Studies of Norovirus in Hong Kong

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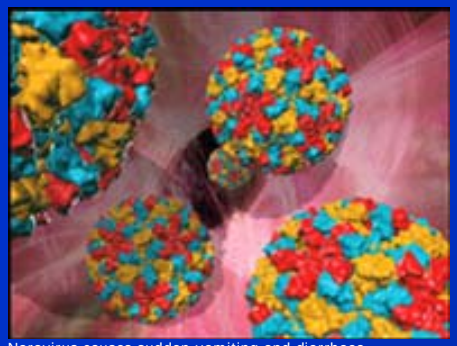
BBC(3.1.08) Stomach bug sweeping the country

People struck down by a stomach bug sweeping the UK have been urged not to go back to work.



Norovirus causes sudden vomiting and diarrhoea

Doctors estimate more than **100,000** people a week are catching norovirus, which causes diarrhoea and vomiting.

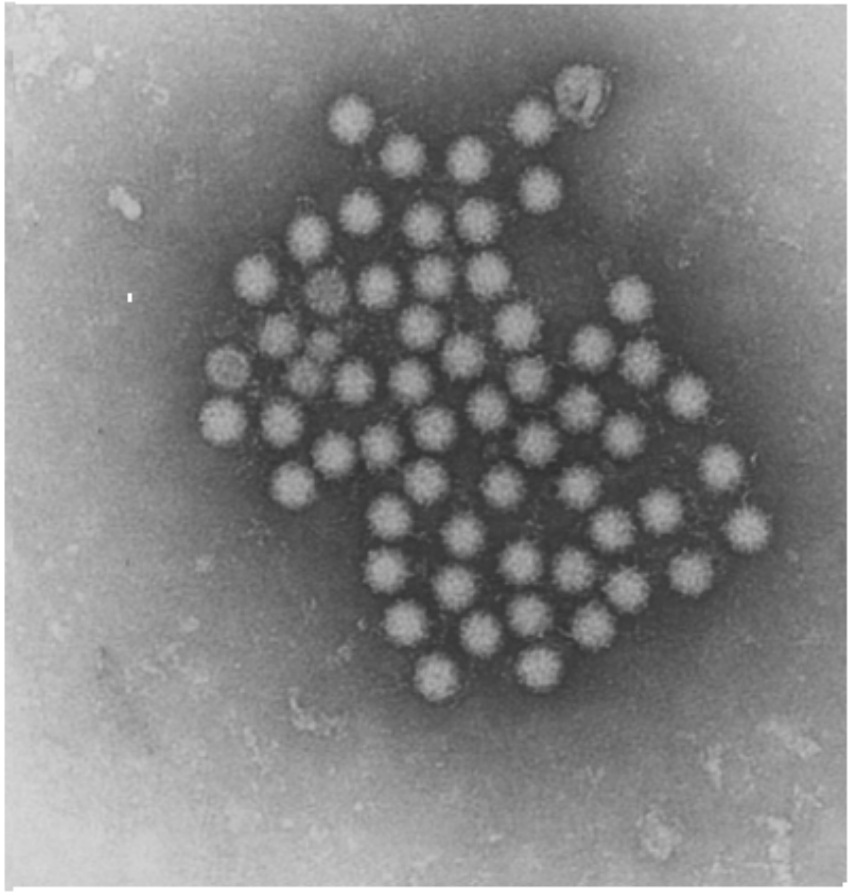
Epidemiology of Norovirus

- Infection occurs worldwide more likely in the winter months.
- UK study showed very high level of unreported infections, estimated to be 1562:1, in contrast to Campylobacter 7.6:1, and Salmonella 3.2:1.
- Highly infectious, faecal-oral or person-to-person spread as well as aerosol spread
- Very small infectious dose: 10-100 particles
- Not inactivated by pH3, ether, 60^{o}C for 60 min; partially inactivated by 70% alcohol; inactivated by chlorine
- Associated with consumption of shellfish or other contaminated foods or water

