Human Metapneumovirus

Molecular Biology and Diagnostic methods

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A newly discovered human pneumovirus isolated from young children with respiratory tract disease

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From 28 young children in the Netherlands, we isolated a paramyxovirus that was identified as a tentative new member of the *Metapneumovirus* genus based on virological data, sequence homology and gene constellation. Previously, avian pneumovirus was the sole member of this recently assigned genus, hence the provisional name for the newly discovered virus: human metapneumovirus. The clinical symptoms of the children from whom the virus was isolated were similar to those caused by human respiratory syncytial virus infection, ranging from upper respiratory tract disease to severe bronchiolitis and pneumonia. Serological studies showed that by the age of five years, virtually all children in the Netherlands have been exposed to human metapneumovirus and that the virus has been circulating in humans for at least 50 years.

The *Paramyxovirinae* and *Pneumovirinae* subfamilies of the *Paramyxoviridae* family include several major pathogens of humans and animals. The *Pneumovirinae* are taxonomically divided in the *Pneumovirus* and the *Metapneumovirus* genera[1]. Human res-

mology and gene constellation, the viruses seemed to be a tentative new member of the *Metapneumovirus* genus that we have provisionally named human metapneumovirus (hMPV). Serological surveys showed that by the age of five years virtually

- CPE in cell cultures tertiary monkey kidney (tMK) cells VERO/A549
- cynomolgus macaques infectivity
- random arbitrarily primer PCR (RAP-PCR) detection
- Full genome sequencing

