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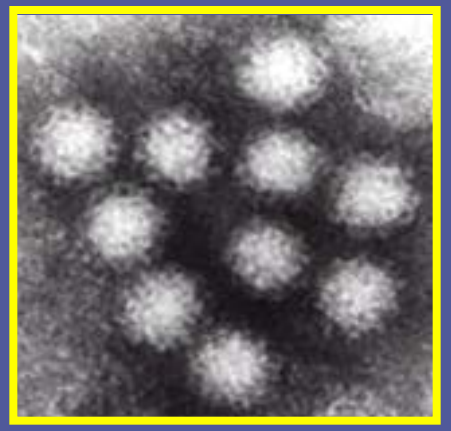
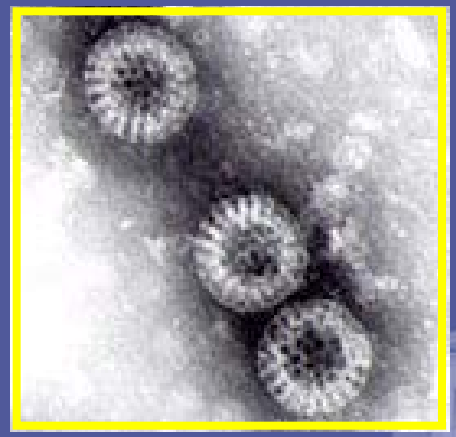




Gastroenteric viruses

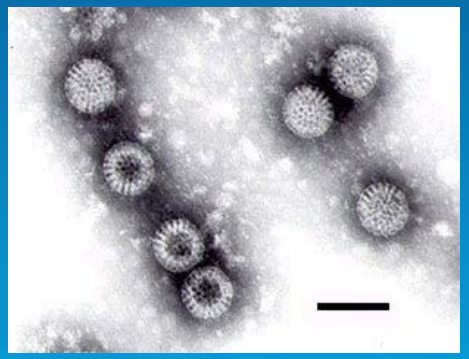
Virus evolution and diversity: Keys to their success.

Jim Gray
Professor of Clinical Virology, UEA &
Consultant Virologist, NNUH

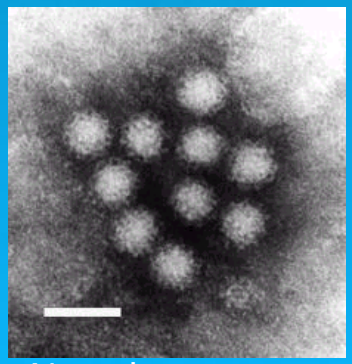
Viruses infecting the gut

Viruses associated with gastroenteritis

- rotaviruses
- caliciviruses
  - noroviruses
  - sapoviruses
- astroviruses
- adenoviruses 40, 41

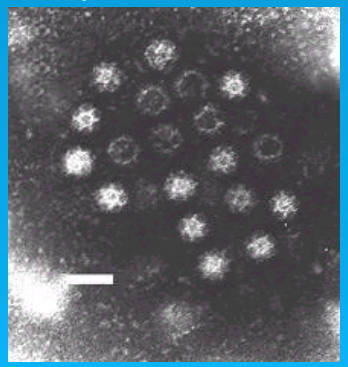


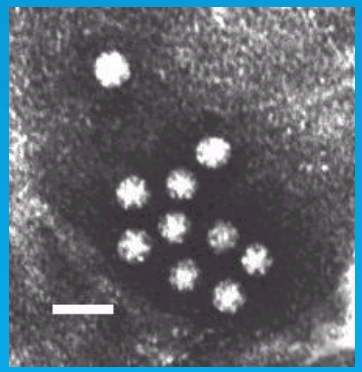
Rotaviruses



Noroviruses

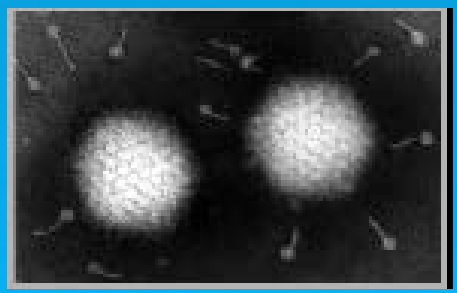
Sapoviruses



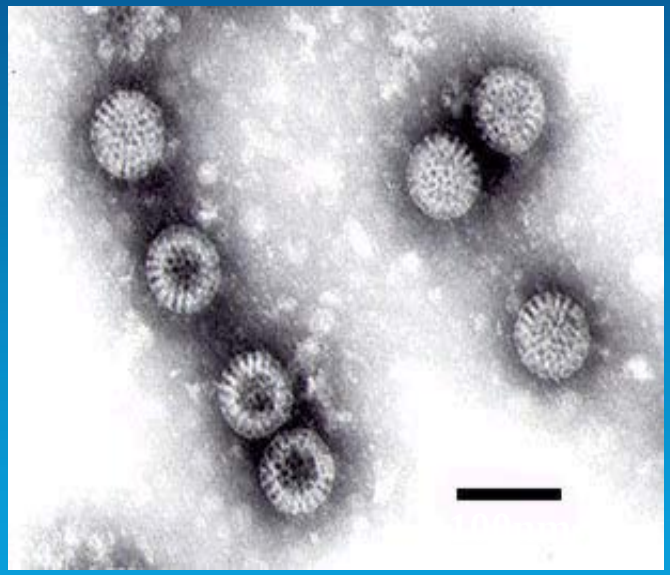


Astroviruses

Adenoviruses



Rotavirus



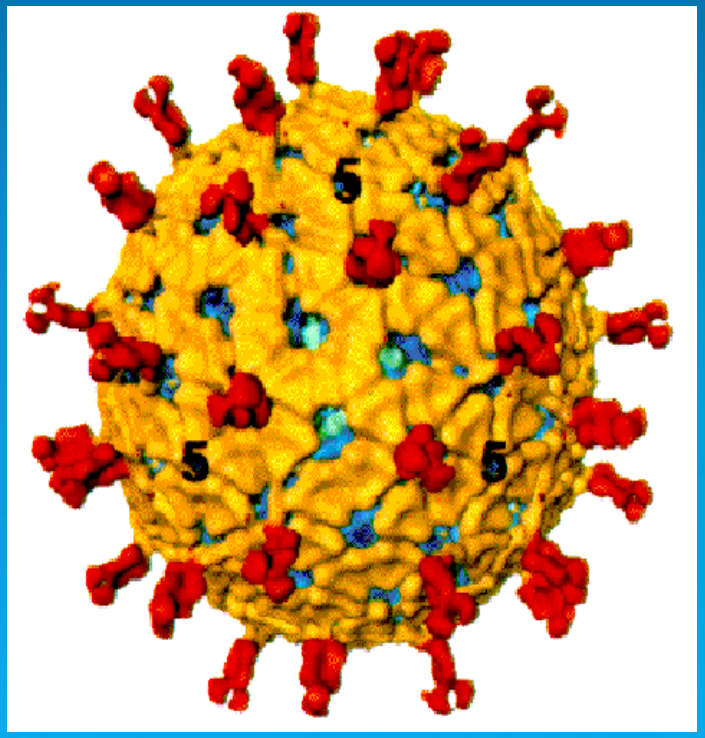
- Family *Reoviridae*
- Unenveloped, icosahedral,

