Medical Science Journal for Advance Research

Vol. 7, No. 3, September 2026
e-ISSN: 2774-4892

Genotypic and Clinical Features of Human Metapneumovirus Infections in Hospitalized Children With Acute Respiratory Infections

Chang Guk Ri [1*], Yong Chan Sin [2], Hyok Kim [3], Yong Chol Pak [4], Yong Su Song [5], Sin Hyok Paek [6] , Mi Rae Han [7], Il Gyong Ri [7], Yon Hui Ri [7], Hyon Song Kang [7], and Song Min Jong [7]

[1-] *Institute for Infection Control, Faculty of Preventive Medicine, Pyongyang University of Medical Sciences, Democratic People's Republic of Korea.*

[2-] *Okryu Children's Hospital, Ministry of Public Health. Democratic People's Republic of Korea.*

[3-] *Department of Pediatrics, Faculty of Clinical Medicine, Pyongyang University of Medical Sciences, Democratic People's Republic of Korea*

[4-] *Institute of Medical Biology, Ministry for Medical Research, Ministry of Public Health. Democratic People's Republic of Korea*

[5-] *Scientific Research Department, Pyongyang University of Medical Sciences, Democratic People's Republic of Korea.*

[6-] *Department of Radiology, Faculty of Clinical Medicine, Pyongyang University of Medical Sciences, Democratic People's Republic of Korea.*

[7-] *Okryu Children's Hospital, Ministry of Public Health. Democratic People's Republic of Korea.*

***e-mail:** cy.kim1992@star-co.net.kp

ABSTRACT

Human metapneumovirus (hMPV) is a newly discovered emerging virus known to cause acute respiratory tract infections (ARTIs) in children and adults. In particular, hMPV is one of the main viral etiological agents that significantly contribute to development of severe respiratory diseases and pneumonia in children. ***Objective****: To illuminate the virological and clinical features in children with respiratory infections caused by hMPV.* ***Methods****: A total of 156 nasopharyngeal aspirate (NPA) samples collected from hospitalized children with acute respiratory tract infections were tested for hMPV by RT-PCR with specific primers to hMPV. In addition to, DNA sequence analysis and Phylogenetic analysis of the specific hMPV amplicons were performed.* ***Results****: In RT-PCR with primers targeting the hMPV fusion glycoprotein (F), nucleoprotein (N), and polymerase (L) genes, single and very sharp amplication bands of the expected length of 0.45Kb, 0.16Kb, and 0.17Kb revealized NPA samples. Overall, hMPV was detected in 8 (5.12%) of analized samples. DNA sequence analysis and Phylogenetic analysis of the specific amplicons of hMPV F, N, and L genes revealed that the virus detected by RT-PCR was hMPV A2 genotype with 83.5~100%, 85.7~100%, and 95.7~97.8% sequence similarity, respectively. The mean ages and mean treatment and hospitalization duration of hMPV positive children was 16.1 months and 6.5 days, respectively. The main symptoms of hMPV positive children were fever (100%), cough (87.5%), rhinorrhea (87.5%), sputum (62.5%), sore throat (62.5%), and dyspnea (37.5%), etc. Also, clinical diagnosis of hMPV positive children were pneumonia (50%), acute bronchiolitis (37.5%), and acute wheezing bronchitis (12.5%).* ***Conclusions:*** *The obtained result revealed that our study provides strong support that hMPV infection is an important cause of severe respiratory disease in children, including pneumonia, bronchiolitis, and acute wheezing attacks, strongly suggesting that hMPV A2 genotype is the major genotype to cause acute and severe respiratory disease associated with hMPV infection.*

Key words: *Human metapneumovirus (hMPV), Acute respiratory tract infections (ARTIs), Bronchiolitis, Wheezy bronchitis, Pneumonitis, RT-PCR, DNA sequence analysis, Phylogenetic analysis, Genotype, Clinical feature.*

Keywords:

Article Information
Received: April 18, 2026; Revised: June 23, 2026; Online: September 2026y

https://doi.org/10.46966/msjar.v7i3.427

Published by: READ INSTITUTE

1.INTRODUCTION

Human metapneumovirus (hMPV) was new emerging virus first isolated and identificated in 2001 by genetic analysis of nasopharyngeal aspirate samples taken from hospitalized children in the Netherlands, which is one of the major viral pathogens of acute respiratory tract infections(ARIs). (4, 16, 17, 20). Human metapneumovirus (hMPV) is virus belonging to the family Paramyxoviridae and genus Metapneumovirus, which has 1 serotype with 2 main genetic lineages, A and B, further them divided into 5 genotypes, including A1, A2a, A2b, B1, and B2, based mainly on variations in the G gene. (5, 12, 15). Human metapneumovirus (hMPV) is an enveloped single-stranded, negative-sense RNA virus with a genome of about 13.2kb, containing 8 genes encoding 9 proteins. (2, 19). hMPV spread worldwidely and generally causes several respiratory infections (1, 4, 11, 12, 15, 17). Many studies showed that hMPV infection occur in nearly most children by the age of 5 years and severe diseases predominantly occur in children below 2 years of age. (7, 11, 13, 14, 23)

Clinical presentation of hMPV infections range from mild upper respiratory tract illness to severe lower respiratory tract diseases needed hospitalization and mechanical ventilation. (16, 18, 24) hMPV generally causes upper respiratory tract symptoms such as cough, fever, sputum, rhinorrhea, dyspnea, sore throat, and wheezing, and lower respiratory tract infections, such as wheezy bronchitis, bronchitis, bronchiolitis, asthma, and pneumonia, in infants and very young children. Even hMPV infections occasionally can result in severe diseases such as bronchiolitis, pneumonitis, and asthma exacerbation including death. (4, 6, 13, 16) In addition, some studies showed that the several clinical characteristics of hMPV infection might be associated with specific hMPV genotype and that disease severity could be increased with specific hMPV subgroup infections. (1, 10, 21, 22)

In this study, we report that we illuminaed the genotypic and clinical features of hMPV infections detected in children hospitalized with various upper respiratory tract illness and severe lower respiratory tract diseases such as wheezy bronchitis, bronchiolitis, and pneumonia.

2. MATERIALS AND METHODS

2.1. Patients and Clinical Specimens

Between November 2024 and May 2025, NPA specimens (n=156) were collected from children (＜ 3 years of age) hospitalized with acute respiratory tract infections at the Okryu Children's Hospital, placed in a viral transfer medium, immediately transferred to the laboratory, and stored at -70℃ before testing.