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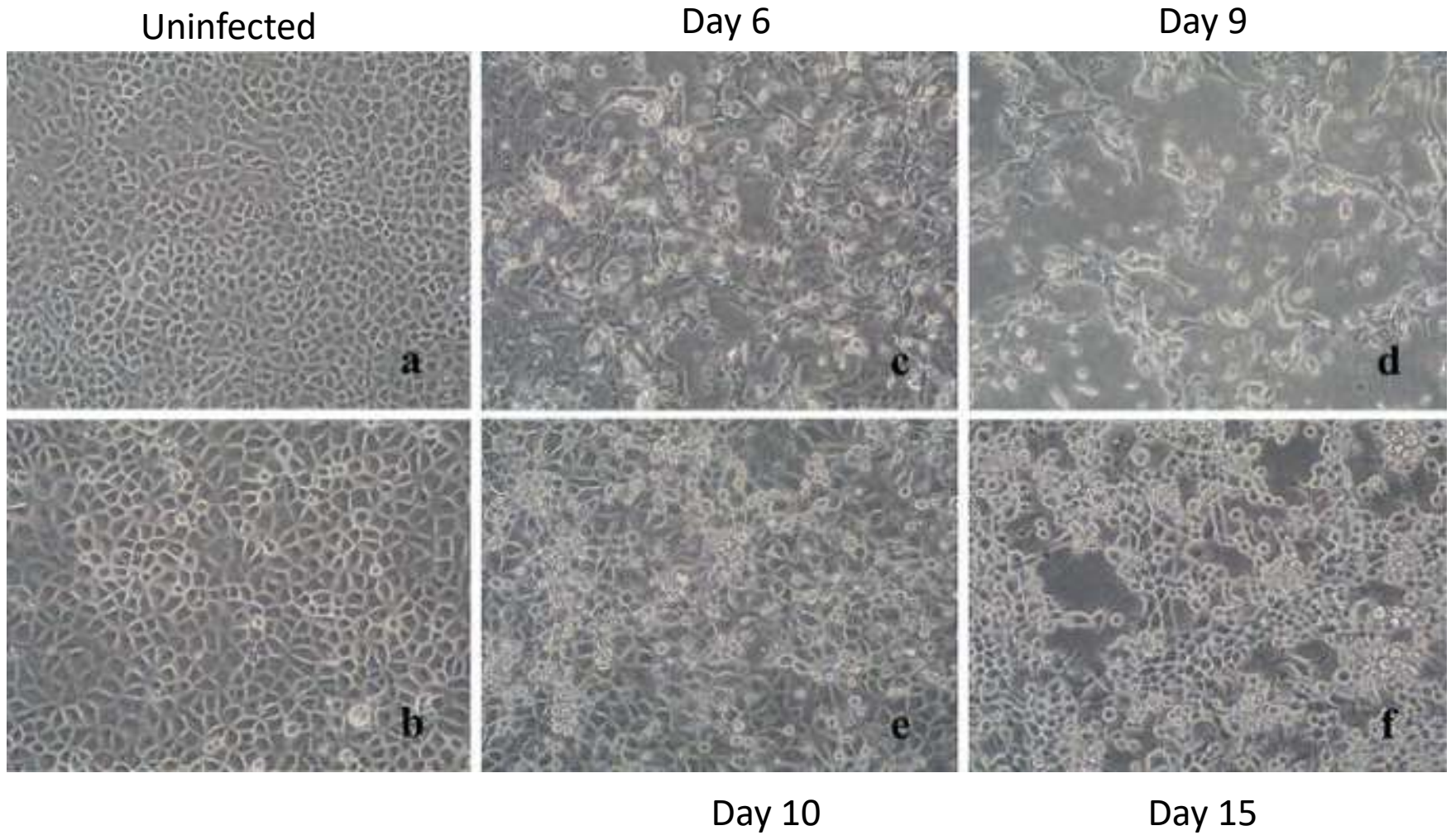