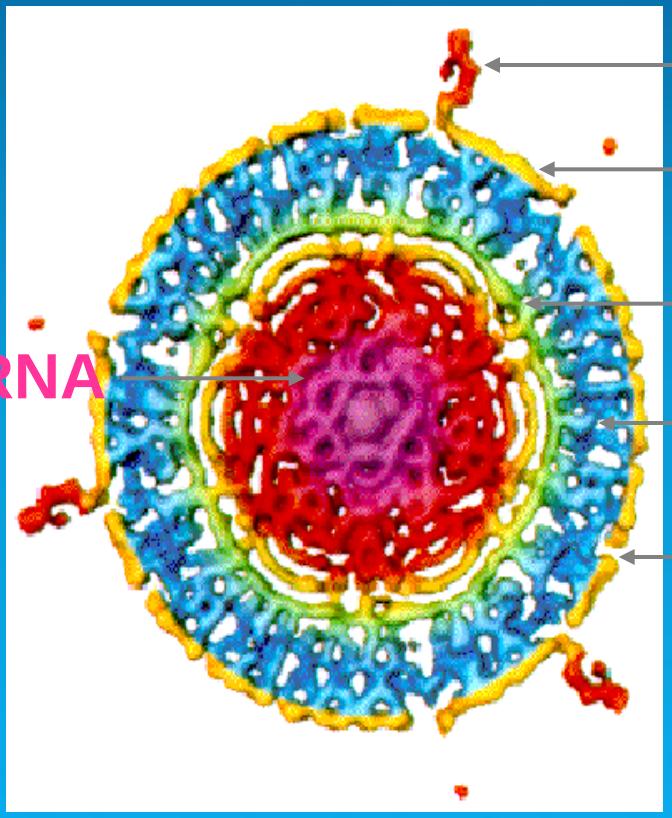
  triple layered capsid (75nm diameter)
- Genome: 11 segments of dsRNA

  ~ 18,550bp (660bp – 3300bp)

- Responsible for more than 500,000 deaths/ year in young children (1 in 285 children)

Rotavirus structure





Rotavirus evolution

Three mechanisms are important for the evolution and diversity of rotaviruses

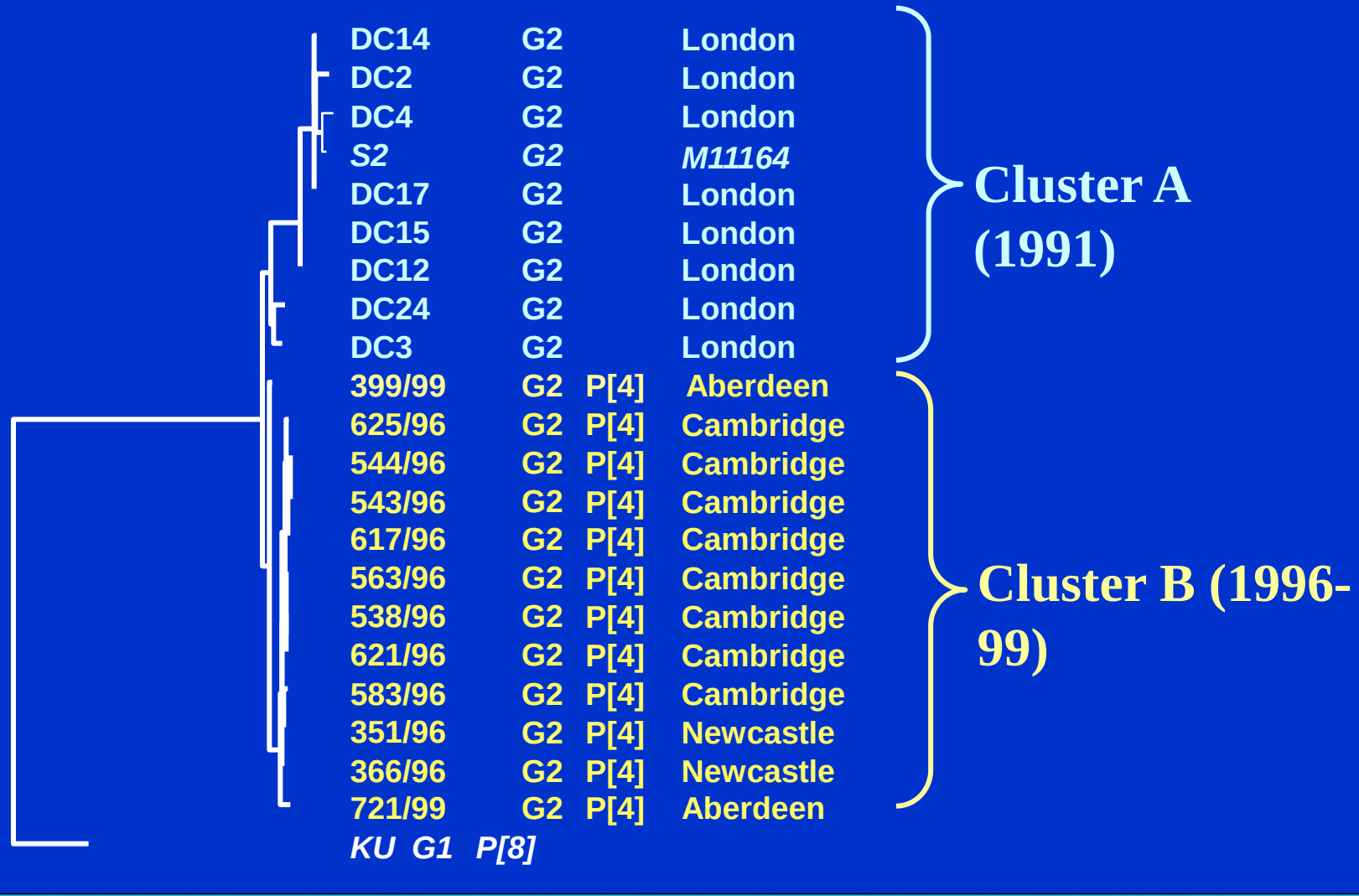
- **Genome rearrangement**
- **Antigenic drift**
- **Antigenic shift**

Rotavirus evolution

Antigenic drift:

- **Rate of mutation within rotavirus genes is relatively high because RNA replication is error-prone.**
- **A rotavirus genome differs from its parental genome by at least one mutation.**
- **Point mutations can accumulate to give rise to intratypic variation.**

Phylogenetic tree constructed with cDNA sequences of 882bp fragments of the VP7 genes of G2 rotavirus strains.



G2 rotavirus strains – VP7

Antigenic Site A

	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	aa position
DSI	A	E	A	K	N	E	I	S	D	D	E	W	E	N	T	
ES2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
HN126	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
DC2/91	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Serotyped
DC3/91	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
DC4/91	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
DC12/91	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
DC14/91	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
DC15/91	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
DC17/91	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
DC24/91	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
DC24/91	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
DC22/91	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
543/96	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	Did not serotype
544/96	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	
536/96	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	
583/96	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	
538/96	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	
621/96	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	
625/96	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	
366/91	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	
617/96	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	
351/96	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	
399/99	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	
721/99	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	

Alanine: threonine

Aspartic acid: asparagine

Rotavirus evolution

Antigenic shift:

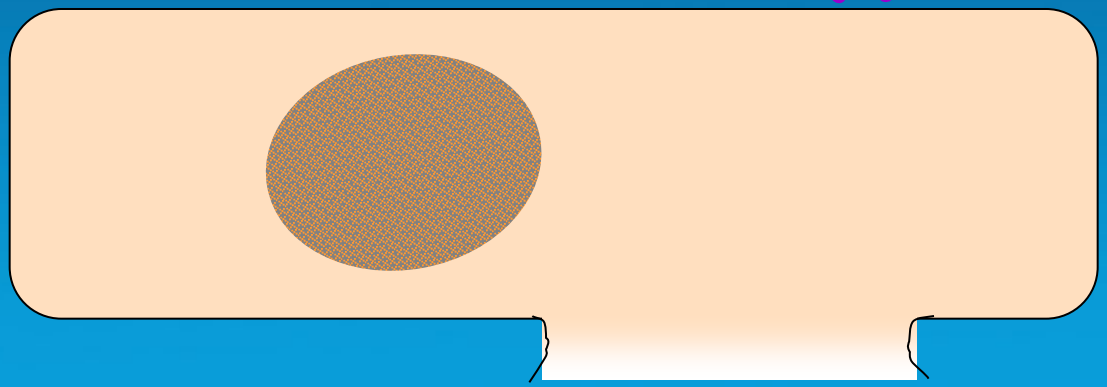
- **Shuffling of gene segments through reassortment can occur during dual infection of one cell.**
- **If reassortment occurs at random, the 11 segments of the 2 parental strains can reassort into 2^{11} possible gene combinations**
- **However, reassortment is not a random phenomenon and is influenced by the selection of progeny with viable gene combinations, the host cell and the immune status of the host.**