2.2. Detection of hMPV by RT-PCR

RT-PCR for the detection of hMPV was performed using specific primers previously described targeting the fusion glycoprotein (F), nucleoprotein (N), and polymerase (L) genes of hMPV [3, 8, 9]. hMPV-1F (5’-TTTGGACTTAATGACAGATG-3’) and hMPV-1R (5’-TCTTCCTGTGCTAACTTTG-3’) primers were targeted to the F gene and the expected product sizes were 448 bp. hMPV-2F (5’-CATATAAGCATGCTATATTAAAAGAGTCTC-3’) and hMPV-2R (5’-CCTATTTCTGCAGCATATTTGTAATCAG-3’) primers were targeted to the N gene and the expected product sizes were 163 bp. hMPV-3F (5’-AAACTTTATGTAGTTGGGGATC-3’) and hMPV-3R (5’-AATTTTACATCATATAACATTAT-3’) primers were targeted to the L gene and the expected product sizes were 170 bp. The primers used for PCR were synthesized by Sangon, Biotech Co, Ltd, China.

Total viral nucleic acid was extracted from 140 μL of NPA specimens using a QIAamp Viral RNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. This kit is useful for the simultaneous extraction of viral RNA and DNA. Subsequently, we used the HotStart PCR kit (Qiagen, Germany) and one-step RT-PCR kit (Qiagen, Germany) to detect and screen the presence of hMPV and other 6 respiratory viruses, namely, human parainfluenza virus 1 (HPIV-1), human parainfluenza virus 3 (HPIV-3), influenza A viruses (A(H1N1) and A(H3N2)), human respiratory syncytial virus

(HRSV), and Human Bocavirus genotype 1 (HBoV1).

Briefly, each RT-PCR mix for hMPV comprised 4 µL of 5× QIAGEN RT-PCR buffer, 0.5 µL of forward and reverse primers (10 µM), 1 µL of Enzyme Mixture, 0.5 µL of dNTPs, 0.5 µL of RNase inhibitor, 2 µL of template RNA, and 11 µL of ddH_2O in a final volume of 20 µL. The RT-PCR conditions were 50°C for 30 minutes and 95°C for 15 minutes, followed by 40 cycles of 94°C for 30 seconds, 52°C for 30 seconds and 72°C for 1 minutes, followed by a final extension of 72°C for 5 min.

In addition to, each PCR mix was comprised 0.4 µM of each primer, 0.5 µL of 10 mM dNTPs, 2.0 µL of 10× Qiagen PCR buffer (Qiagen), 1.0 U of Qiagen HotStart Taq polymerase (Qiagen), and 2 µL of nucleic acid extract in a final volume of 20 µL. PCR was performed on an DNA Engine thermal cycler (Bio-Rad, Gladesville, Australia) with the following thermocycling conditions; a 12 min incubation at 95°C, followed by 40 cycles of 94°C for 30 sec, 52°C for 30 sec, and 72°C for 60 sec, followed by a final extension of 72°C for 5 min.

PCR and RT-PCR products were visualized by electrophoresis on 1.5% agarose gel with ethidium bromide staining.

2.3. DNA sequencing of RT-PCR product for hMPV and Phylogenetic analysis

The amplified products of hMPV F, N, and L genes were purified using a DNA Gel Extraction kit (MicroDNA Gel Extraction Kit, Sangon Biotech) and sequenced in forward and reverse directions using a BigDye terminator cycle sequencing ready reaction kit (Perkin-Elmer Applied Biosystems, Tokyo, Japan) with an ABI Prism 3500 Genetic Analyzer DNA sequencer (Perkin-Elmer Applied Biosystems). Nucleotide sequences of amplified hMPV F, N, and L gene products were aligned by using CLUSTAL W software. All nucleotide sequences were compared to those of reference strains available in the NCBI GenBank database using the BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic and molecular evolutionary analysis was performed by the neighbor-joining algorithm and Kimura two-parameter model, supported by bootstrapping with 1000 replicates in MEGA software (ver. 7.0). Phylogenetic trees of the partial hMPV F, N, and L genes were constructed by the clustal method using Megalign (Lacergene, DNA STAR, Inc., Madison, WI, USA).

2.4. Coinfections with other respiratory viruses

All NPA specimens with positive hMPV RNA in RT-PCRwere tested for coinfections with other respiratory viruses such as HPIV-1, HPIV-3, IFV-A(H1N1), IFV-A(H3N2), RSV, and HBoV1 using specific primers, respetively.

2.5. Analysis for Clinical features and outcomes of patients

Basic conditions and clinical features of the patients at admission such as sex, age, diagnosis and clinical severity in hospitalization, major symptoms and signs, body temperature, white blood count, chest X-ray imagings, result after treatment, and length of treatment and hospitalization were recorded and analyzed.

3. RESULTS AND DISCUSSIONS

3.1. Detection of hMPV

3.1.1. Detection of hMPV by fusion glycoprotein (F) gene specific primer

Firstly, we performed RT-PCR with primer pair targeting F gene of hMPV and nucleic acid extracts extracted from NPA specimens of patients with severe lower respiratory tract diseases such as wheezy bronchitis, bronchiolitis, and pneumonia as template (Fig. 1).

As a result, sharp amplication bands of the expected length of 0.45Kb revealized from 8 of 156 nucleic acid extracts extracted from NPA specimens of patients with acute respiratory tract infections (ARIs).

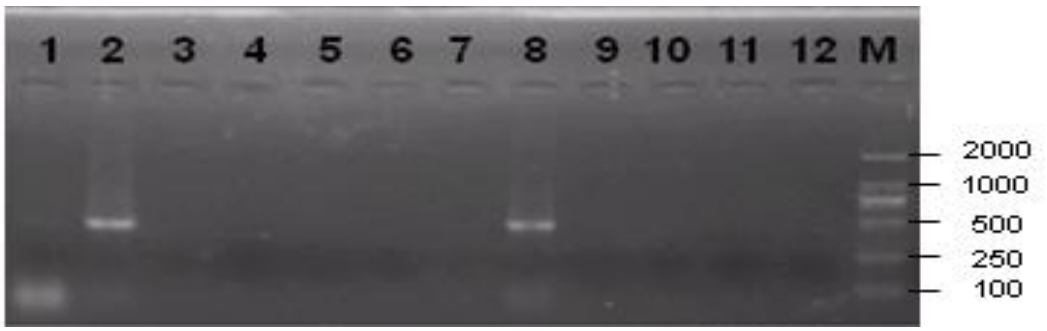


Fig. 1. RT-PCR for detection of hMPV by F gene specific primer : lane 1~12: RT-PCR products specific to hMPV F gene , lane M: DL2000 molecular marker (1.5% AGE)

Also, specific and sharp amplication bands of the expected length (0.45Kb) revealized in all RNAs extracted from NPA specimens positive hMPV infection (Fig. 2).