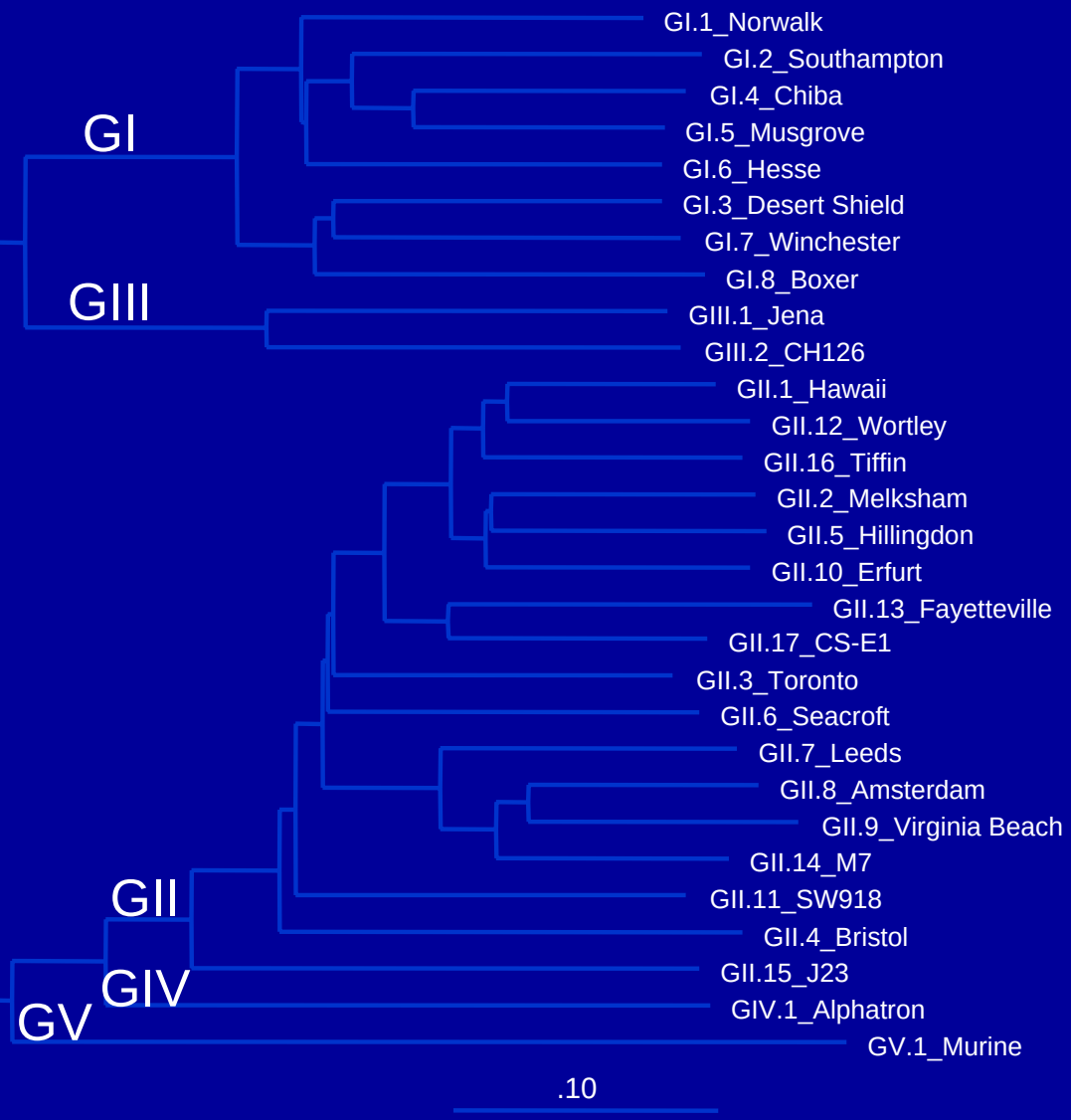
Caliciviruses

- Four genera of Caliciviruses are recognized.
  - Norovirus (Norwalk-like viruses)
  - Sapovirus (Sapporo-like viruses)
  - Lagovirus (Rabbit)
  - Vesivirus (Feline)
- Virions 27-40nm in diameter with the characteristic “Star of David” appearance
- Single-stranded RNA genome with 3 reading frames
- Although different serotypes had been ascribed to norovirus and sapovirus, the exact antigenic relationships have yet to be determined.

