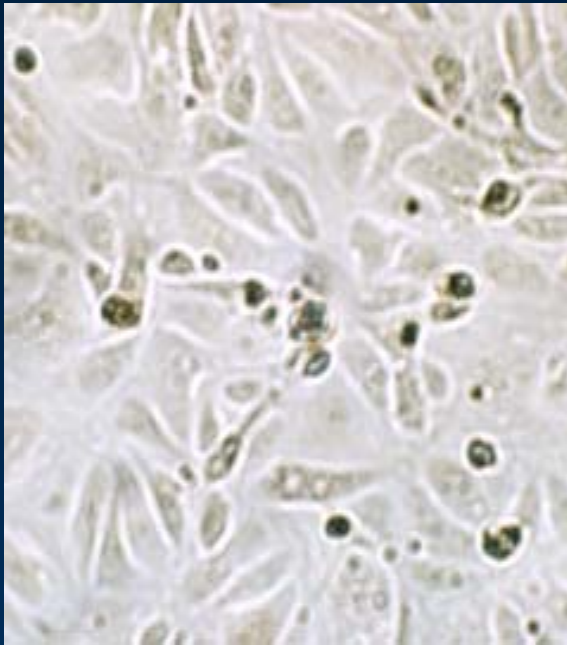


A newly discovered human pneumovirus isolated from young children with respiratory tract disease

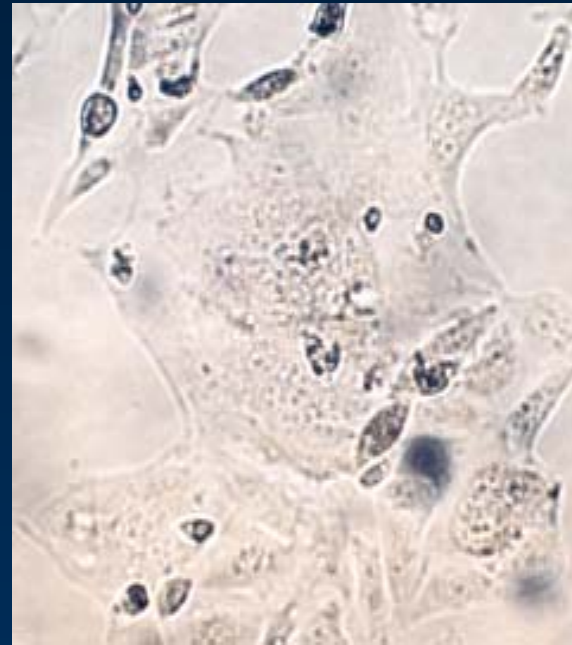
Bernadette G. van den Hoogen, Jan C. de Jong, Jan Groen, Thijs Kuiken, Ronald de Groot, Ron A.M. Fouchier and Albert D.M.E. Osterhaus