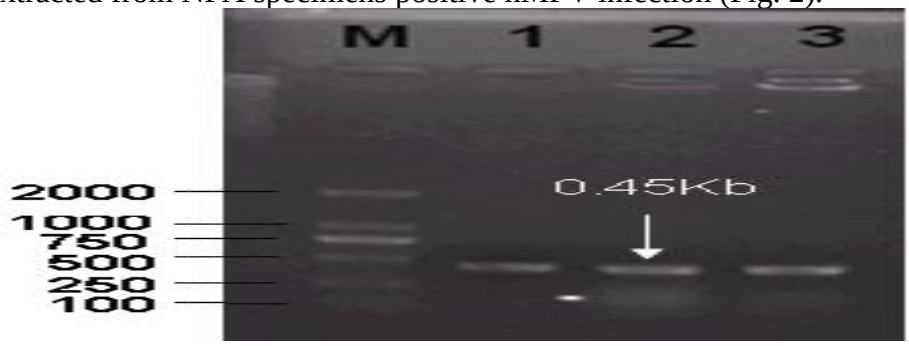


Fig. 2. RT-PCR for detection of hMPV by F gene specific primer : lane 1~3: RT-PCR products specific to hMPV F gene , lane M: DL2000 molecular marker (1.5% AGE).

3.1.2. Detection of hMPV by nucleoprotein (N) gene specific primer

Secondary, we conducted RT-PCR to all nucleic acid extracts including specimen RNAs positive in hMPV F gene-based RT-PCR with primer pair targeting the nucleoprotein (N) gene of hMPV (Fig. 3).

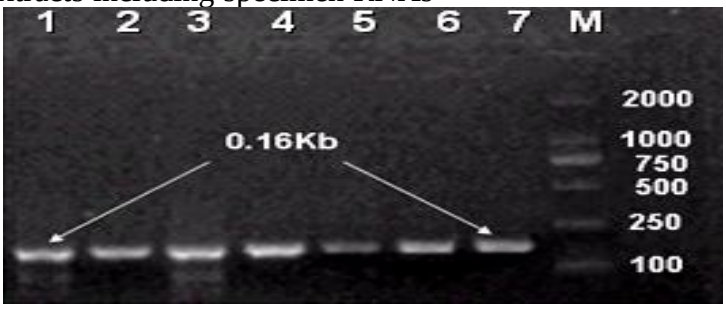


Fig. 3. RT-PCR for detection of hMPV by N gene specific primer : lane 1~7: RT-PCR products specific to hMPV N gene , lane M: DL2000 molecular marker (1.5% AGE) .

As a result, single and very sharp amplication bands of the expected length of 0.16Kb revealized from 8 specimen nucleic acid extracts in hMPV N gene-specific RT-PCR.

3.1.3. Detection of hMPV by polymerase (L) gene specific primer

Thirdly, we performed RT-PCR to all nucleic acid extracts including specimen RNAs positive in hMPV F and N genes-based RT-PCR with primer pair targeting the polymerase (L) gene of hMPV (Fig. 4).

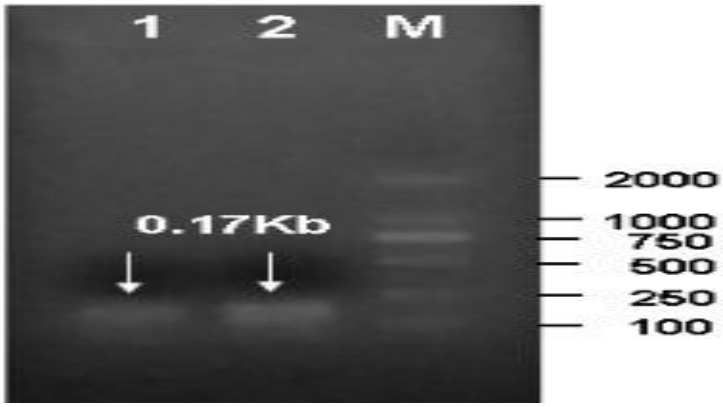


Fig. 4. RT-PCR for detection of hMPV by L gene specific primer : lane 1~2: RT-PCR products specific to hMPV L gene , lane M: DL2000 molecular marker (1.5% AGE).

As a result, relatively single amplication bands of the expected length of 0.17Kb revealized from previously positive 8 all specimen RNAs in hMPV L gene-specific RT-PCR, although obtained amplication bands had not very sharp shape.

3.1.4. Detection of hMPV by F, N, and L genes specific primers

At the end, we performed comprehensive RT-PCR to 1 specimen RNA (number 3) positive in hMPV infection and 3 specimen RNAs (number 1, 2, 4) negative in hMPV infection with primer pairs targeting the fusion glycoprotein (F), nucleoprotein (N), and polymerase (L) genes of hMPV (Fig. 5).