Rotavirus gene reassortment

G9P[6]

G1P[8]

Dual Infection of an enterocyte by 2 parent viruses (A, B)



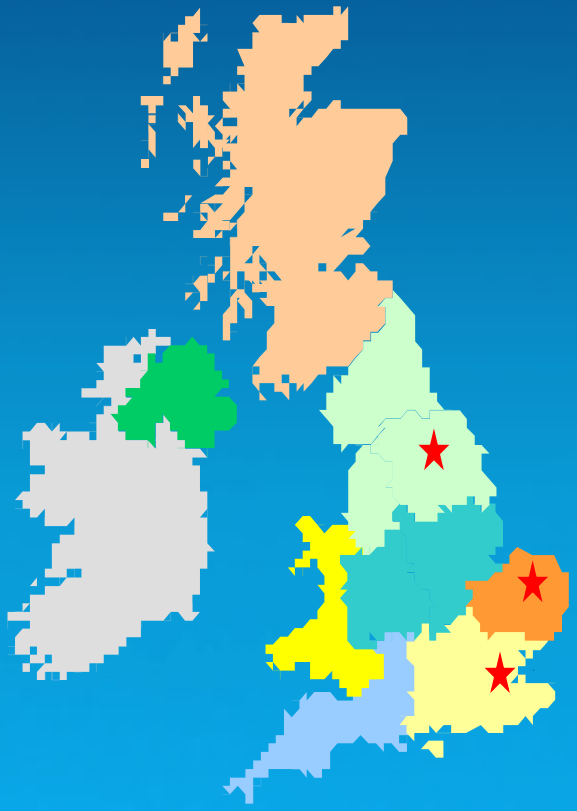
Progeny viruses

G9P[6] G9P[8] G1P[6] G1P[8]

Parental and daughter stains and multiple type combinations of rotavirus strains 1995/96

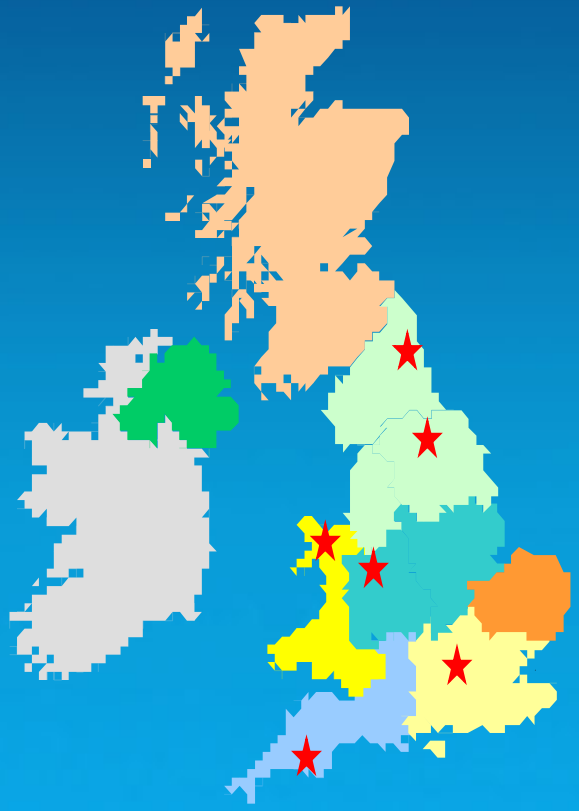
	1995/96
G1P[8]	407
G1P[6]	2
G9P[6]	18
G9P[8]	1
G1+G9/P[8]	5
G1+G9/P[6]	2

Geographical Distribution of Rotavirus G9 Strains in the UK during Three Consecutive Seasons



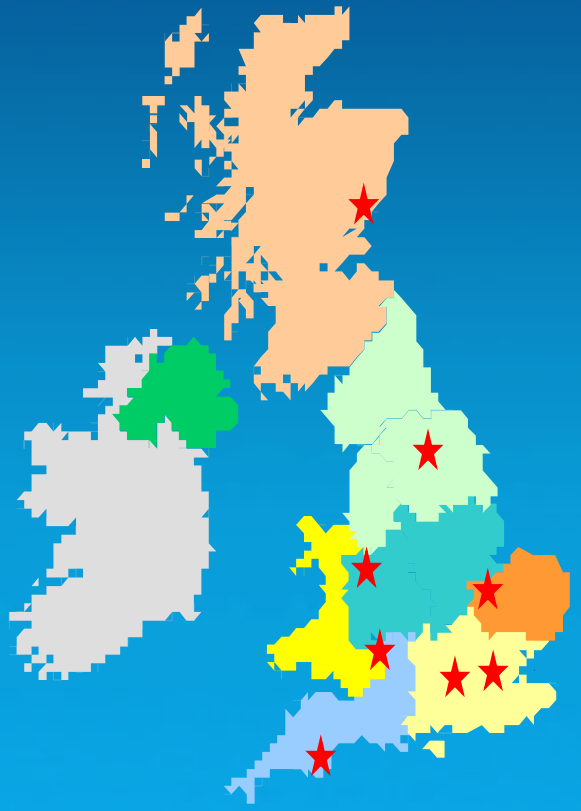
1995/96

3 locations



1996/97

6 locations



1997/98

8 Locations

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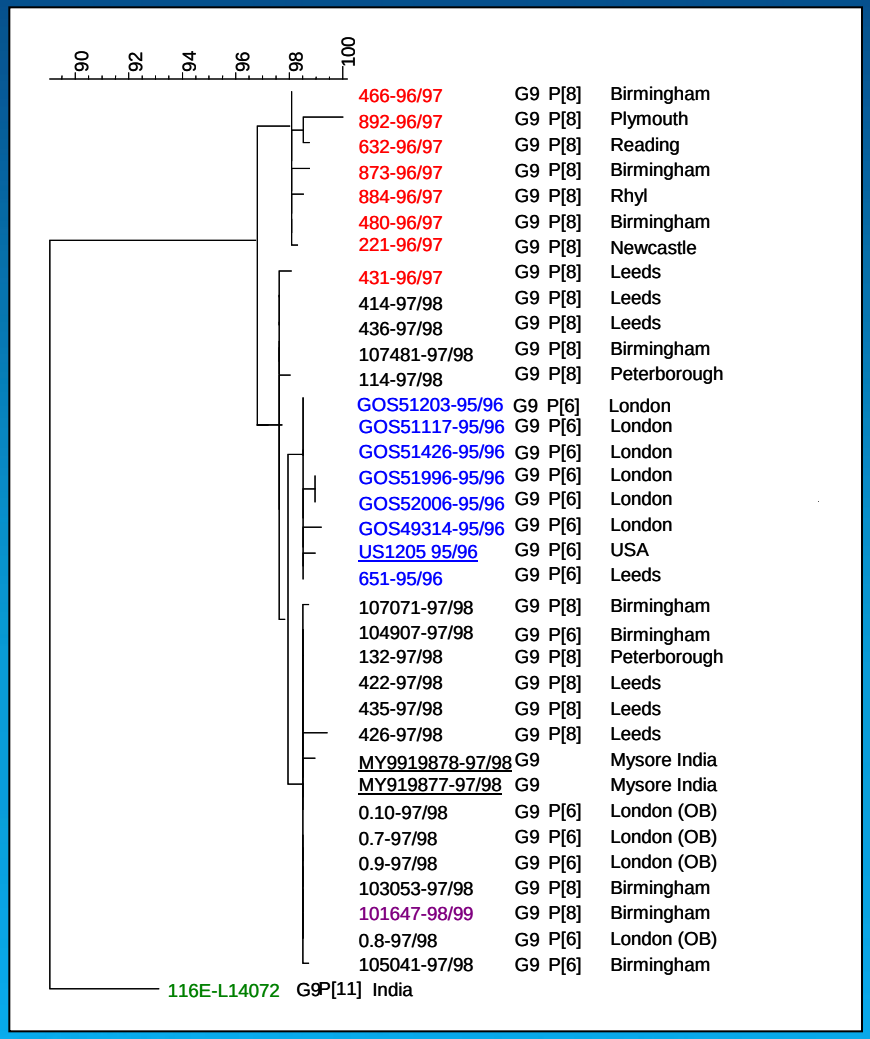
Several findings suggest that rotavirus G9P[6] strains were introduced into the human population recently