Cytopathic effects in tMK cells

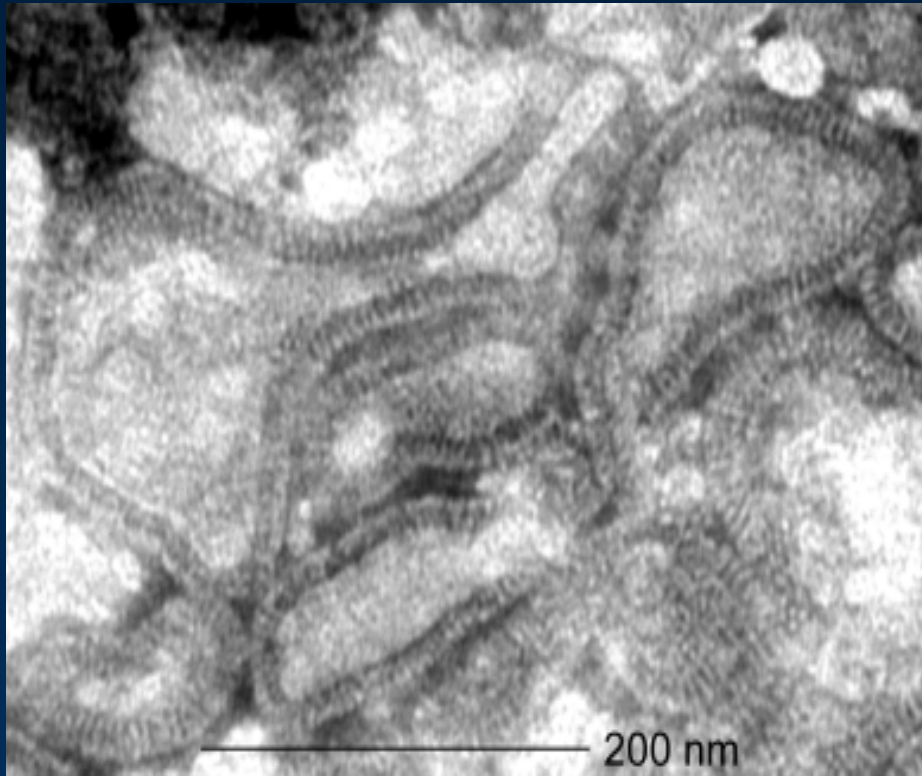
Control



hMPV-infected



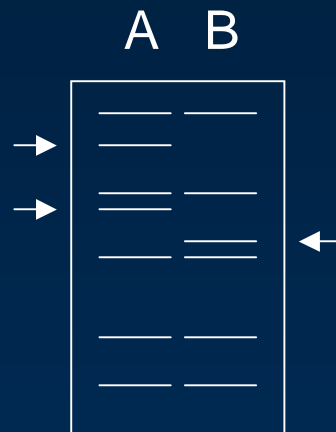
Electron microscopy



- Pleiomorph
- Average size 100 - 600 nm
- Nucleocapsids rarely observed
- Envelope projections of 13 -17 nm
- Paramyxovirus