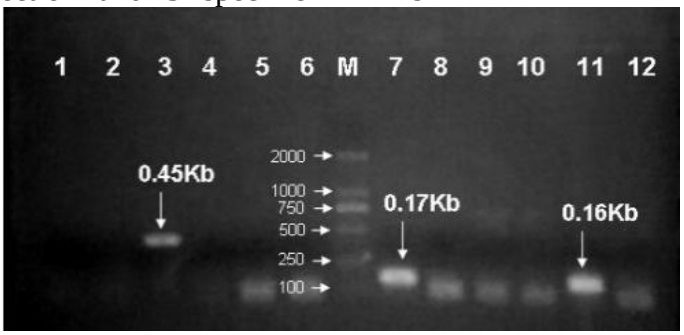


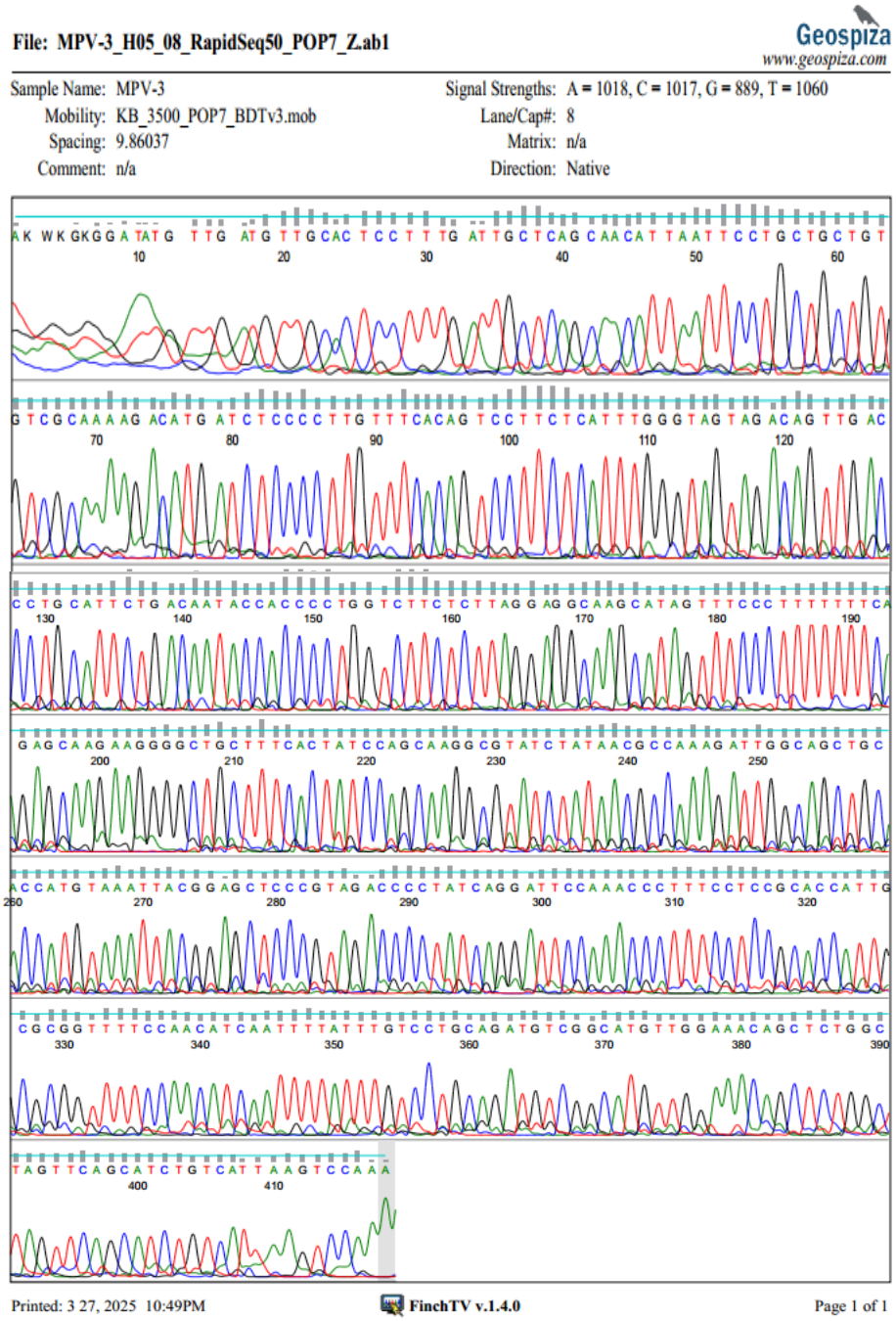
Fig. 5. RT-PCR for detection of hMPV by F, N, and L gene specific primers : lane 1~4: RT-PCR products specific to hMPV F gene, lane 5~8: RT-PCR products specific to hMPV L gene, lane 9~12: RT-PCR products specific to hMPV N gene, lane M: DL2000 molecular marker (1.5% AGE).

As a result, single and very sharp amplication bands of the expected length of 0.45kb, 0.16kb, and 0.17kb specific to F, N, and L genes of hMPV revealized from only 1 specimen (number 3) nucleic acid extract identified with positive hMPV infection.

3.2. Identification of hMPV genotype by DNA Sequencing and Phylogenetic analysis.

3.2.1. Identification of hMPV genotype based on F gene specific amplication bands

The amplication bands (0.45kb) obtained from positive hMPV F gene specific RT-PCR were purified and sequenced using Big Dye terminator chemistry and the ABI Prism 3500 Genetic Analyzer DNA sequencer (Perkin-Elmer Applied Biosystems). As a result, 411 nucleotide sequences were analyzied from the amplified products (0.45kb) of hMPV F gene. The analyzied nucleotide sequences data were as followed.



TTGGACTTAATGACAGATGCTGAACTAGCCAGAGCTGTTTCCAACATGCCGACATCTGCAGGACAAATAAAATTG
ATGTTGGAAAACCGCGCAATGGTGCGGAGGAAAGGGTTTGGAATCCTGATAGGGGTCTACGGGAGCTCCGTAATTTAC
ATGGTGCAGCTGCCAATCTTTGGCGTTATAGATACGCCTTGCTGGATAGTGAAAGCAGCCCCTTCTTGCTCTGAAAAAA
AGGGAAACTATGCTTGCCTCCTAAGAGAAGACCAGGGGTGGTATTGTCAGAATGCAGGGTCAACTGTCTACTACCCAA
ATGAGAAGGACTGTGAAACAAGGGGAGATCATGTCTTTTGCGACACAGCAGCAGGAATTAATGTTGCTGAGCAATCA
AAGGAGTGCAACATCAACATATCC

Phylogenetic tree showed that hMPV strain detected by F gene-based RT-PCR and identified by sequencing and phylogenetic analysis have the most closest phylogenetic relationship with hMPV genotypes A (Fig. 6).