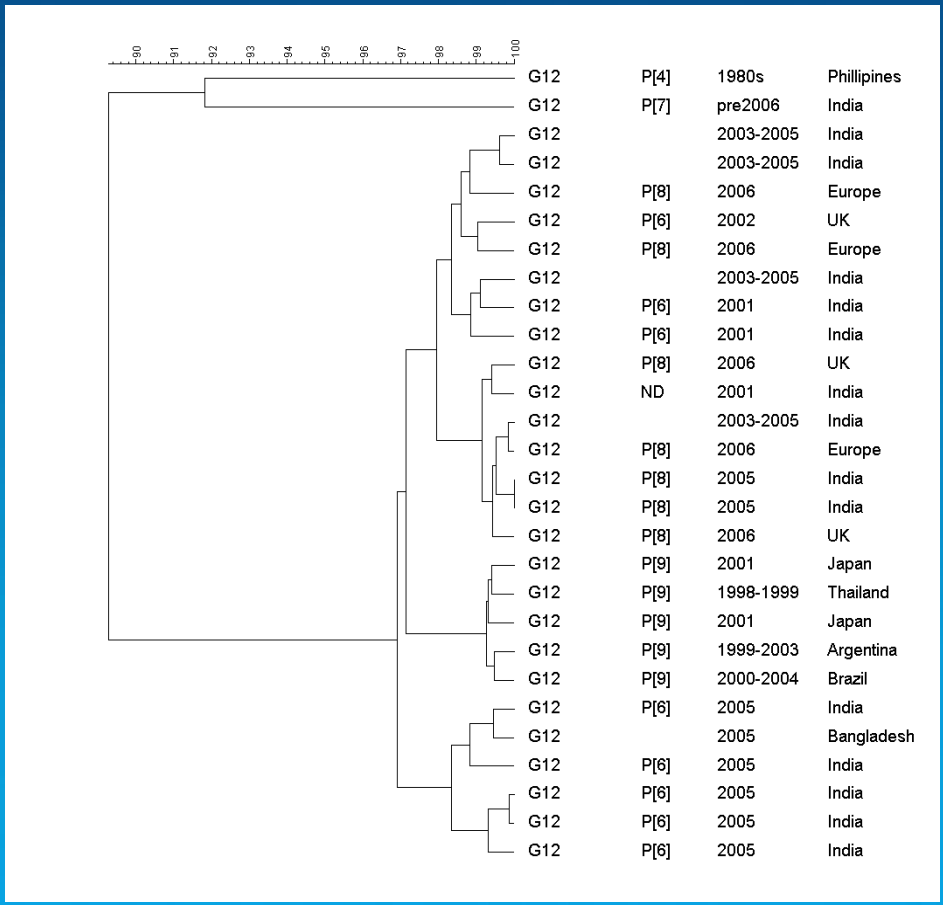
- Relative lack of diversity between the VP7 genes and the chronological clustering of all G9 strains.
- The associations between infection with G9P[8] strains and;
  - Symptomatic infections in older individuals (older children and adults)
  - More severe disease requiring hospitalisation and iv-rehydration
  - Infection in a neonatal unit (lack of maternal protection)
  - Infections predominantly in the urban population

G9 rotavirus strains



- Relative lack of diversity between the VP7 genes and the chronological clustering of all G9 strains.
- May suggest single introduction

G12 rotavirus strains



- G12 found in association with many P-types
- G12 found throughout the world
- Does this represent multiple introductions?
- Can we identify an animal reservoir?

G and P Genotype Combinations (%)

	1995/96	1996/97	1997/98	1998/99	2005/06	2006/07
G1P[8]	56.9	86.5	73.1	92.1	51.8	59.7
G2P[4]	17.9	1.9	15.9	3.9	5.1	13.0
G3P[8]	6.3	4.2	0.4	0.3	9.8	5.2
G4P[8]	11.6	4.2	4.7	2.0	1.5	1.4
G1P[4]	1.5	1.2	0.8	0.1	0.0	0.3
G2P[8]	0.8	0.0	0.3	0.0	0.5	1.0
G4P[4]	0.1	0.2	0.2	0.0	0.2	0.0
G1P[6]	0.3	0.0	0.0	0.0	0.0	0.0
G1P[9]	0.0	0.1	0.2	0.3	0.0	0.0
G3P[6]	1.5	0.0	0.0	0.0	0.0	0.0
G3P[9]	0.1	0.0	0.0	0.0	0.0	0.0
G4P[6]	0.1	0.0	0.0	0.0	0.2	0.0
G8P[8]	0.0	0.0	0.2	0.0	0.0	0.3
G9P[8]	0.1	1.7	3.7	1.2	22.7	8.8
G9P[4]	0.0	0.0	0.0	0.0	0.0	0.4
G10P[4]	0.0	0.0	0.0	0.0	0.0	0.1
G12P[8]	0.0	0.0	0.0	0.0	2.4	1.0
G9P[6]	2.5	0.0	0.7	0.0	0.0	0.1
G12P[6]	0.0	0.0	0.0	0.0	0.0	0.1