RAP-PCR procedure

1. RNA isolation
2. RAP-PCR
3. Gel electrophoresis



4. Cloning (pCR2.1)
5. Sequencing
6. BLAST

PCR and sequence analysis of vRNA



Genomic organisation of pneumoviruses



APV, 13.373 bp

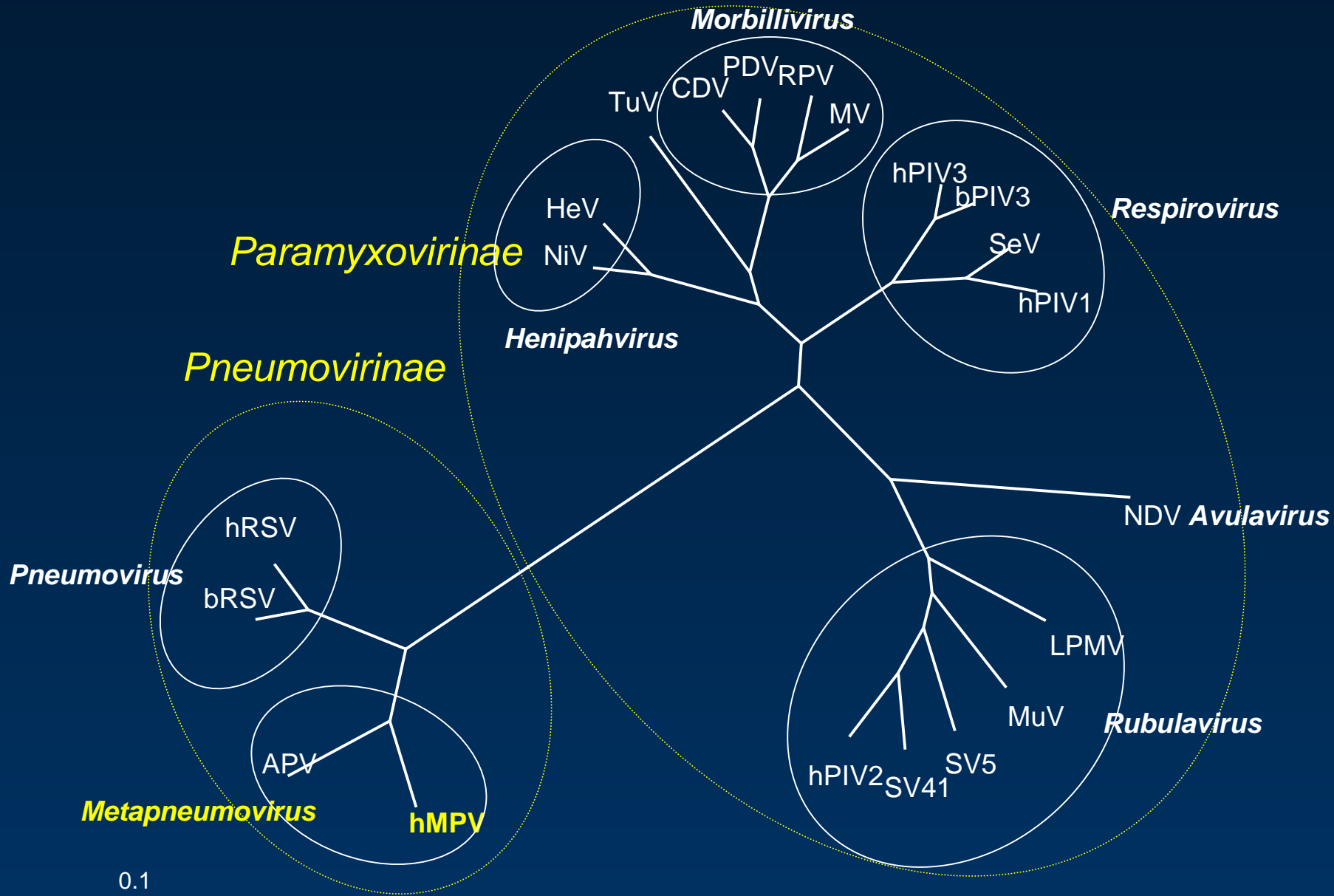


hMPV, 13.378 bp



hRSV, 15.225 bp

Order Mononegavirales, family Paramyxoviridae

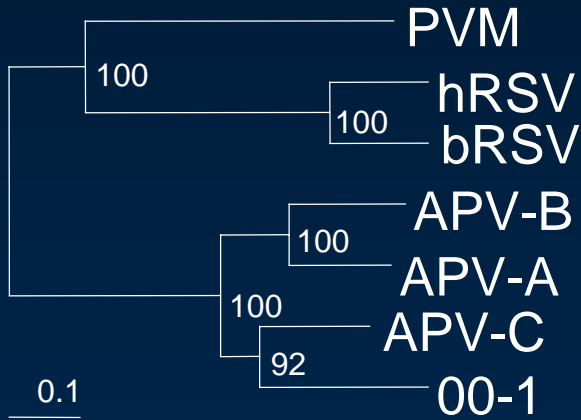


