2 proteins (M2-1 and M2-2), small hydrophobic (SH) protein, glycoprotein (G), and large (L) polymerase protein.

Epidemiology and Clinical Features

hMPV exhibits a seasonal distribution and has been isolated on every continent. The spring and winter seasons—January to March in the northern hemisphere and June to July in the southern hemisphere—are when outbreaks generally happen.[21] According to a recent study, the RSV and influenza infection seasons are followed by March and April, when the peak of hMPV seasonal cases occurs.[22] The hMPV infection season and the RSV infection season coincide, according to another study.[23] According to global epidemiological research, the majority of children under the age of five will already have a hMPV infection. Ten percent of all hospitalized respiratory viral infections will be caused by hMPV, forty percent by hRSV, and five percent by adenoviruses. Between 4% and 5% of severe acute respiratory disorders in adults are brought on by hMPV.[24] The human respiratory tract's ciliated epithelial cells are the specific target of hMPV. The virus takes four to six days to incubate, then it excretes over the course of five to two weeks.[25] In children, HMPV often causes 5–15% of hospitalizations for lower respiratory tract infections, and its clinical manifestations frequently mimic those of bronchiolitis.[26] Young adults who are infected typically just exhibit flu-like symptoms; however, older or immune compromised patients may experience

more serious infection. There have been reports of significant hMPV infection outbreaks in long-term care facilities, with case fatality rates close to 10%. Interestingly, it has also been discovered that older mice have significantly more clinical severity.[27,28] Although children are diagnosed with hMPV more frequently, recurrences are more common in the elderly. This would imply that immune responses that are not fully protective and/or infection by distinct virus genotypes could be the cause of hMPV re-infection in adulthood. On the other hand, it has also been proposed that excessive levels of glycosylation of the G protein are the cause of the weakened immunological response.[29] Although asymptomatic children exhibited considerably lower virus loads than symptomatic children, hMPV was nevertheless detected by real-time RT-PCR in these children. Regardless of genotype, there was a strong correlation between higher hMPV viral loads and the severity of the sickness and the duration of illness.[30,31] Acute sickness was followed by one to two weeks of high hMPV virus shedding. A kid undergoing chemotherapy for acute lymphoblastic leukemia may be at risk for hMPV-associated deadly pneumonia.[32,33,34] A patient who received an allogeneic hematopoietic stem cell transplant and died from an infection that caused severe alveolar cell destruction along with interstitial and intra-alveolar pneumonitis. An increased risk of morbidity and death may be linked to hMPV infection in the first week following a hematopoietic stem cell transplant.[35,36] Lung transplant recipients may experience a variety of infections as a result of hMPV, ranging from a minor upper respiratory tract infection to a serious lower respiratory tract infection.[37] A modest to moderate increase in C-reactive protein (CRP) levels, decreased peripheral blood lymphocytes, and an elevated monocyte ratio were the hallmarks of the early phases of hMPV infection in a prospective trial involving patients with severe physical and intellectual impairments. As symptoms subsided, the ratio of peripheral blood lymphocytes to monocytes returned to normal, although the CRP levels remained elevated for a while. Some hospitalized children infected with hMPV were also observed to exhibit leukopenia and leukocytosis in addition to high serum CRP levels.[38,39]

Pathogenesis

A limited and delayed immune response, as well as delayed cytotoxic T cell activity and compromised viral clearance after primary infection, may be the cause of persistent hMPV infection.By infecting dendritic cells, hMPV prevents superantigen-induced T cell activation. As a result, the development of long-term immunity is hampered and the proliferation of antigen-specific CD4+ T cells is limited.[40,41] Cytokine responses are known to be modulated