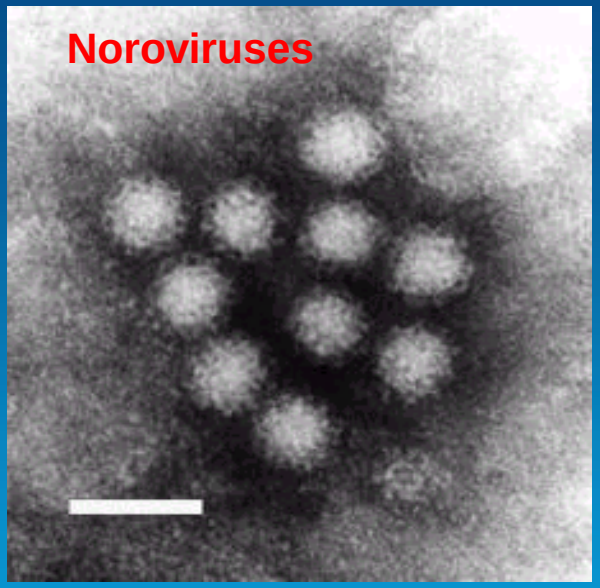
Common genotypes

Reassortment among common genotypes

Reassortment between animal and human genotypes

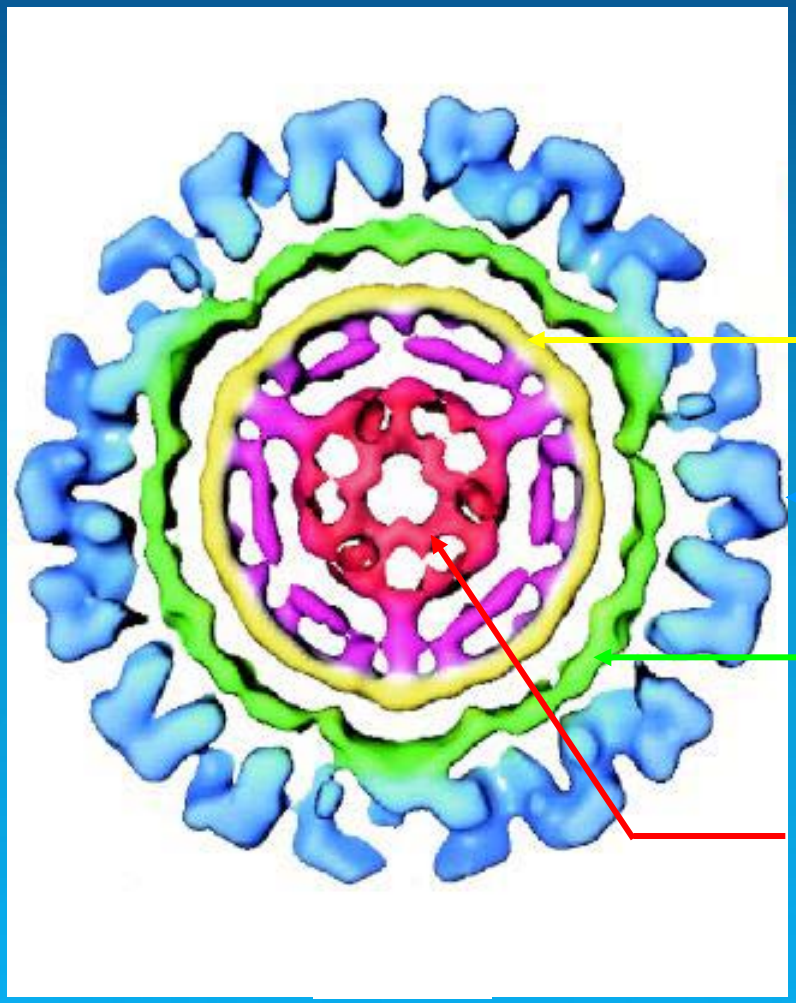
Zoonotic introduction

Noroviruses



- Family : *Caliciviridae*
- Non-enveloped small round structured viruses (27-32 nm diameter)
- Genome: pos sense ssRNA ~ 7.5kb
- Predominantly epidemic
- The most common cause of outbreaks of gastroenteritis