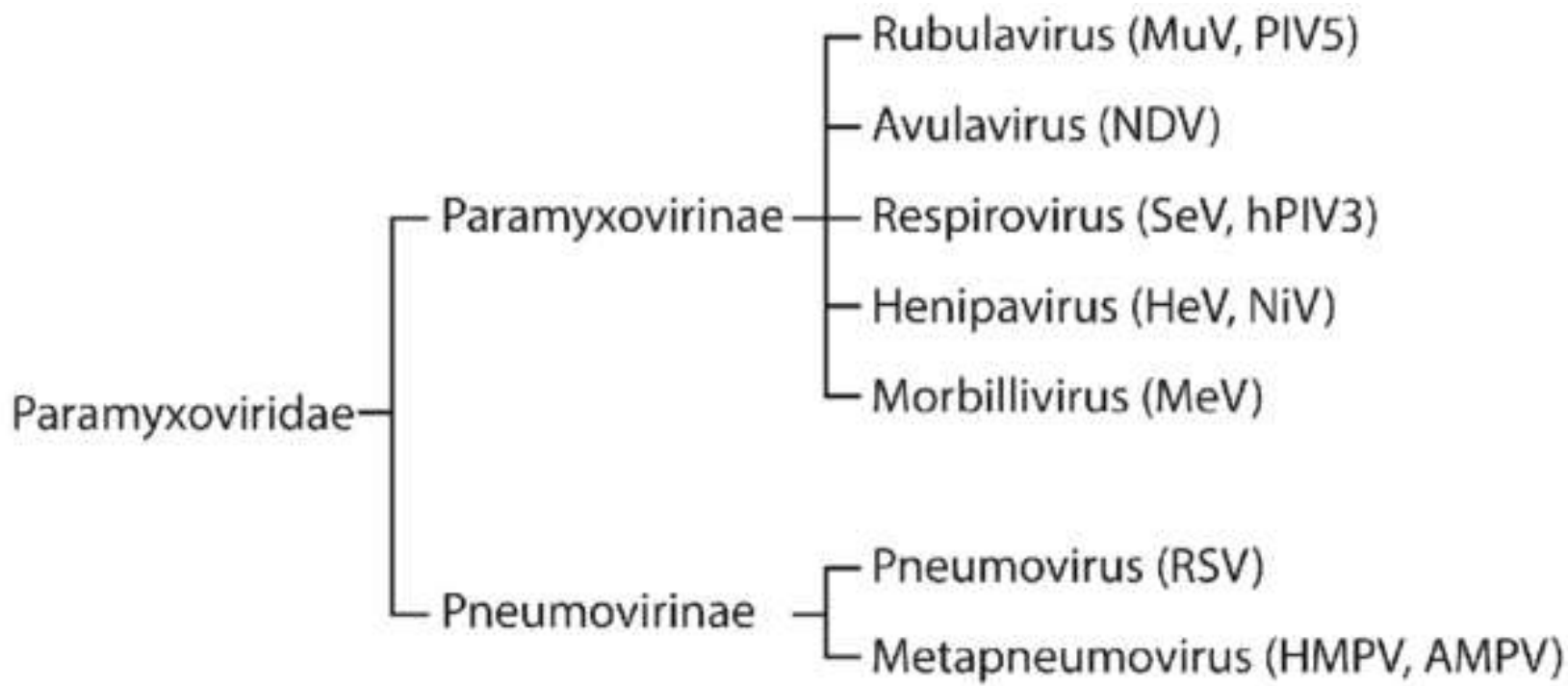
Metapneumovirus Isolation