by respiratory viruses. Several cytokines, including interleukin (IL)-12, tumor necrosis factor alpha (TNF-a), IL-6, IL-1b, IL-8, and IL-10, are less effectively induced by hMPV than by RSV and influenza.[42] BALB/c mice and cotton rats that are infected with hMPV experience pulmonary inflammatory changes, which raise the levels of interleukins (IL-2, IL-8, and IL-4), interferon (IFN-a), macrophage inflammatory protein 1a, and monocyte chemotactic proteins in the lungs and bronchoalveolar lavage fluid. Perivascular and peribronchiolar infiltration and inflammation are further consequences of these alterations.[43,44] Immunological and histological studies reveal the development of intra-alveolar foamy and hemosiderin-loaded macrophages, smudge cells, alveolar damage, and hyaline membrane disease. Cellular signaling that is dependent on toll-like receptors is known to be induced by hMPV infection. It is uncertain, therefore, how toll-like receptor-mediated signaling contributes to the host's defense against pulmonary hMPV infection and pathogenesis. A recent study found that following intranasal infection with hMPV, MyD88-deficient mice exhibited considerably lower levels of pulmonary inflammation and related illness in comparison to wild-type C57BL/6 mice.[45] Figure 4 depicts the molecular processes involved in the etiology of hMPV. As of yet, it is unclear if hMPV is confined to the respiratory system during infection or if it can spread throughout the body. Although further research is required, there is some indication that the latter is feasible. For example, one study found hMPV in middle ear fluid, and another found hMPV RNA in the brain tissue of a patient who passed away from encephalitis[figure 2].[46]

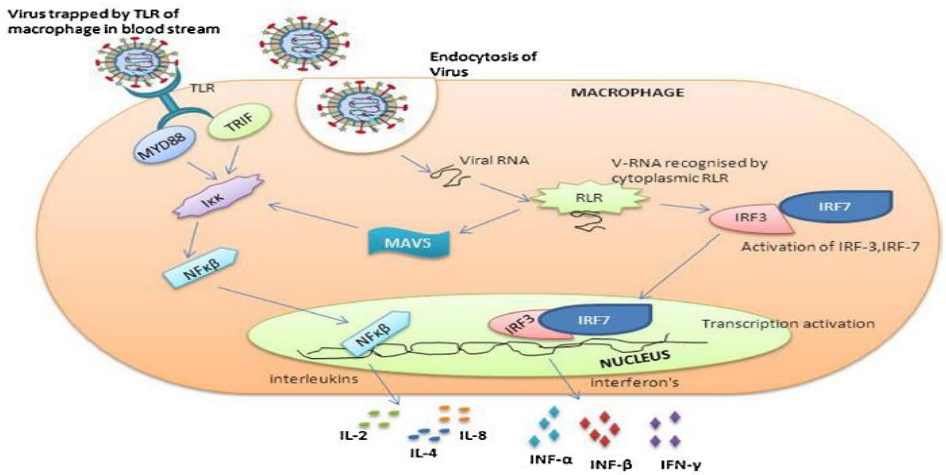

Figure 2: Molecular processes in the hMPV infection pathogenesis. TRF and MYD88 are two immune system adaptor molecules that are activated when a virus attaches to the toll-like receptors (TLR) of macrophages and/or dendritic cells. This, in turn, activates nuclear factor kappa beta (NFkb). When the cytoplasmic RIG1-like receptor

(RLR) detects the RNA of an internalized virus, it activates the transcription activators interferon regulatory factors 3 and 7 (IRF-3 and IRF-7) and mitochondrial antiviral signaling protein (MAVS), which in turn activates NFkb. Lastly, the production of several interleukins and interferons is induced by NFkb and IRFs.[46]

Diagnosis

The African green monkey kidney cell line (Vero) has since been used to produce hMPV, which was initially isolated and grown on a rhesus monkey cell line (LLC-MK2). Certain hMPV strains can be supported in their reproduction by other cells (Hep2 and HepG2).[14] It has a long and challenging growth period in cell culture, and its cytopathic effects (CPEs) can vary. The rounding of infected cells, their subsequent separation from the cell culture matrix, and sporadically the appearance of tiny syncytia—which often do not show up before the second week of culture in LLC-MK2 cells—are the hallmarks of hMPV-induced CPE.[47] The comparatively late finding of hMPV can be partially explained by the common requirement for early blind passages before such virological traits can be recognized. It is also possible to employ quicker diagnostic methods like ELISA-based antigen detection or direct immunofluorescence (DFA).[48,49] Upon entering the cytoplasm, proteins P, N, and L separate from the viral RNA and subsequently bond to one another to form the polymerase complex. Therefore, in the cytoplasm of infected cells, the genomic RNA can act as a matrix for viral transcription and replication.[50] At the cell membrane surface, the freshly generated proteins P, N, L, and M2 combine with neosynthesized viral genomes to create new nucleocapsids, which are then integrated into the virions during budding. The envelope glycoproteins (F, G, and SH) travel to these areas of membranous accumulation through the golgi apparatus and join them to become visible on the surface of the infected cells. Viral-induced syncytia development has also been used to track the infection's progression in cell culture.[51,52] By this mechanism Viral genomes can spread when infected cells combine with neighboring cells through the action of viral fusion proteins that are visible on their surface.