Calicivirus structure



Inner shell

P domain

S domain

RNA genome

Mechanisms generating diversity among noroviruses

Genetic Recombination

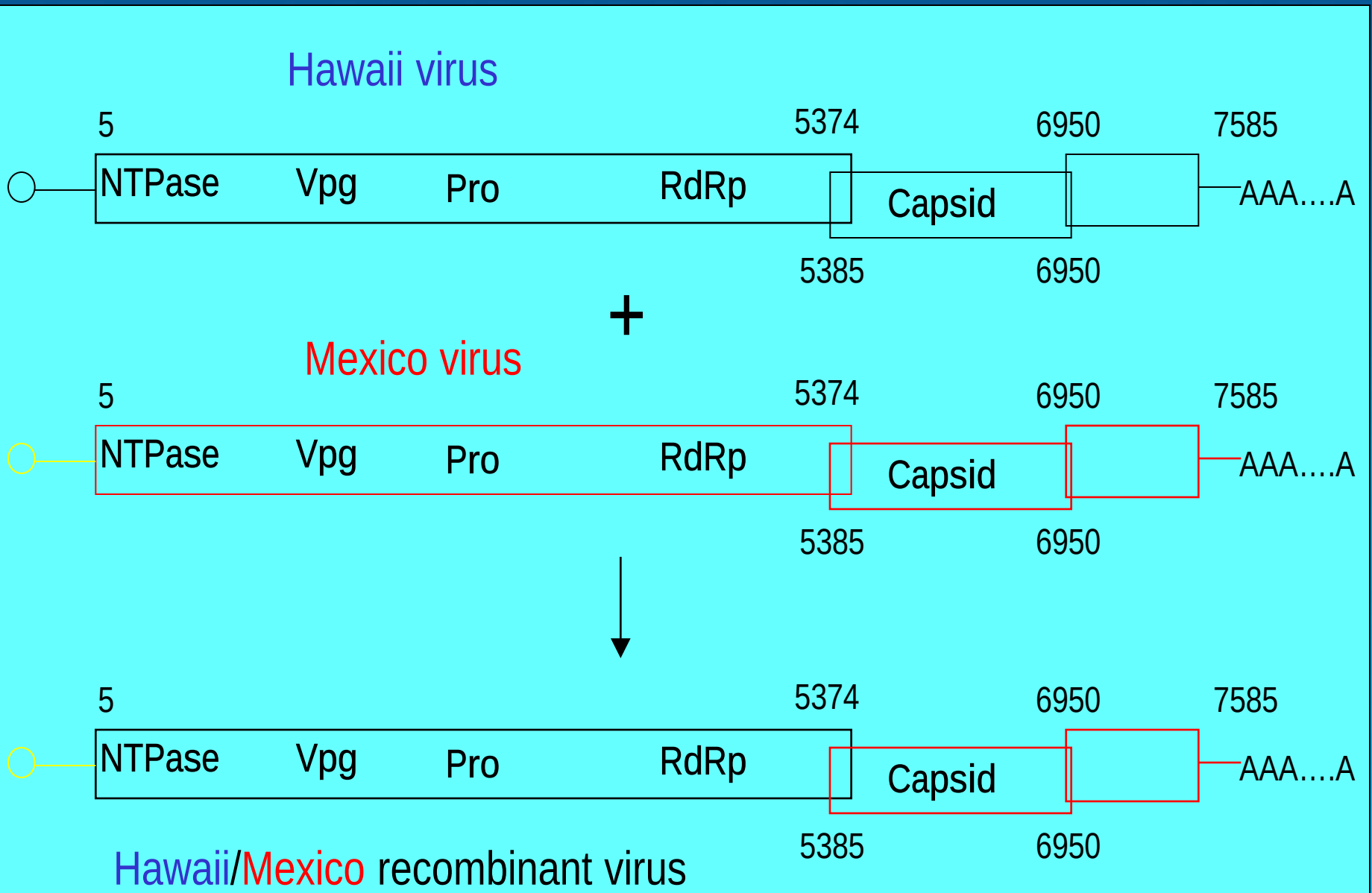
Requirements

- co-infection of a single cell
- relatedness of parental strains

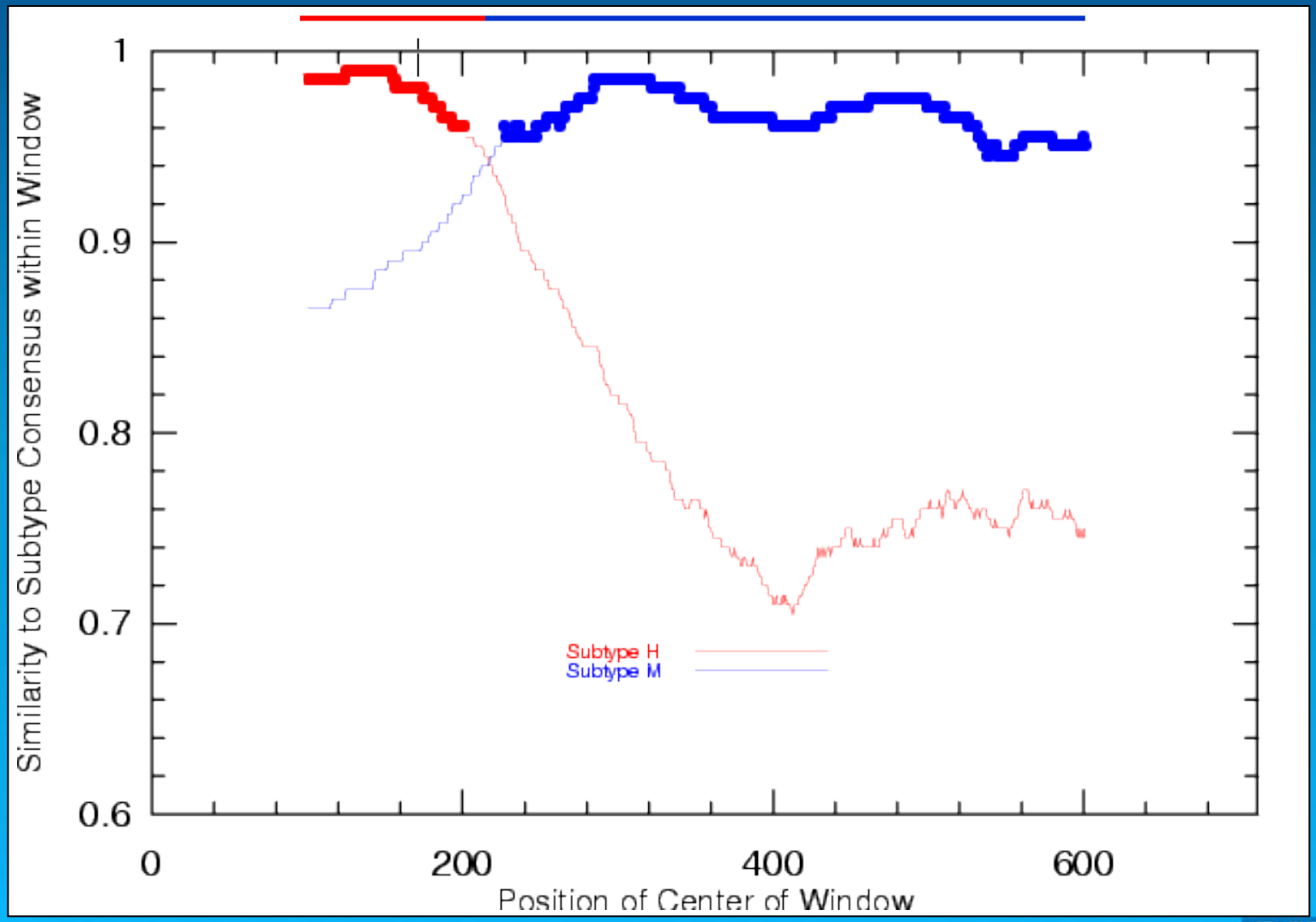
Noroviruses

- endemic co-circulation of genotypes
- multiple infections associated with food and water borne spread
- faecal-oral route of transmission
- limited heterotypic protection
- absence of long term immunity

Norovirus recombination



GII-1 and GII-3 Recombinant norovirus



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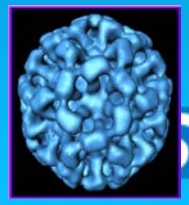
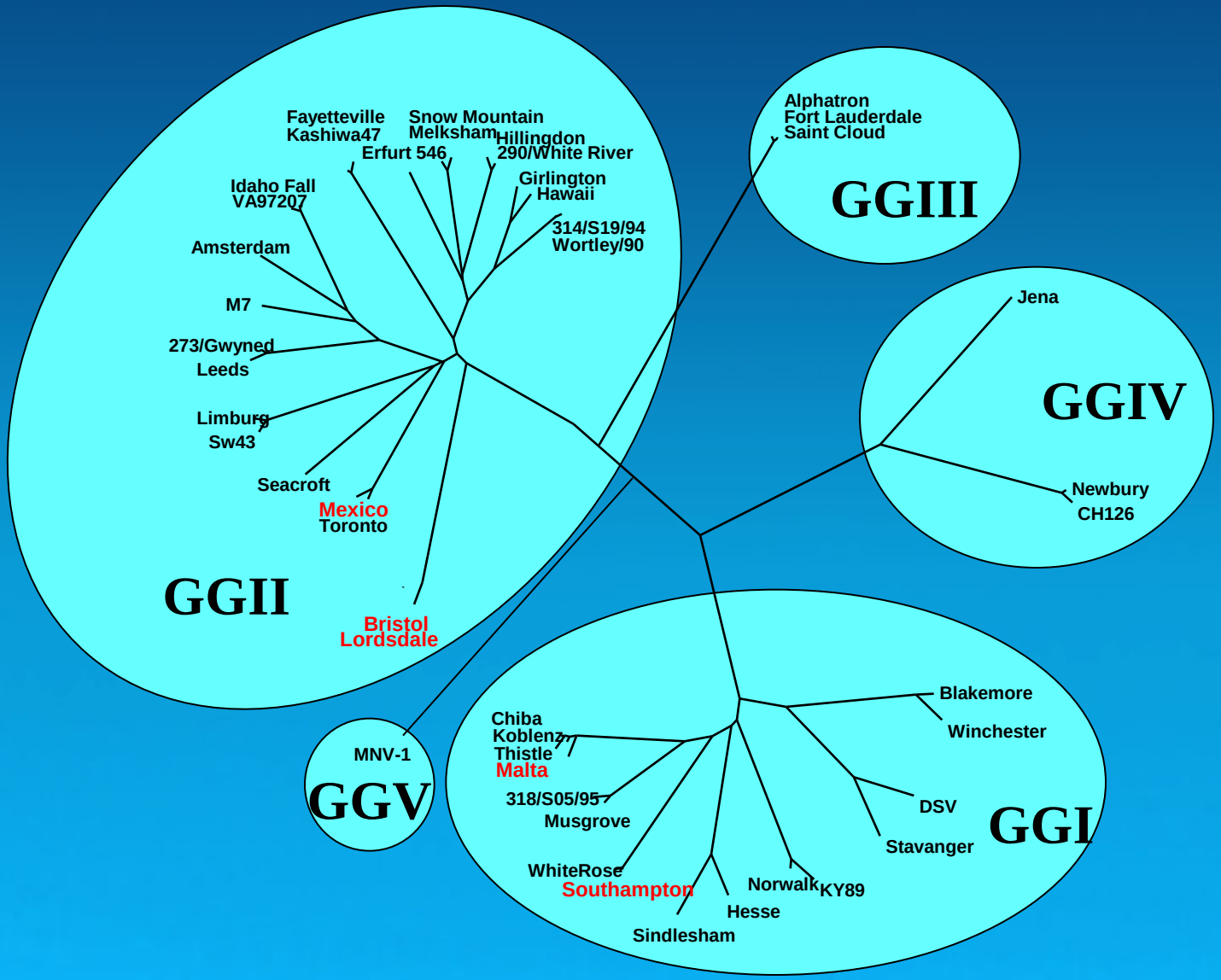
Mechanisms generating diversity among noroviruses

- Accumulation of point mutations
  - emergence of variants
  - antibody escape mutants