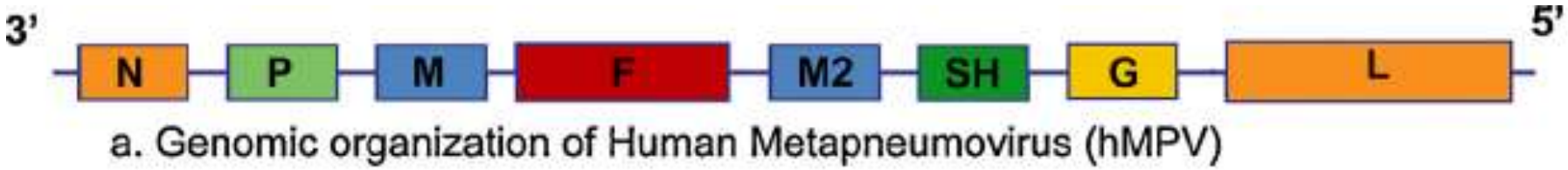
Li, Xy., Chen, Jy., Kong, M. *et al.* Prevalence of human metapneumovirus in hospitalized children with respiratory tract infections in Tianjin, China. *Arch Virol* **154**, 1831–1836 (2009)

Paramyxoviridae- Metapneumovirus



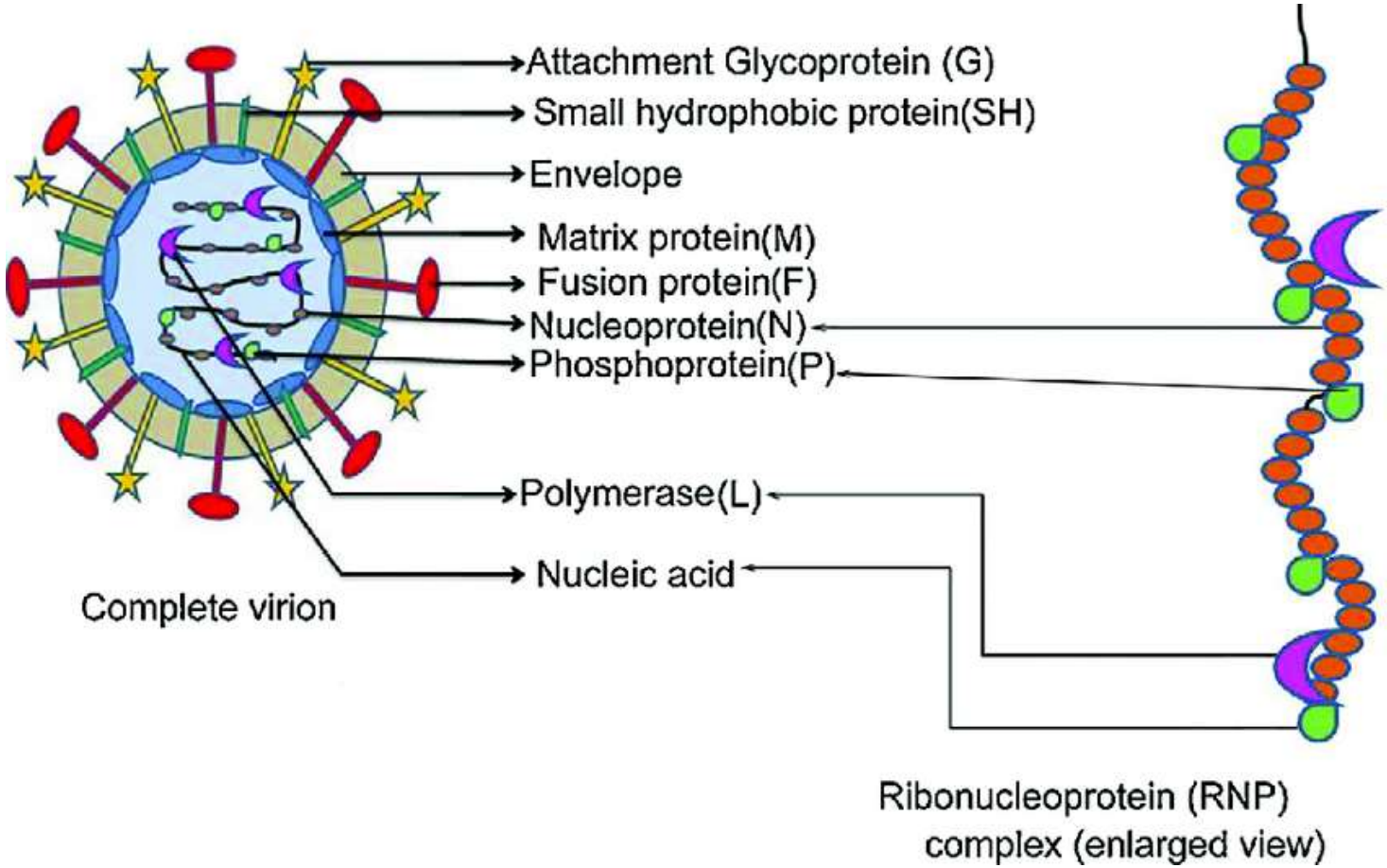
Metapneumovirus

-ve sense RNA Genome

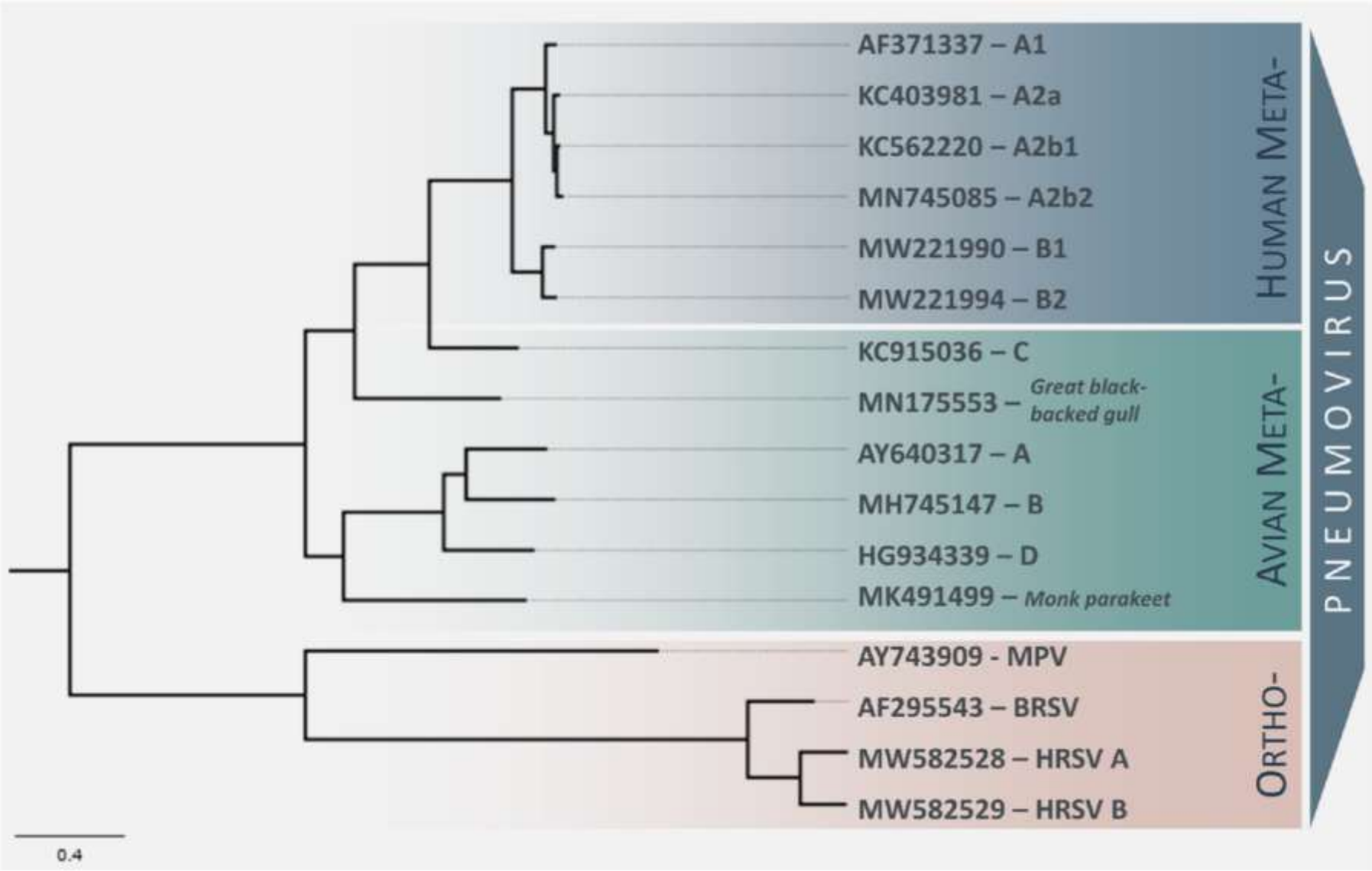


a. Genomic organization of Human Metapneumovirus (hMPV)

13.3kb



Paramyxoviridae- Metapneumovirus



Variant A2b strains display increased rates of virus transmission