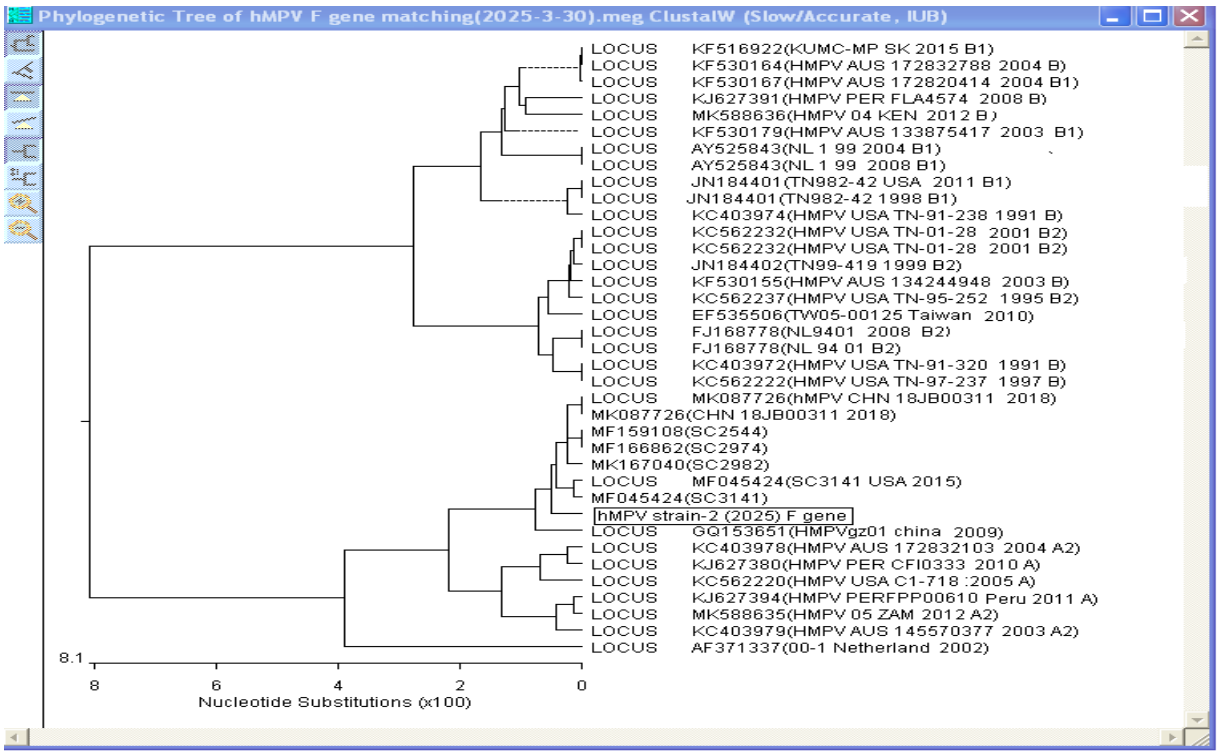
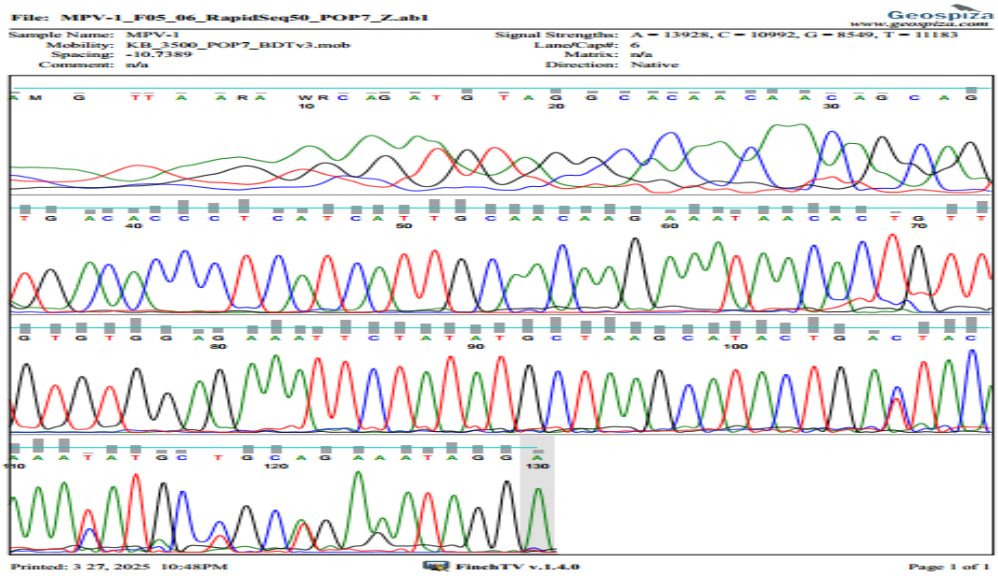


Fig. 6. Phylogenetic tree of the F gene coding regions of hMPV strains constructed by the clustal method.

In addition to, hMPV strain detected and identified on basis of F gene region showed very high sequence homology of 83.5~100% with different hMPV genotypes, and phylogenetic analysis also showed that this virus belonged to the hMPV genotype A2.

3.2.2. Identification of hMPV genotype based on N gene specific amplication bands

The amplication bands (0.16kb) obtained from positive hMPV N gene specific RT-PCR were purified and sequenced. As result, 118 nucleotide sequences were analyzied from the amplified products (0.16kb) of hMPV N gene. The analyzied nucleotide sequences data were as followed.



CAGATGTAGGCACAACAACAGCAGTGACACCCTCATCATTGCAACAAGAAATAACACTGTTGTGTGGAGAAATTCTAT
ATGCTAAGCATACTGACTACAAATATGCTGCAGAAATAGG

Phylogenetic tree showed that hMPV strain detected by hMPV N gene-based RT-PCR have the most closest phylogenetic relationship with hMPV genotype A2 (Fig. 7).