Phylogenetic grouping among noroviruses

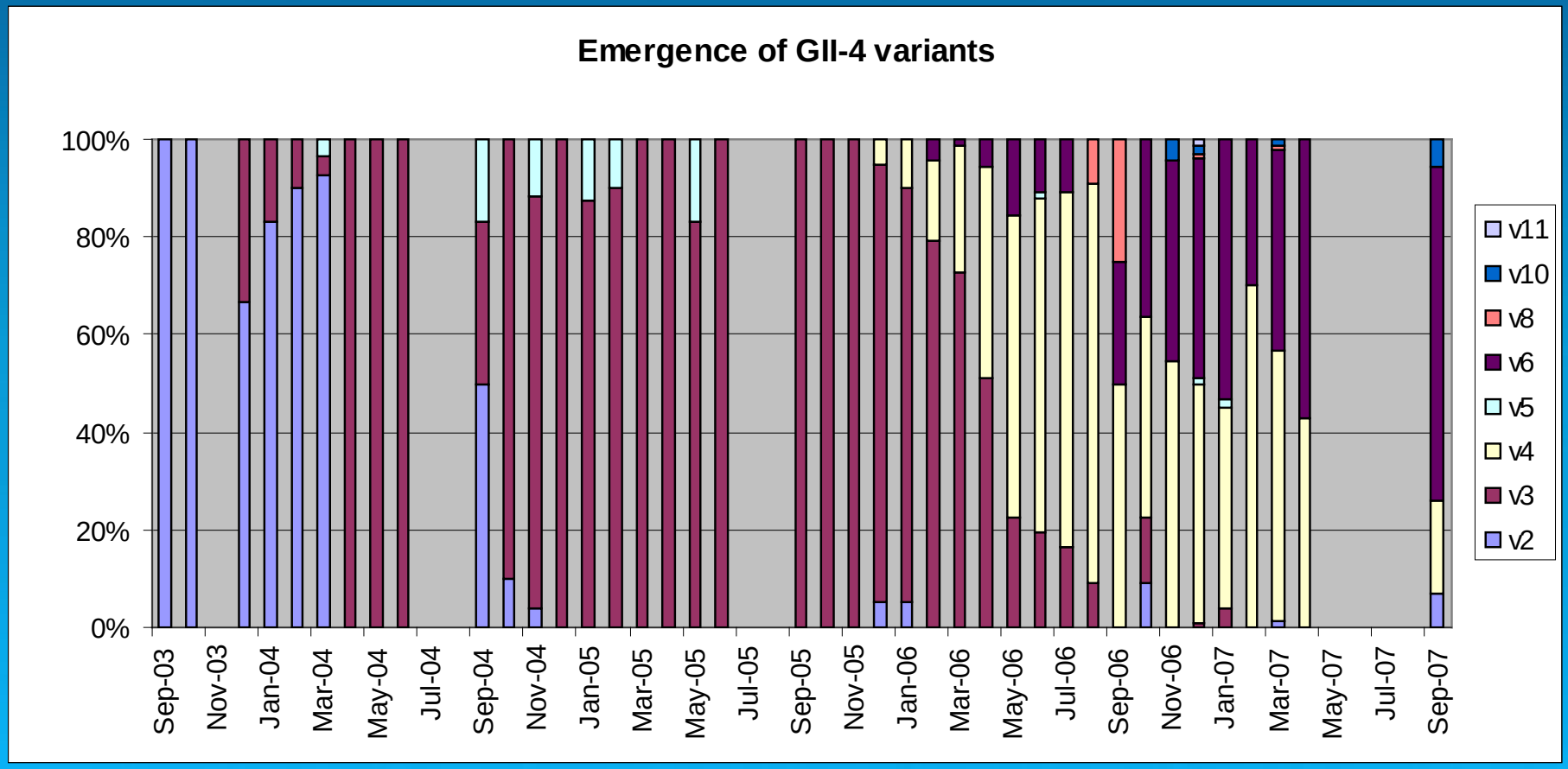


Inter- and Intra-seasonal diversity of NoV genotypes during 2003 to 2006. Early, mid and late season outbreaks characterised.

Genotype	2003/04			2004/05			2005/06		
	Early	Mid	Late	Early	Mid	Late	Early	Mid	Late
GI-1	0	0	0	0	0	0	0	1 (5%)	0
GI-2	1 (5%)	0	0	0	0	0	0	0	0
GI-3	1 (5%)	0	0	0	0	0	0	1 (5%)	0
GI-4	0	0	0	0	0	0	0	3 (15%)	0
GI-6	2 (10%)	0	0	0	0	0	0	0	0
GII-1	0	0	0	0	0	1 (5%)	0	1 (5%)	0
GII-2	3 (15%)	1 (5%)	0	4 (20%)	2 (10%)	0	0	1 (5%)	0
GII-3	7 (35%)	3 (15%)	2 (10%)	0	0	0	0	2 (10%)	0
GII-4	1 (5%)	14 (70%)	18 (90%)	14 (70%)	18 (90%)	18* (90%)	9 (45%)	7 (35%)	18 (90%)
GII-6	1 (5%)	0	0	2 (10%)	1 (5%)	0	2 (10%)	0	1 (5%)
GII-7	3 (15%)	2 (10%)	0	0	0	1(5%)	6 (30%)	3 (15%)	1 (5%)
GII-8	1 (5%)	0	0	0	0	0	0	1 (5%)	0
Total	20	20	20	20	20	20	20	20	20
Total of genotypes	3 GI 6 GII	4 GII	2 GII	3 GII	3 GII	2 GII	4 GII	3 GI 6 GII	3 GII

Early = September/October, Middle = December, Late = March, GII = Genogroup II, GI = Genogroup I, * = February and March

GII-4 variants: September 2003 to September 2007



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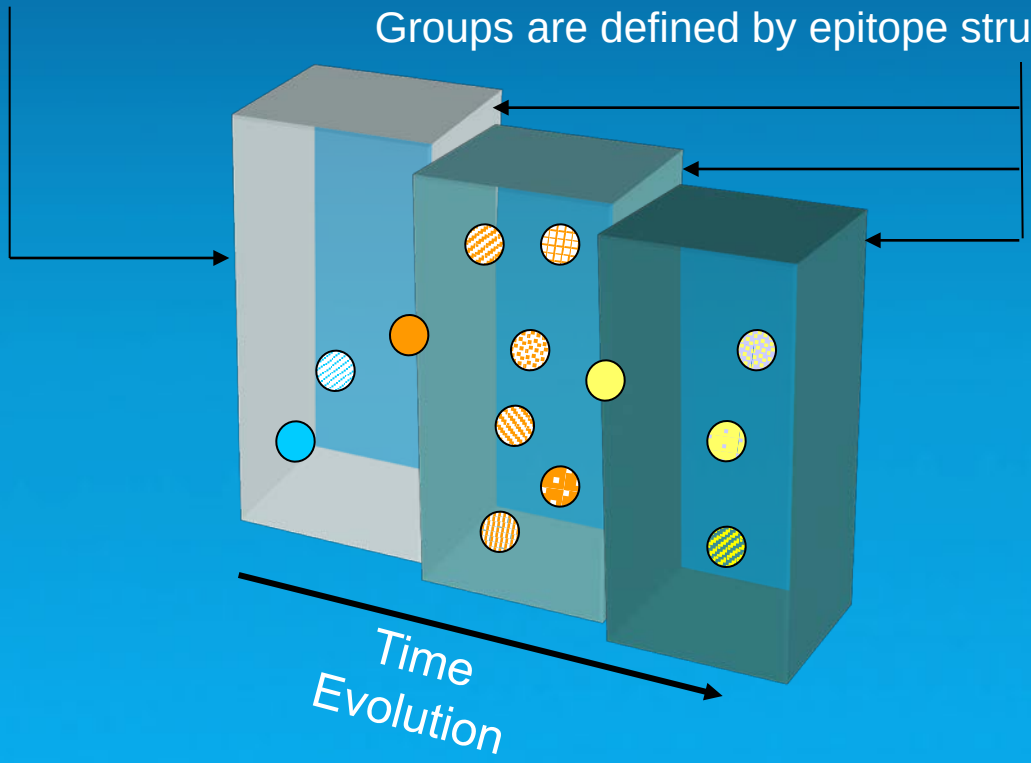
Neutral Networks: A model for NoV evolution

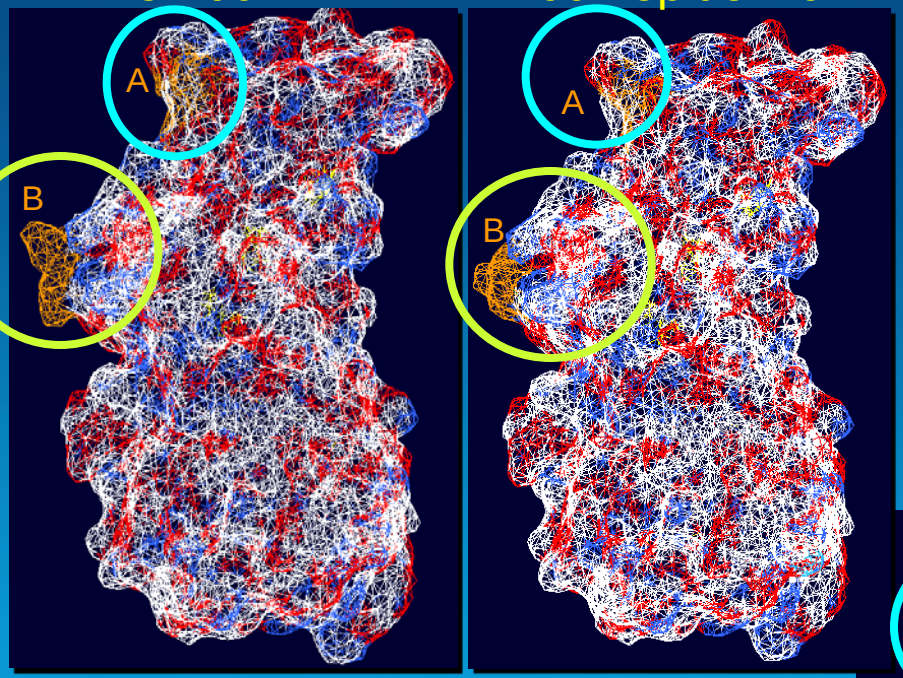
- Method of representing random neutral drift between related proteins