Treatment and Control

The majority of hMPV infection therapies now on the market are supportive. However, several studies have suggested that ribavirin, immunoglobulin, fusion inhibitors, and tiny interfering ribonucleic acids could be used to treat and manage hMPV infection.[2] Various approaches taken to treat hMPV infection. Numerous hMPV vaccination candidates have been tested in non-human primate and rodent models. None have been tested on human

volunteers yet, despite their encouraging results. There might be issues: when tested in mice, a heat-inactivated viral vaccination against hMPV exacerbated pulmonary illness.[53] Immunomodulation by hMPV challenge has been demonstrated to be decreased by T cell epitope vaccinations. Following a hMPV challenge, mice inoculated with a hMPV cytotoxic T cell epitope vaccine generated fewer Th1 and Th2 type cytokines than mice who were not immunized. A few studies have also assessed the effectiveness of chimeric vaccines in preventing hMPV infection. Chimeric vaccines for hMPV have been demonstrated to produce neutralizing antibodies and provide protection against a challenge with the wildtype in hamsters and African green monkeys.[54,55] It has been demonstrated that a subunit vaccination that uses the hMPV fusion protein can give hamsters cross-protective immunity against hMPV challenge. When tested on rats, hamsters, and non-human primates, a number of hMPV F subunit vaccines have demonstrated high levels of protection.[2,56] A recent study examined hMPV virus-like particles (VLPs) as a potential vaccination candidate by simulating the characteristics of the viral surface of both subgroups A and B. These VLPs demonstrated the ability to elicit a robust humoral immune response in mice against both homologous and heterologous pathogens. Even though a hMPV-VLP vaccine appears to be a promising strategy, further study is necessary to create a vaccine that will work against every hMPV subgroup. The development of plasmid-based reverse genetics techniques has greatly accelerated the search for a live vaccination to prevent hMPV infection.[57,58] The virus replication levels of recombinant hMPVs with deletions in the SH, G, or M2-2 genes have been assessed, and it has been demonstrated that the deletion of these genes has no effect on the virus's immunogenicity or antigenicity. A live attenuated vaccine strain of hMPV was created in a recent study by altering the F protein's glycosylation location. Even with a challenge 56 days after inoculation, this vaccine was found to provide full protection against homologous virus challenge and some protection against heterologous viral challenge.[59,60] All of these results point to the need for a more thorough understanding of the molecular pathophysiology of hMPV before an effective vaccine against it can be created.

CONCLUSIONS

Despite being a relatively new virus, human metapneumovirus (hMPV) seems to be just as harmful as hRSV in terms of morbidity and mortality. Knowing the pathophysiology of hMPV and the molecular limitations causing severe disease is crucial for both treating infections and creating an efficient vaccine against this significant respiratory virus. We can now access live vaccination candidates and gain some insight into hMPV pathogenesis thanks

to recent research employing reverse genetics technologies and animal models for hMPV infection. We must now start clinical trials to assess the various hMPV infection therapy techniques.

DECLARATIONS

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Author's Contribution

Mir Irfan Soyel and Malay Besra Design this study, conducted the data analysis and prepares manuscript.

Biplab Kumar Chakra have done Proof reading.

Sumit Maji, Mousumi Das, Deepayan Kar, Monalisa Malakar, Manonayan Singha, Nilanjan Adhikari, Dipyaman Chattaraj, Shyam Sundar Kundu have supply resource compilations.

All authors read and approved the final manuscript.

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Data availability

The data that support the findings of this study are available from the corresponding author, Mir Irfan Soyel, upon reasonable request.