No animal reservoirs

Small animal models
(c57/BL6 or Balb/c mice
Non-human primates

Jesse, S.T.; Ludlow, M.; Osterhaus, A.D.M.E. Zoonotic Origins of Human Metapneumovirus: A Journey from Birds to Humans. Viruses 2022, 14, 677

Possible Avian origin. (Turkey Rhinotracheitis virus) **80% aa identity**

HMPV Pandemic Potential

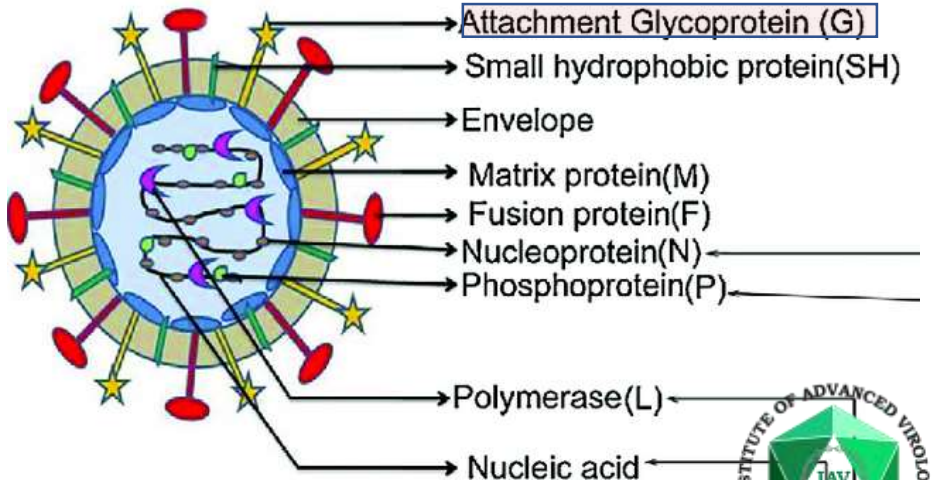
HMPV G protein : Receptor binding protein
Displays the highest level of inter-strain diversity
Mean 63% aa identity between HMPV subgroups