0.1

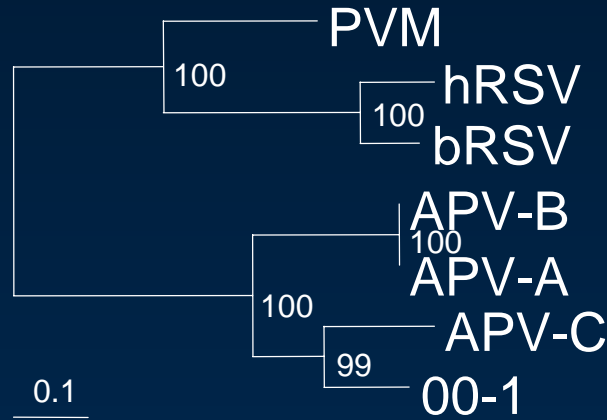
DNA Maximum likelihood, Polymerase ORF

Phylogenetic analysis

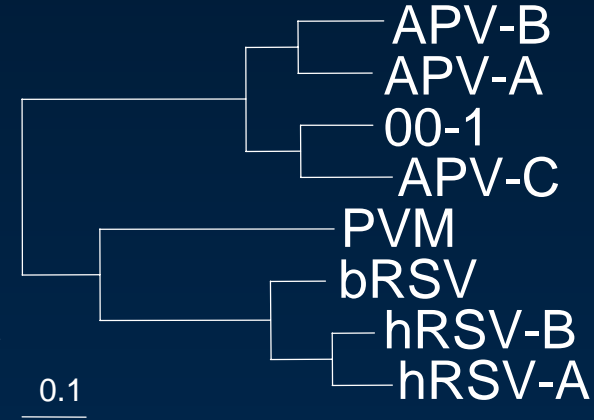
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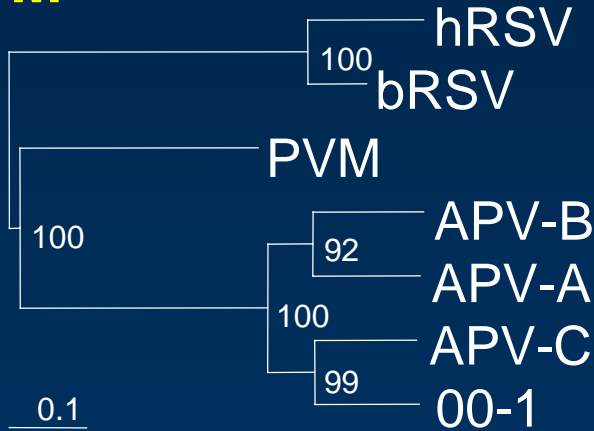
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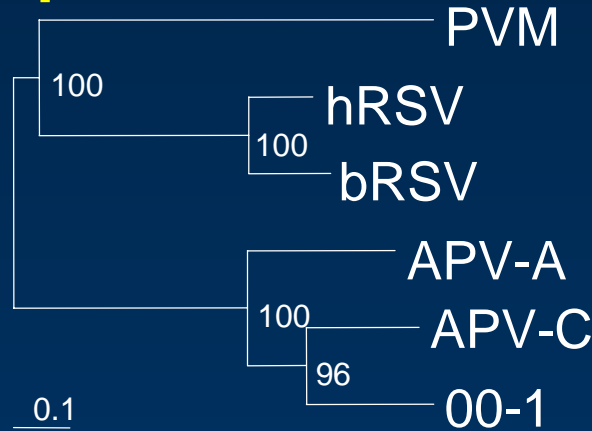
M2-1