Competing Interests

The authors declare that they have no competing interests.

REFERENCE

1. Williams BG, Gouws E, Boschi-Pinto C, Bryce J, Dye C. Estimates of world-wide distribution of child deaths from acute respiratory infections. The Lancet infectious diseases. 2002 Jan 1; 2(1): 25-32.
2. Panda S, Mohakud NK, Pena L, Kumar S. Human metapneumovirus: review of an important respiratory pathogen. International journal of infectious diseases. 2014 Aug 1; 25: 45-52.
3. Shapiro ED. Epidemiology of acute respiratory infections. InSeminars in Pediatric Infectious Diseases 1998 Jan 1 (Vol. 9, No. 1, pp. 31-36). WB Saunders.

4. Campbell H. Acute respiratory infection: a global challenge. Archives of disease in childhood. 1995 Oct; 73(4): 281.
5. Van den Hoogen BG, de Jong JC, Groen J, Kuiken T, de Groot R, Fouchier RA, Osterhaus AD. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nature medicine. 2001 Jun; 7(6): 719-24.
6. Van den Hoogen BG, Herfst S, Sprong L, Cane PA, Forleo-Neto E, De Swart RL, Osterhaus AD, Fouchier RA. Antigenic and genetic variability of human metapneumoviruses. Emerging infectious diseases. 2004 Apr; 10(4): 658.
7. Williams JV, Harris PA, Tollefson SJ, Halburnt-Rush LL, Pingsterhaus JM, Edwards KM, Wright PF, Crowe Jr JE. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. New England Journal of Medicine. 2004 Jan 29; 350(5): 443-50.
8. Boivin G, Mackay I, Sloots TP, Madhi S, Freymuth F, Wolf D, Shemer-Avni Y, Ludewick H, Gray GC, LeBlanc É. Global genetic diversity of human metapneumovirus fusion gene. Emerging infectious diseases. 2004 Jun; 10(6): 1154.
9. Stockton J, Stephenson I, Fleming D, Zambon M. Human metapneumovirus as a cause of community-acquired respiratory illness. Emerging infectious diseases. 2002 Sep; 8(9): 897.
10. Lo MS, Brazas RM, Holtzman MJ. Respiratory syncytial virus nonstructural proteins NS1 and NS2 mediate inhibition of Stat2 expression and alpha/beta interferon responsiveness. Journal of virology. 2005 Jul; 79(14): 9315-9.
11. Ditt V, Lüsebrink J, Tillmann RL, Schildgen V, Schildgen O. Respiratory infections by HMPV and RSV are clinically indistinguishable but induce different host response in aged individuals. PloS one. 2011 Jan 26; 6(1): e16314.
12. Biacchesi S, Murphy BR, Collins PL, Buchholz UJ. Frequent frameshift and point mutations in the SH gene of human metapneumovirus passaged in vitro. Journal of virology. 2007 Jun 1; 81(11): 6057-67.
13. Chang A, Masante C, Buchholz UJ, Dutch RE. Human metapneumovirus (HMPV) binding and infection are mediated by interactions between the HMPV fusion protein and heparan sulfate. Journal of virology. 2012 Mar 15; 86(6): 3230-43.
14. Feuillet F, Lina B, Rosa-Calatrava M, Boivin G. Ten years of human metapneumovirus research. Journal of clinical virology. 2012 Feb 1; 53(2): 97-105.