HMPV Mutation rate

G gene	3.5×10^{-3} nucleotide substitution per site per year
F gene	$7.1 - 8.5 \times 10^{-4}$ nucleotide substitution per site per year
N gene	9×10^{-4} nucleotide substitution per site per year

SARS Cov-2 Mutation Rate during initial pandemic time

Spike protein: 2.19×10^{-3} substitution per site per year



HMPV-Global scenario

- HMPV is found globally and has been identified on every continent.
- In temperate climates, HMPV circulates predominantly in late winter and spring.
- The peak of HMPV activity often coincides with or follows the peak of Respiratory Syncytial Virus (RSV) activity.
- Sub lineage A2b -prevalent worldwide for several years
- Prevalence of HMPV worldwide is estimated to be between 5 and 10%

HMPV-Indian Scenario

HMPV has been identified in India as a respiratory pathogen in 2004

Significant cause of respiratory illness in all age groups

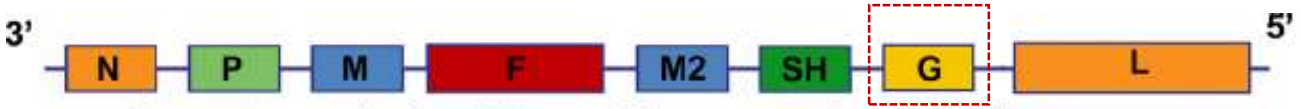
HMPV activity peaks globally during winter and spring, in India, infections vary regionally and often coincide with the post-monsoon season.

Average prevalence of HMPV varies from 4-12%

Circulating lineages identified in India include B1, B2, A2b and A2c

Rao BL, Gandhe SS, Pawar SD, Arankalle VA, Shah SC, Kinikar AA. 2004. First Detection of Human Metapneumovirus in Children with Acute Respiratory Infection in India: a Preliminary Report. J Clin Microbiol 42:.

Laboratory Diagnosis



a. Genomic organization of Human Metapneumovirus (hMPV)

Table 1 Summary of HMPV molecular diagnostic approaches and their characteristics

Methods	Operation time	Limit of detection	Experimental cost	Characteristics
RT-PCR [24, 25]	3–5 h	1000 copies/reaction	Low	RT-PCR has been widely used for performing epidemiological investigation of HMPV, while which requires complex instruments and trained workers
RT-qPCR [27, 28]	1–3 h	10 ~ 100 copies/reaction	Low	RT-qPCR has been widely amplified in HMPV monitoring in clinical samples
LAMP [34, 35]	~ 1.5 h	< 10 copies/reaction	High	Advantages: High sensitivity and specificity, rapid diagnosis with simple reaction procedure, and constant temperature Disadvantages: High requirements of primers, high false positive rate, and high cost
Recombinase-aided amplification RAA [38]	15 ~ 30 min	100 copies/reaction	High	Advantages: High sensitivity and specificity, and rapid diagnosis of virus infection Disadvantages: High cost and high positive rate
recombinase polymerase amplification RT-RPA combined with CRISPR-Cas12a [45]	< 30 min	< 700 copies/reaction	High	Advantages: High sensitivity and specificity, and rapid diagnosis of virus infection Disadvantages: High cost and high positive rate
mNGS [48] Metagenomics	5 ~ 10 days	Not determined	High	Advantages: High sensitivity and specificity, and rapid diagnosis of unknown pathogens Disadvantages: High cost and time-consuming
Virus isolation [51]	3 ~ 4 days or more time	Not determined	High	Advantages: The "gold standard" for pathogen detection Disadvantages: High cost and time-consuming, and low isolation rate, and requires complex instruments and trained workers