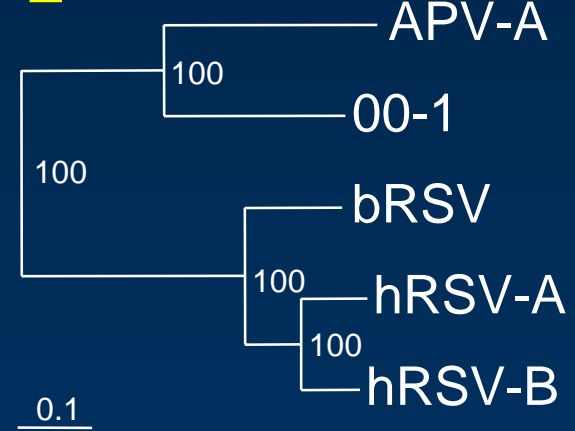
M



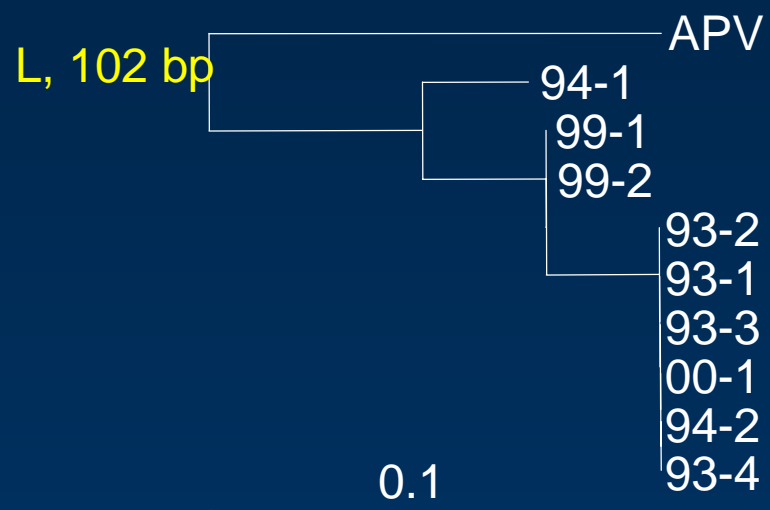
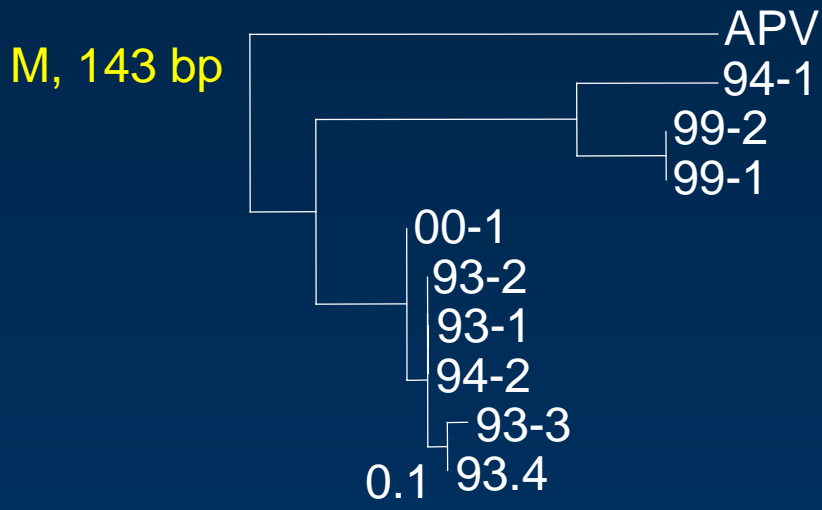
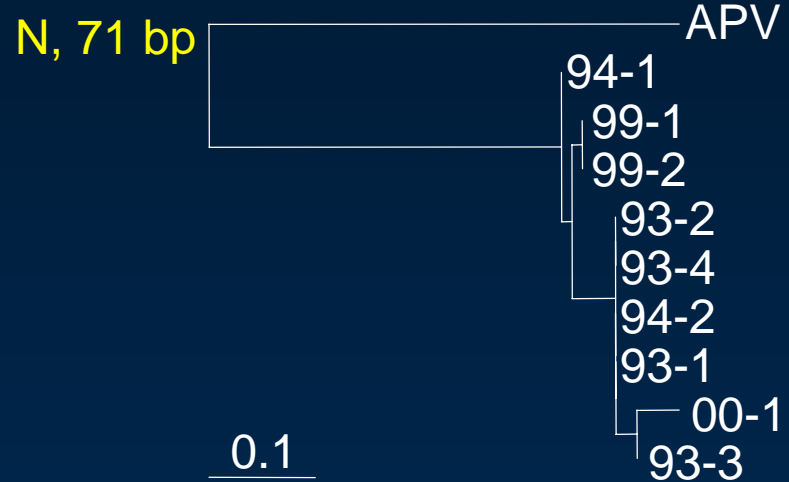
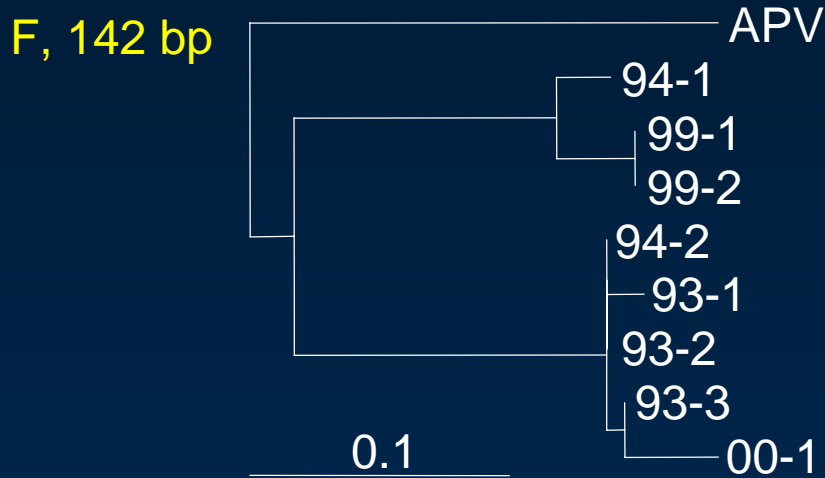
P