15. Ren J, Wang Q, Kolli D, Prusak DJ, Tseng CT, Chen ZJ, Li K, Wood TG, Bao X. Human metapneumovirus M2-2 protein inhibits innate cellular signaling by targeting MAVS. Journal of virology. 2012 Dec 1; 86(23): 13049-61.
16. Schickli JH, Kaur J, MacPhail M, Guzzetta JM, Spaete RR, Tang RS. Deletion of human metapneumovirus M2-2 increases mutation frequency and attenuates growth in hamsters. Virology journal. 2008 Dec; 5: 1-4.
17. Céspedes PF, Gonzalez PA, Kalergis AM. Human metapneumovirus keeps dendritic cells from priming antigen-specific naive T cells. Immunology. 2013 Jul; 139(3): 366-76.
18. Kolli D, Bao X, Casola A. Human metapneumovirus antagonism of innate immune responses. Viruses. 2012 Dec 7; 4(12): 3551-71.
19. Kumar P, Srivastava M. Prophylactic and therapeutic approaches for human metapneumovirus. Virusdisease. 2018 Dec; 29(4): 434-44.
20. Ballegeer M, Saelens X. Cell-Mediated Responses to Human Metapneumovirus Infection. Viruses. 2020 May 14; 12(5): 542.
21. Pilger DA, Cantarelli VV, Amantea SL, Leistner-Segal S. Detection of human bocavirus and human metapneumovirus by real-time PCR from patients with respiratory symptoms in Southern Brazil. Memorias do Instituto Oswaldo Cruz. 2011; 106: 56-60.
22. Mizuta K, Abiko C, Aoki Y, Ikeda T, Matsuzaki Y, Itagaki T, Katsushima F, Katsushima Y, Noda M, Kimura H, Ahiko T. Seasonal patterns of respiratory syncytial virus, influenza A virus, human metapneumovirus, and parainfluenza virus type 3 infections on the basis of virus isolation data between 2004 and 2011 in Yamagata, Japan. Japanese journal of infectious diseases. 2013; 66(2): 140-5.
23. Chan PC, Wang CY, Wu PS, Chang PY, Yang TT, Chiang YP, Kao CL, Chang LY, Lu CY, Lee PI, Chen JM. Detection of human metapneumovirus in hospitalized children with acute respiratory tract infection using real-time RT-PCR in a hospital in northern Taiwan. Journal of the Formosan Medical Association. 2007 Jan 1; 106(1): 16-24.
24. Freymuth F, Vabret A, Legrand L, Lebon P, Bach N, Brouard J, Guillois B. Le métapneumovirus humain. Virologie. 2004 Nov 1; 8(6): 413-23.
25. Louie JK, Schnurr DP, Pan CY, Kiang D, Carter C, Tougaw S, Ventura J, Norman A, Belmusto V, Rosenberg J, Trochet G. A summer outbreak of human metapneumovirus infection in a long-term-care facility. The Journal of infectious diseases. 2007 Sep 1; 196(5): 705-8.
26. Papenburg J, Boivin G. The distinguishing features of human metapneumovirus and respiratory syncytial virus. Reviews in medical virology. 2010 Jul; 20(4): 245-60.

27. Boivin G, Serres GD, Hamelin ME, Côté S, Argouin M, Tremblay G, Maranda-Aubut R, Sauvageau C, Ouakki M, Boulianne N, Couture C. An outbreak of severe respiratory tract infection due to human metapneumovirus in a long-term care facility. Clinical Infectious Diseases. 2007 May 1; 44(9): 1152-8.
28. Darniot M, Pitoiset C, Petrella T, Aho S, Pothier P, Manoha C. Age-associated aggravation of clinical disease after primary metapneumovirus infection of BALB/c mice. Journal of virology. 2009 Apr 1; 83(7): 3323-32.
29. de Graaf M. Metapneumovirus: determinants of host range and replication. Erasmus MC: University Medical Center Rotterdam; 2009 Jan 15.
30. Bosis S, Esposito S, Osterhaus AD, Tremolati E, Begliatti E, Tagliabue C, Corti F, Principi N, Niesters HG. Association between high nasopharyngeal viral load and disease severity in children with human metapneumovirus infection. Journal of clinical virology. 2008 Jul 1; 42(3): 286-90.
31. Peng D, Zhao X, Liu E, Huang Y, Yang X, Zhao Y, Chen X, Zhang Z. Analysis of viral load in children infected with human metapneumovirus. Iranian journal of pediatrics. 2010 Dec; 20(4): 393.
32. Talaat KR, Karron RA, Thumar B, McMahon BA, Schmidt AC, Collins PL, Buchholz UJ. Experimental infection of adults with recombinant wild-type human metapneumovirus. The Journal of infectious diseases. 2013 Nov 15; 208(10): 1669-78.
33. Englund JA, Boeckh M, Kuypers J, Nichols WG, Hackman RC, Morrow RA, Fredricks DN, Corey L. Brief communication: fatal human metapneumovirus infection in stem-cell transplant recipients. Annals of internal medicine. 2006 Mar 7; 144(5): 344-9.
34. Pelletier G, Déry P, Abed Y, Boivin G. Respiratory tract reinfections by the new human metapneumovirus in an immunocompromised child. Emerging infectious diseases. 2002 Sep; 8(9): 976.
35. Englund JA, Boeckh M, Kuypers J, Nichols WG, Hackman RC, Morrow RA, Fredricks DN, Corey L. Brief communication: fatal human metapneumovirus infection in stem-cell transplant recipients. Annals of internal medicine. 2006 Mar 7; 144(5): 344-9.
36. Dokos C, Masjosthusmann K, Rellensmann G, Werner C, Schuler-Lüttmann S, Müller KM, Schiborr M, Ehlert K, Groll AH. Fatal human metapneumovirus infection following allogeneic hematopoietic stem cell transplantation. Transplant Infectious Disease. 2013 Jun; 15(3): E97-101.
37. Hopkins MJ, Redmond C, Shaw JM, Hart IJ, Hart CA, Smyth RL, Semple MG. Detection and characterisation of human metapneumovirus from children with acute respiratory