Genotype populations that are linked by point mutation but are selectively neutral

Groups are defined by epitope structure, not sequence diversity

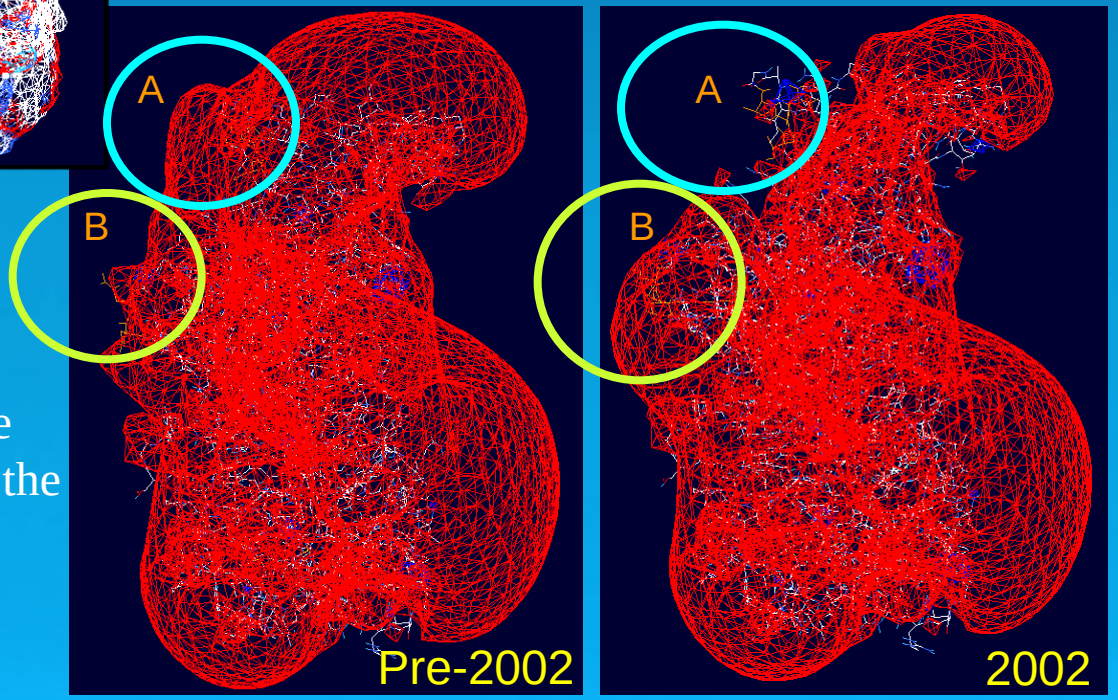


Structural changes on the P2 domain between GII-4 variants



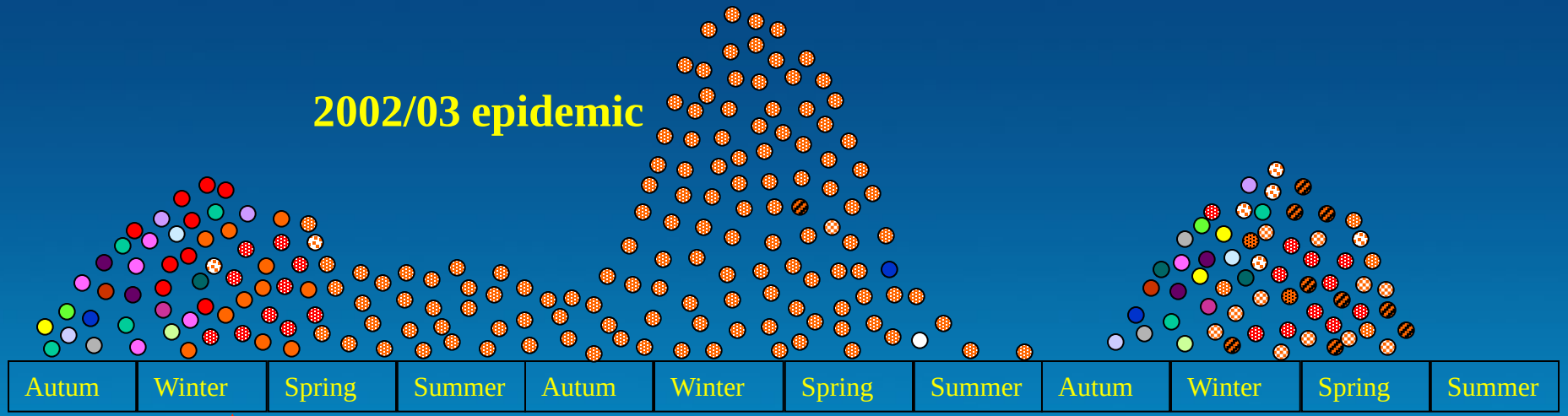
Molecular surface

Electrostatic surface



Monoclonal antibodies raised against the pre-2002 GII-4 strains do not react with the 2002 GII-4 strain and *vice versa*.

2002/03 epidemic



Normal winter season

Unusual summer activity

Epidemic winter season

Normal summer activity

Normal winter season

Narrowing diversity: GII4 predominates

GII4 variants emerge

GII4 variant is selected, out of season outbreaks occur, becomes epidemic

Return to normal season, wide diversity at the beginning, narrowing as season progresses.

Lack of short-term herd immunity to a new variant

GII4 dominate and have an advantage over other co-circulating genotypes.

- replicative advantage
- greater transmissibility associated with a lower infectious dose
- larger proportion of the population susceptible through inherited genetic factors,
- better survival of the virus in the environment,
- a mechanism that allows the virus to evade immune surveillance to some degree.

•Population protected in the short term against variant GII4

•Population susceptible to other genotypes due to short-term immune protection.

Keys to the success of gastroenteric viruses

- Low infectious dose ~ 10-100 virus particles
  - Noroviruses 10^7 particles per gram/ml
  - Rotaviruses 10^{11} particles per gram/ml
- Stability in the environment
  - Norovirus survives up to 80^{o}C
  - Rotavirus survives in the environment for months
- Protected by the matrix – faeces and vomit, which also inactivate chlorine-based disinfectants
- Non-enveloped viruses – resistant to many disinfectants and alcohol
- Short term immunity ~ 6 months
- 16% of the population excrete NoV in the absence of symptoms
- 14% of the population excrete rotavirus in the absence of symptoms

- Multiple routes of transmission - person to person contact, through ingestion of contaminated water or food or by contact with contaminated environmental surfaces
- RNA genome replication results in the accumulation of point mutations
- Segmented rotavirus genome replication results in reassortment
- Dual infections can result in recombination or reassortment
- Rotaviruses are associated with zoonotic infection
- Hospital-acquired infections are predominantly associated with GII-4
- There are multiple introductions into hospitals, of variants of GII-4, throughout the NoV season and many rotavirus strains co-circulate in the human population
- Antibody-escape mutants and rotavirus reassortants are selected and driven by herd immunity resulting in epidemics in an immunologically naive population

Acknowledgements

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Stephen Brolley
Emma Meader

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