L



Genetic diversity of hMPV isolates



Amino acid sequence homology of metapneumovirus ORFs

Sequence identity with ORFs of hMPV isolate 00-1

	N	P	M	F	M2.1	M2.2	SH	G
hMPV 99-1	95	86	98	94	95	90	57	33
APV-C	88	68	87	81	84	56	N.A.	N.A.
APV-A	69	55	78	68	72	25	18	9

Seroprevalence of hMPV in The Netherlands

Age (Years)	Immunofluorescence assays		Virus neutralization assays		
	<i>n</i> tested	<i>n</i> positive (%)	<i>n</i> tested	<i>n</i> positive (%)	Titre range
0.5 - 1	20	5 (25)	12	3 (25)	16-32
1 - 2	20	11 (55)	13	4 (31)	16-32
2 - 5	20	14 (70)	8	3 (38)	16-512
5 - 10	20	20 (100)	4	4 (100)	32-256
10 - 20	20	20 (100)	4	3 (75)	32-128
> 20	20	20 (100)	4	3 (75)	32-128
8 - 99 ¹	72	72 (100)	11	11 (100)	16-128

Conclusions-1

a newly discovered human metapneumovirus (hMPV)

- hMPV is associated with respiratory tract illnesses:
 - In young children (& elderly, immunocompromised, others)
 - Primarily in the winter season
 - Symptoms similar to those caused by RSV (ranging from mild respiratory symptoms to severe bronchiolitis and pneumonia)
 - From children infected with hMPV, no other human pathogens were isolated
 - hMPV vRNA is not detected in throat swabs from healthy children
- Experimental infections of animals with hMPV:
 - hMPV does not replicate in chickens and turkeys
 - hMPV replicates in macaques, and causes mild respiratory symptoms
 - hMPV replicates in guinea pigs and ferrets

Conclusions-2

a newly discovered human metapneumovirus (hMPV)

- hMPV is a putative new member of the *pneumovirinae*, genus *metapneumovirus*
 - Virus characteristics (EM, CPE, HA⁻, CHCl₃-sensitive, trypsin-dependence)
 - Sequence homology
 - Genomic organisation
- hMPV is a virus that commonly infects humans
 - Sero-prevalence reaches 100 % of humans by the age of five years
 - In the 2000 winter season, hMPV was isolated from 10 % of children with respiratory tract illnesses (negative for other pathogens)
 - Human sera collected in 1958 were all positive for antibodies to hMPV
 - Reinfection with homologous/heterologous virus strains?
- hMPV diversity
 - Two genetic clusters of hMPV isolates were found in the Netherlands
 - The genetic clusters may represent different serotypes of hMPV

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J. de Jong

Paediatric

R. de Groot

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