symptoms in north-west England, UK. Journal of Clinical Virology. 2008 Jul 1; 42(3): 273-9.

38. van den Hoogen BG, van Doornum GJ, Fockens JC, Cornelissen JJ, Beyer WE, Groot RD, Osterhaus AD, Fouchier RA. Prevalence and clinical symptoms of human metapneumovirus infection in hospitalized patients. The Journal of infectious diseases. 2003 Nov 15; 188(10): 1571-7.
39. Wei HY, Tsao KC, Huang CG, Huang YC, Lin TY. Clinical features of different genotypes/genogroups of human metapneumovirus in hospitalized children. Journal of Microbiology, Immunology and Infection. 2013 Oct 1; 46(5): 352-7.
40. Alvarez R, Tripp RA. The immune response to human metapneumovirus is associated with aberrant immunity and impaired virus clearance in BALB/c mice. Journal of virology. 2005 May 15; 79(10): 5971-8.
41. Céspedes PF, Gonzalez PA, Kalergis AM. Human metapneumovirus keeps dendritic cells from priming antigen-specific naive T cells. Immunology. 2013 Jul; 139(3): 366-76.
42. Laham FR, Israele V, Casellas JM, Garcia AM, Lac Prugent CM, Hoffman SJ, Hauer D, Thumar B, Name MI, Pascual A, Taratutto N. Differential production of inflammatory cytokines in primary infection with human metapneumovirus and with other common respiratory viruses of infancy. The Journal of infectious diseases. 2004 Jun 1; 189(11): 2047-56.
43. Baños-Lara MD, Ghosh A, Guerrero-Plata A. Critical role of MDA5 in the interferon response induced by human metapneumovirus infection in dendritic cells and in vivo. Journal of virology. 2013 Jan 15; 87(2): 1242-51.
44. Williams JV, Martino R, Rabella N, Otegui M, Parody R, Heck JM, Crowe Jr JE. A prospective study comparing human metapneumovirus with other respiratory viruses in adults with hematologic malignancies and respiratory tract infections. The Journal of infectious diseases. 2005 Sep 15; 192(6): 1061-5.
45. Ren J, Kolli D, Deng J, Fang R, Gong B, Xue M, Casola A, Garafalo RP, Wang T, Bao X. MyD88 controls human metapneumovirus-induced pulmonary immune responses and disease pathogenesis. Virus research. 2013 Sep 1; 176(1-2): 241-50.
46. Schildgen O, Glatzel T, Geikowski T, Scheibner B, Simon A, Bindl L, Born M, Viazov S, Wilkesmann A, Knöpfle G, Roggendorf M. Human metapneumovirus RNA in encephalitis patient. Emerging Infectious Diseases. 2005 Mar; 11(3): 467.
47. Boivin G, Abed Y, Pelletier G, Ruel L, Moisan D, Côté S, Peret TC, Erdman DD, Anderson LJ. Virological features and clinical manifestations associated with human

metapneumovirus: a new paramyxovirus responsible for acute respiratory-tract infections in all age groups. The Journal of infectious diseases. 2002 Nov 1; 186(9): 1330-4.