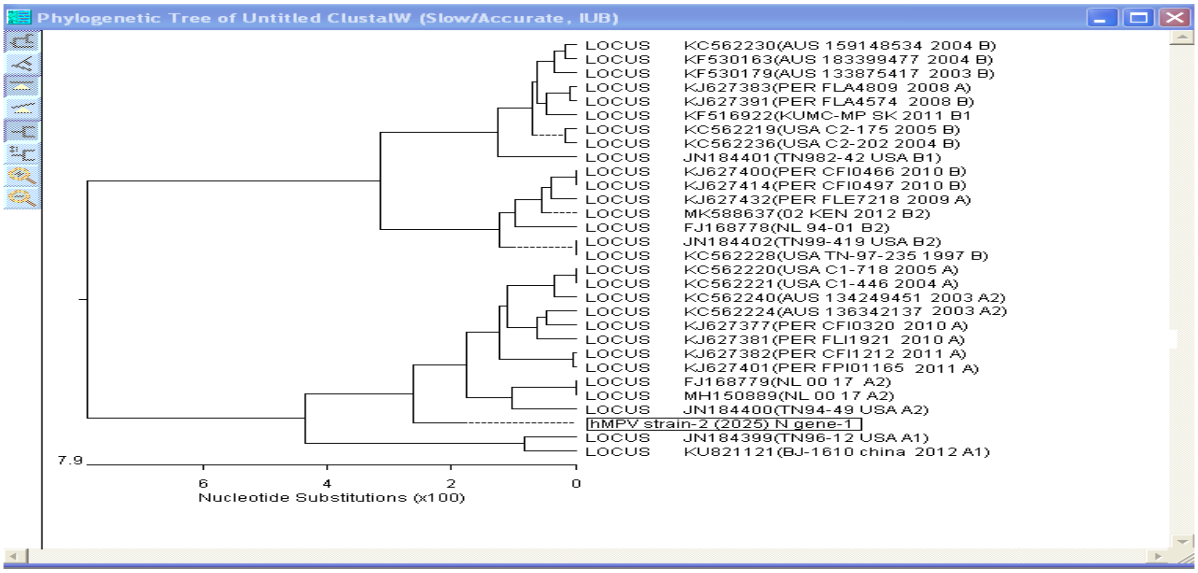
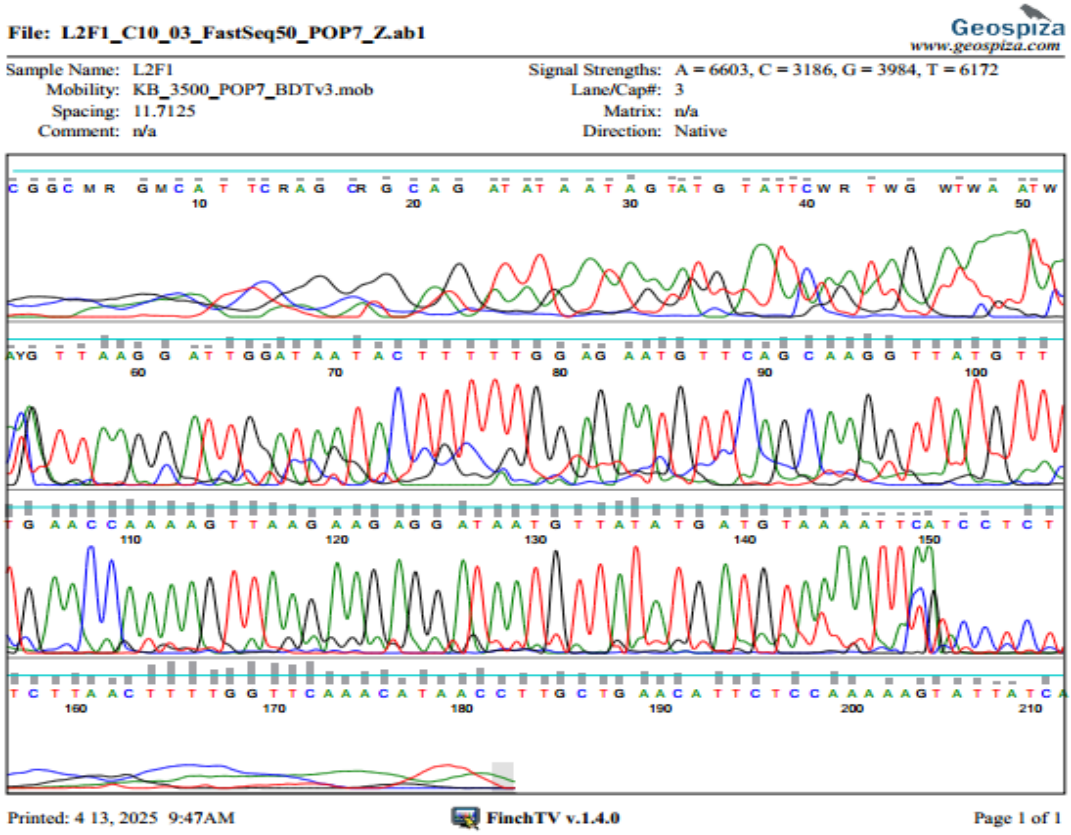


Fig. 7. Phylogenetic tree of the N gene coding regions of hMPV strains constructed by the clustal method.

In addition to, hMPV strain detected and identified on basis of N gene region showed very high sequence homology of 85.7~100% with different hMPV genotypes, and phylogenetic analysis also showed that this virus belonged to the hMPV genotype A2.

3.2.3. Identification of hMPV genotype based on L gene specific amplication bands

The amplication bands (0.17kb) obtained from positive hMPV L gene specific RT-PCR were purified and sequenced. As result, 93 nucleotide sequences were analyzied from the amplified products (0.17kb) of hMPV L gene. The analyzied nucleotide sequences data were as followed.



GTTAAGGATTGATAATACTTTTTGGAGAATGTTCAGCAAGGTTATGTTTGAACCAAAAGTTAAGAAGAGGATAATGTT ATATGATGTAAAATT

Phylogenetic tree showed that hMPV strain detected by hMPV L gene-based RT-PCR and identified by sequencing and phylogenetic analysis have the most closest phylogenetic relationship with hMPV genotype A2 (Fig. 7).

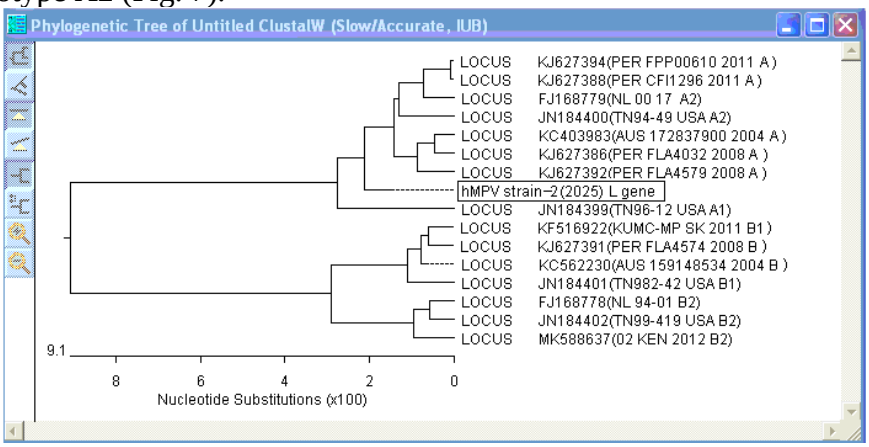


Fig. 8. Phylogenetic tree of the L gene coding regions of hMPV strains constructed by the clustal method.

In addition to, hMPV strain detected and identified on basis of L gene region showed very high sequence homology of 95.7~97.8% with different hMPV genotypes, and phylogenetic analysis also showed that this virus belonged to the hMPV genotype A2.

3.3. Co-infection with other viruses

To determine the co-infection with other viruses, we conducted RT-PCR detections to all nucleic acid extracts positive in hMPV F, N, and L genes-based RT-PCRs using primers specific for influenza viruses A (H1N1) and A (H3N2), human respiratory syncytial virus (HRSV), human parainfluenza virus (HPIV)1 and 3 types (Fig. 9).

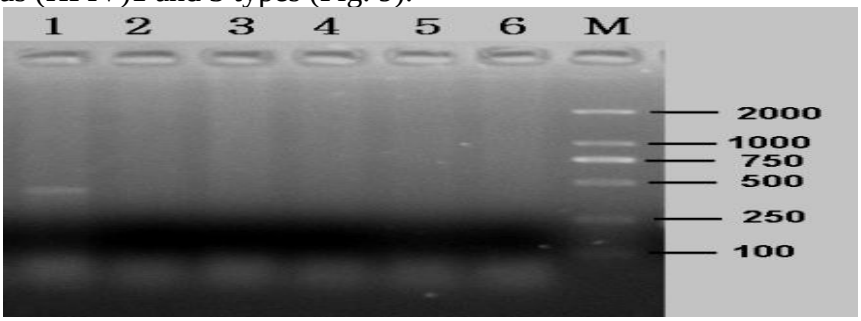


Fig. 9. RT-PCR for detection of co-infection with other viruses : lane 1: RT-PCR products specific to hMPV F gene, lane 2: RT-PCR products specific to influenza virus A(H1N1), lane 3: RT-PCR products specific to influenza virus A(H3N2), lane 4: RT-PCR product specific to HRSV, lane 5: RT-PCR product specific to HPIV 1, lane 6: RT-PCR product specific to HPIV 3, lane M: DL2000 molecular marker (1.5% AGE).