Feng et al. Virology Journal (2024) 21:59

Laboratory Diagnosis

SL.No	AETIOLOGY/PATHOGEN	SPECIMEN	ASSAY TYPE	RESULT
1	Enterovirus	NASOPHARYNGEL SWAB	Real Time PCR	Negative
2	Hemophilus influenzae	NASOPHARYNGEL SWAB	Real Time PCR	Negative
3	Human Adenovirus	NASOPHARYNGEL SWAB	Real Time PCR	Negative
4	Human Bocavirus	NASOPHARYNGEL SWAB	Real Time PCR	Negative
5	**Human Metapneumovirus A/B**	**NASOPHARYNGEL SWAB**	**Real Time PCR**	**POSITIVE**
6	Human Parechovirus	NASOPHARYNGEL SWAB	Real Time PCR	Negative
7	Human Respiratory syncytial virus	NASOPHARYNGEL SWAB	Real Time PCR	Negative
8	Human Rhinovirus	NASOPHARYNGEL SWAB	Real Time PCR	Negative
9	Human coronavirus 229E	NASOPHARYNGEL SWAB	Real Time PCR	Negative
10	Human coronavirus HKU1	NASOPHARYNGEL SWAB	Real Time PCR	Negative
11	Human coronavirus NL63	NASOPHARYNGEL SWAB	Real Time PCR	Negative
12	Human coronavirus OC43	NASOPHARYNGEL SWAB	Real Time PCR	Negative
13	Human parainfluenza virus -1	NASOPHARYNGEL SWAB	Real Time PCR	Negative
14	Human parainfluenza virus -2	NASOPHARYNGEL SWAB	Real Time PCR	Negative
15	Human parainfluenza virus -3	NASOPHARYNGEL SWAB	Real Time PCR	Negative
16	Human parainfluenza virus -4	NASOPHARYNGEL SWAB	Real Time PCR	Negative
17	Influenza A Virus	NASOPHARYNGEL SWAB	Real Time PCR	Negative
18	Influenza B virus	NASOPHARYNGEL SWAB	Real Time PCR	Negative
19	Mycoplasma pneumoniae	NASOPHARYNGEL SWAB	Real Time PCR	Negative
20	SARS CoV2	NASOPHARYNGEL SWAB	Real Time PCR	Negative
21	Staphylococcus aureus	NASOPHARYNGEL SWAB	Real Time PCR	Negative
22	Streptococcus pneumoniae	NASOPHARYNGEL SWAB	Real Time PCR	Negative

Disclaimer: PCR done by institute of Advanced Virology is on Research mode. Please correlate the cases clinically.

In 2022, IAV started testing HMPV along with other respiratory viruses

Specimen :Nasopharyngeal swab and Throat swab

Method: Real Time RT-PCR

Target gene : G gene

Courtesy: Dr.Aswathyraj, Scientist C & In-charge, Molecular Diagnostic Facility, IAV

IAV – HMPV Diagnosis

YEAR	TOTAL SAMPLES TESTED	TOTAL POSITIVE CASES
2022	191	3
2023	380	1
2024	3888	8
2025	24	0

	HMPV CASES (n=12)
Clinical parameters	N (%)
Cough	11 (91.6%)
Coryza	10 (83.3%)
Breathlessness	8 (66.6%)
Headache	4 (33.3%)
Mechanical ventilation	3. (25%)
Altered sensorium	2 (16.6%)
Vomiting	1 (8.3%)

Age group (In Years)	HMPV CASES (n=12) N (%)
0-2	4 (33.3%)
2-5	2 (16.6%)
5-15	1 (8.3%)
15-50	1 (8.3%)
50-70	4 (33.3%)

Courtesy: Dr.Aswathyraj, Scientist C & In-charge, Molecular Diagnostic Facility, IAV

Thank You