48. Kukavica-Ibrulj I, Boivin G. Detection of human metapneumovirus antigens in nasopharyngeal aspirates using an enzyme immunoassay. Journal of Clinical Virology. 2009 Jan 1; 44(1): 88-90.
49. Jun KR, Woo YD, Sung H, Kim MN. Detection of human metapneumovirus by direct antigen test and shell vial cultures using immunofluorescent antibody staining. Journal of virological methods. 2008 Sep 1; 152(1-2): 109-11.
50. Easton AJ, Domachowske JB, Rosenberg HF. Animal pneumoviruses: molecular genetics and pathogenesis. Clinical Microbiology Reviews. 2004 Apr; 17(2): 390-412.
51. Mackay IM, Jacob KC, Woolhouse D, Waller K, Syrmis MW, Whiley DM, Siebert DJ, Nissen M, Sloots TP. Molecular assays for detection of human metapneumovirus. Journal of clinical microbiology. 2003 Jan; 41(1): 100-5.
52. Vargas SO, Kozakewich HP, Perez-Atayde AR, McAdam AJ. Pathology of human metapneumovirus infection: insights into the pathogenesis of a newly identified respiratory virus. Pediatric and Developmental Pathology. 2004 Sep; 7(5): 478-86.
53. Hamelin MÈ, Couture C, Sackett MK, Boivin G. Enhanced lung disease and Th2 response following human metapneumovirus infection in mice immunized with the inactivated virus. Journal of general virology. 2007 Dec; 88(12): 3391-400.
54. Herd KA, Mahalingam S, Mackay IM, Nissen M, Sloots TP, Tindle RW. Cytotoxic T-lymphocyte epitope vaccination protects against human metapneumovirus infection and disease in mice. Journal of virology. 2006 Feb 15; 80(4): 2034-44.
55. Tang RS, Schickli JH, MacPhail M, Fernandes F, Bicha L, Spaete J, Fouchier RA, Osterhaus AD, Spaete R, Haller AA. Effects of human metapneumovirus and respiratory syncytial virus antigen insertion in two 3′ proximal genome positions of bovine/human parainfluenza virus type 3 on virus replication and immunogenicity. Journal of virology. 2003 Oct 15; 77(20): 10819-28.
56. Herfst S, Schrauwen EJ, de Graaf M, van Amerongen G, van den Hoogen BG, de Swart RL, Osterhaus AD, Fouchier RA. Immunogenicity and efficacy of two candidate human metapneumovirus vaccines in cynomolgus macaques. Vaccine. 2008 Aug 5; 26(33): 4224-30.
57. Lévy C, Aerts L, Hamelin MÈ, Granier C, Szécsi J, Lavillette D, Boivin G, Cosset FL. Virus-like particle vaccine induces cross-protection against human metapneumovirus infections in mice. Vaccine. 2013 Jun 7; 31(25): 2778-85.

58. Smith KA. The use of plasmid-based reverse genetics to generate influenza virus strains for improved vaccine production. Warning: get_class () expects parameter 1 to be object, array given in/home/vhosts/ejournal/user-dir/htdocs/classes/cache/GenericCache. inc. php on line 63 MMG 445 Basic Biotechnology eJournal. 2007 May 11; 3(1): 123-30.
59. Biacchesi S, Pham QN, Skiadopoulos MH, Murphy BR, Collins PL, Buchholz UJ. Infection of nonhuman primates with recombinant human metapneumovirus lacking the SH, G, or M2-2 protein categorizes each as a nonessential accessory protein and identifies vaccine candidates. Journal of virology. 2005 Oct 1; 79(19): 12608-13.
60. Liu P, Shu Z, Qin X, Dou Y, Zhao Y, Zhao X. A live attenuated human metapneumovirus vaccine strain provides complete protection against homologous viral infection and cross-protection against heterologous viral infection in BALB/c mice. Clinical and Vaccine Immunology. 2013 Aug; 20(8): 1246-54.