As a result, only hMPV-specific amplicons were visualized in the hMPV positive NPA specimens, whereas influenza virus A (H1N1) and A (H3N2) types, HRSV, and HPIV 1 and 3 types were not detected.

3.4. Clinical findings and outcomes of patients infected with hMPV

Clinical presentation and outcomes of the children infected with hMPV newly discovered in 2001 were very interesting (Tab 1). hMPVs were detected in 5.12% (8/156) of the children hospitalized with various upper respiratory tract illness and severe lower respiratory tract diseases such as wheezy bronchitis, bronchiolitis, and pneumonia and all detected hMPV strains belonged to the hMPV genotype A2. The majority (75.0%, 6/8) of hMPV positive children were male in gender and the rest (25.0%, 2/8) were female. All Children infected with hMPV ranged from 10 month to 34 months of age (median 16.1 months), and 87.5% (7/8) of hMPV positive children were under 2 years of age. Predominant symptoms in the 8 hMPV positive children included fever (100%, 8/8), cough (87.5%, 7/8), rhinorrhea (87.5%, 7/8), sputum (62.5%, 5/8), sore throat

(62.5%, 5/8), dyspnea (37.5%, 3/8), vomiting (12.5%, 1/8), and diarrhea (12.5%, 1/8).

In addition to, children infected with hMPV presented physical examination findings including the mean body temperature (38.2℃,37.4~38.9), the mean pulse rate (128 beats/min, 122~132), the mean respiratory rate (29 breaths/min, 26~31), Rale (75.0%, 6/8), Wheezing (12.5%, 1/8), abnormal chest X-ray radiographs (100%, 8/8). Leukocyte counts were normal or moderately elevated (9.0~10.2×10^{3}/μL) and oxygen saturation were fellen remarkedly. At admission, the clinical diagnoses of children infected with hMPV were predominantly pneumonia (50%, 4/8), acute bronchiolitis (37.5%, 3/8), and acute wheezy bronchitis (12.5%, 1/8). All hMPV positive children were admitted to the hospital during January–March. The administration of antibiotics and treatment for the hMPV positive children were judged according to physical findings and laboratory data. Oxygen saturation of the all patients was monitored during the hospital stay and on basis of this data Oxygen therapy was provided to achieve oxygen saturation ≥95%. One hMPV infection positive child with 11 month of age had intensive treatment including oxygen therapy and mechanical ventilation during hospitalization. All hMPV positive children showed good prognosis and the median length of hospitalization was 6.5 days (5~8).

Overall, We demonstrated that all hMPV positive children confirmed during investigation were infected with the hMPV genotype A2, and that the clinical diagnoses of children infected with hMPV were predominantly pneumonia, acute bronchiolitis, and acute wheezing bronchitis and that the clinical outcome and prognosis of hMPV positive children were good relatively. These findings demonstrated an very close association between hMPV infection and occurrances of the acute lower respiratory tract infections such as pneumonia, bronchiolitis, and acute wheezing. The results observed in our study were similar to that reported in previous studies (4, 18, 20, 24). The obtained result also strongly suggests that Human metapneumovirus (hMPV) is one of the causative agents that causes acute respiratory tract diseases especially among children. We expect that results obtained from our study could aid understanding hMPV-attributable acute lower respiratory infection (ALRI) burden and with regard to the involvement of this virus in causing childhood ALRI

Table 1. Clinical characteristics of children infected with hMPV at admission.

No	Age (mo)	Sex	Diagnosis at admissio	Clinical Severity	Clinical findings					Clinical outcome	
					Body temperature (℃)	Leukocytes (×10^{3}/μL)	PR/RR(frequency/min)	Main Symptoms and signs	Abnormal chest X-ray	Result after treatment	Days in hospital (day)
1	34	Females	Pneumonia	Moderate	38.9	10.2	126/26	F, C, R, RH	+	good	7
2	11	Males	Acute bronchiolitis	Severe	37.8	9.4	132/31	F, C, R, S, ST, D, RH	+	good	8
3	13	Males	Acute bronchiolitis	Severe	38.0	9.2	122/30	F, C, R, S, D, RH	+	good	6
4	12	Males	Acute pneumonia	Moderate	38.5	9.8	126/28	F, C, R, ST, RH	+	good	5
5	21	Females	Acute pneumonia	Moderate	38.8	9.8	130/28	F, R, ST	+	good	5
6	14	Males	Acute pneumonia	Moderate	38.0	10.2	128/30	F, C, R, S, RH	+	good	7
7	10	Males	Acute bronchiolitis	Severe	37.4	9.2	130/29	F, C, R, S, ST, V, DI, RH	+	good	8
8	11	Males	Wheezy bronchitis	Moderate	38.0	9.0	132/30	F, C, S, ST, D, W	+	good	6

Abbreviation ⌈F: Fever, C: Cough, R: Rhinorrhea, S: sputum, ST: Sore Throat, D: Dyspnea, V: Vomiting, DI: diarrhea, RH: Rhonchi, W: Wheezing, PR: Pulse rate, RR: Respiratory rate, +: exist ⌋

CONCLUSION

In this study, we detected human metapneumovirus genotype A2 (hMPV A2) in 8 children hospitalized with acute respiratory tract diseases by RT-PCR using specific primers targeting the hMPV F, N, and L genes and confirmed that the viruse detected by hMPV specific RT-PCR were human metapneumovirus genotype A2 through DNA sequence analysis and Phylogenetic analysis of specific amplicons. In these children, human metapneumovirus genotype A2 is the only causative virus that cause symptoms and signs of acute upper and lower respiratory tract infections such as fever, cough, rhinorrhea, sore throat, sputum, wheezing, hypoxia, and cyanosis, suggesting that hMPV infection is the decisive cause of acute lower respiratory tract diseases such as pneumonia, bronchiolitis, and acute wheezy bronchitis in children.

Declaration of competing interests:

The authors declare that they have no conflict of interest.

Funding:

This research received no external funding.

Author Contribution

Chang Guk Ri: Overall design, primer design, RT-PCR detection, Yong Chan Sin: Overall analysis of clinical characteristics, Hyok Kim: analysis of clinical characteristics, Yong Chol Pak and Yong Su Song: DNA sequence analysis and Phylogenetic analysis, Sin Hyok Paek: Analysis of Chest X ray Imaging, Mi Rae Han and Il Gyong Ri: RT-PCR detection, Yon Hui Ri: Analysis of Laboratory data, Hyon Song Kang and Song Min Jong: Collection of samples and statistical analysis of clinical findings.

Abbreviation:

- *hMPV - Human metapneumoviru*
- *RT-PCR - Reverse Transcription - Polymerase Chain Reaction*
- *DNA - deoxyribonucleic acid*
- *NPA - Nasopharyngeal aspirate*
- *F - fusion glycoprotein*
- *N - nucleoprotein*
- *L - polymerase*
- *NCBI - National Center of BioInformation*
- *ARTIs - acute respiratory tract infections*
- *URTIs - upper respiratory tract infections*
- *LRTIs - lower respiratory tract infections*
- *IFV-A(H1N1) - influenza A viruses (H1N1)*
- *IFV-A(H3N2) - influenza A viruses (H3N2)*
- *HRSV - human respiratory syncytial virus*
- *HPIV-1 - human parainfluenza virus type 1*
- *HPIV-3 - human parainfluenza virus type 3*

REFERENCES

1. Agapov E, Sumino K.C, Gaudreault-Keener M, Storch G.A, Holtzman M.J., Genetic variability of human metapneumovirus infection: evidence of a shift in viral genotype without a change in illness. J Infect Dis. 2006. 193. 396–403.
2. Biacchesi S, Murphy B.R, Collins P.L, et al., Frequent frameshift and point mutations in the SH gene of human metapneumovirus passaged in vitro. J Virol. 2007. 81. 6057-6067.
3. Chun-mei Yu. et al., Replication and pathogenicity of attenuated human metapneumovirus F mutants in severe combined immunodeficiency mice. Vaccine. 2012. 30. 231–236.
4. Feuillet F, Lina B, Rosa-Calatrava M, Boivin G. Ten years of human metapneumovirus research. J Clin Virol. 2012. 53. 97–105.
5. Huck B.; Scharf G., Neumann-Haefelin, D., Puppe W., Weigl J., Falcone V., Novel human metapneumovirus sublineage. Emerg. Infect. Dis. 2006. 12. 147–150.
6. Kahn J.S., Epidemiology of human metapneumovirus. Clin Microbiol Rev. 2006. 9. 546–557.
7. Mackay I.M, Bialasiewicz S, Jacob K.C, et al., Genetic diversity of human metapneumovirus over 4 consecutive years in Australia. J Infect Dis. 2006. 193. 1630–1633.

8. Maertzdorf J., et al., Real-Time Reverse Transcriptase PCR Assay for Detection of Human Metapneumoviruses from All Known Genetic Lineages. Journal of Clinical Microbiology. 2004. 42(3) 981-986.
9. Maertzdorf J., et al., Analysis of Replication Kinetics of the Human Metapneumovirus in Different Cell Lines by Real-Time PCR. Journal of Clinical Microbiology. 2005. 43(1) 488–490.
10. Matsuzaki Y, Itagaki T, Abiko C, Aoki Y, Suto A, Mizuta K., Clinical impact of human metapneumovirus genotypes and genotype-specific seroprevalence in Yamagata, Japan. J Med Virol. 2008. 80. 1084–1089.
11. Moe N, Stenseng I.H, Krokstad S, Christensen A, Skanke L.H, Risnes K.R, Nordbø S.A, Døllner H., The burden of human metapneumovirus and respiratory syncytial virus infections in hospitalized Norwegian children. J Infect Dis. 2017. 216. 110–116. doi:10.1093/infdis/jix262.
12. Nao, N., Saikusa M., Sato K., Sekizuka T., Usuku S., Tanaka N., Nishimura H., Takeda M., Recent Molecular Evolution of Human Metapneumovirus (HMPV): Subdivision of HMPV A2b Strains. Microorganisms. 2020. 8. 1280.
13. Panda S, Mohakud N.K, Pena L, Kumar S., Human metapneumovirus: review of an important respiratory pathogen. Int J Infect Dis. 2014. 25. 45–52.
14. Principi N, Bosis S, Esposito S., Human metapneumovirus in paediatric patients. Clin Microbiol Infect. 2006. 12. 301–308.
15. Rima B, Collins P, Easton A, Fouchier R, Kurath G, Lamb R.A, Lee B, Maisner A, Rota P, Wang L., ICTV Report Consortium. ICTV virus taxonomy profile: Pneumoviridae. J Gen Virol. 2017. 98. 2912–2913. doi:10.1099/jgv.0.000959.
16. Schildgen V, van den Hoogen B, Fouchier R, et al., Human metapneumovirus: lessons learned over the first decade. Clin Microbiol Rev. 2011. 24. 734–754.
17. Shahda S, Carlos W.G, Kiel P.J, Khan B.A, Hage C.A. The human metapneumovirus: a case series and review of the literature. Transpl Infect Dis. 2011. 13. 324–328.
18. Stockton, J, Stephenson I, Fleming D, and Zambon M., Human metapneumovirus as a cause of community-acquired respiratory illness. Emerg. Infect. Dis. 2002. 8. 897–901.
19. van den Hoogen B.G, Bestebroer T.M, Osterhaus A.D, et al., Analysis of the genomic sequence of a human metapneumovirus. Virology. 2002. 295. 119-132.
20. van den Hoogen B.G, de Jong J.C, Groen J, Kuiken T, de Groot R, Fouchier R.A, et al., A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat Med. 2001. 7. 719–724.
21. Vicente D, Montes M, Cilla G, Perez-Yarza E.G, Perez-Trallero E,. Differences in clinical severity between genotype A and genotype B human metapneumovirus infection in children. Clin Infect Dis. 2006. 42. e111–113.
22. Wei H.Y, Tsao K.C, Huang C.G, Huang Y.C, Lin T.Y., Clinical features of different genotypes/genogroups of human metapneumovirus in hospitalized children. J Microbiol Immunol Infect. 2013. 46. 352–357.
23. Williams J.V, Edwards K.M, Weinberg G.A, Griffin M.R, Hall C.B, Zhu Y, Szilagyi P.G, Wang C.K, Yang C.F, Silva D, Ye D., Population-Based Incidence of Human Metapneumovirus in Hospitalized Children. J Infect Dis. 2010. 201(12) 1890–1898.
24. Yang Z, Suzuki A, Watanabe O, et al., Outbreak of Human Metapneumovirus Infection in a Severe Motor-and-Intellectual Disabilities Ward in Japan. Jpn J Infect Dis. 2014. 67. 318-321.