Electron Micrograph of Norovirus



Genogroups and Clusters of Norovirus



Clusters differ by ≥ 20% amino acid pairwise distance

Genogroups differ by 44-55% amino acid pairwise distance

From Steve Monroe, US CDC

Laboratory Diagnosis

- Viruses are non-culturable
- Electron microscopy
  - Direct EM
  - IEM
- Antigen detection
- Virus detection by RT-PCR

Constrains on Designing an enzyme immunoassay for Norovirus Detection

- Norovirus is highly diversified
  - 8 genotypes/clusters in G.I,
    17 genotypes/clusters in G.II
  - Recombination among the capsid genes has been reported
  - Difficult to identified a conserved epitope to produce antibodies for EIA detection

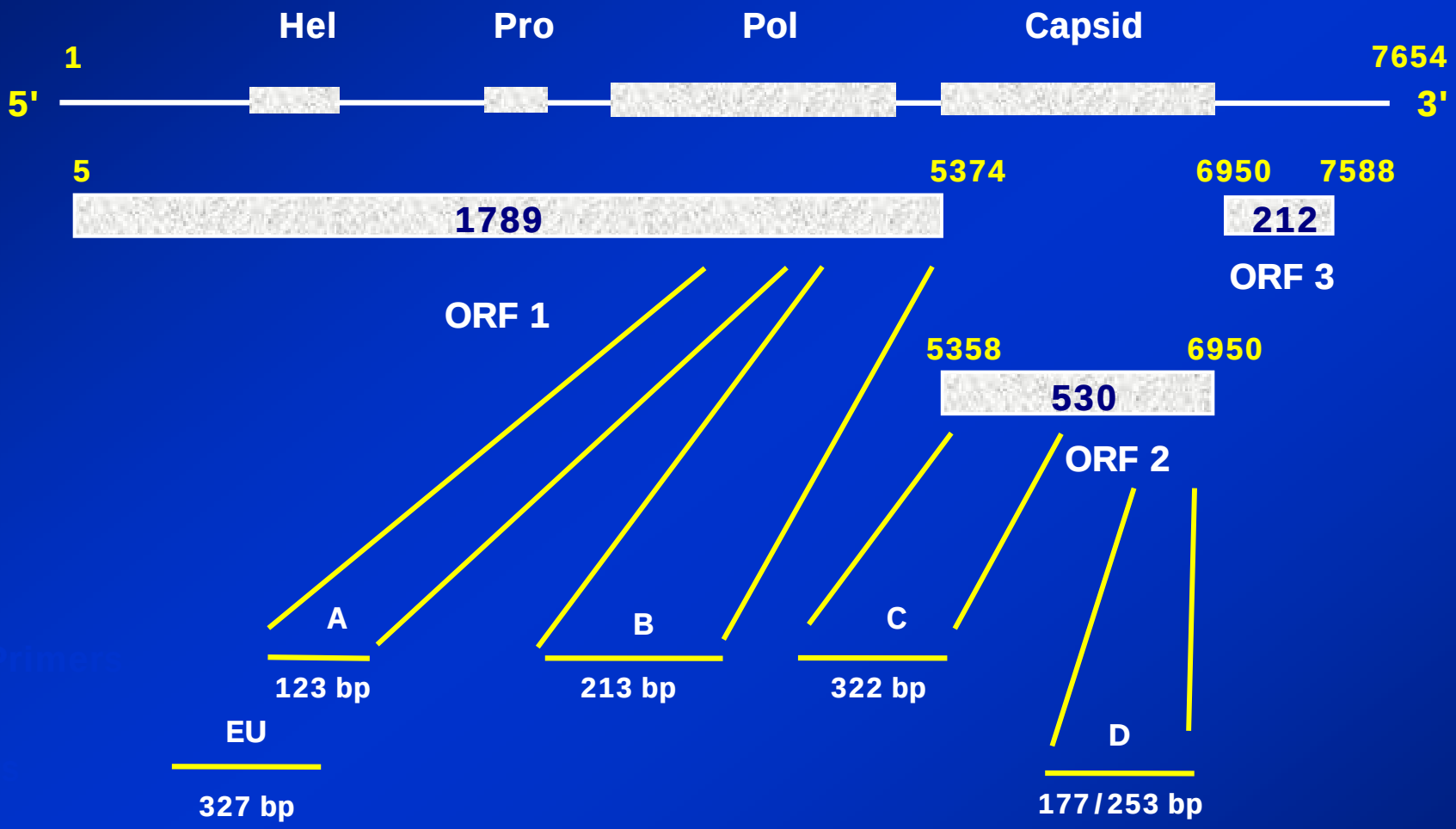
Comparison Between the Detection of Norovirus by EIA and RT-PCR

	EIA*	RT-PCR**
Sensitivity	30/68=44%	68/68=100%
Specificity	19/20=95%	20/20=100%

* R-Biopharm Ridascreen Norwalk-like virus EIA kit was used.

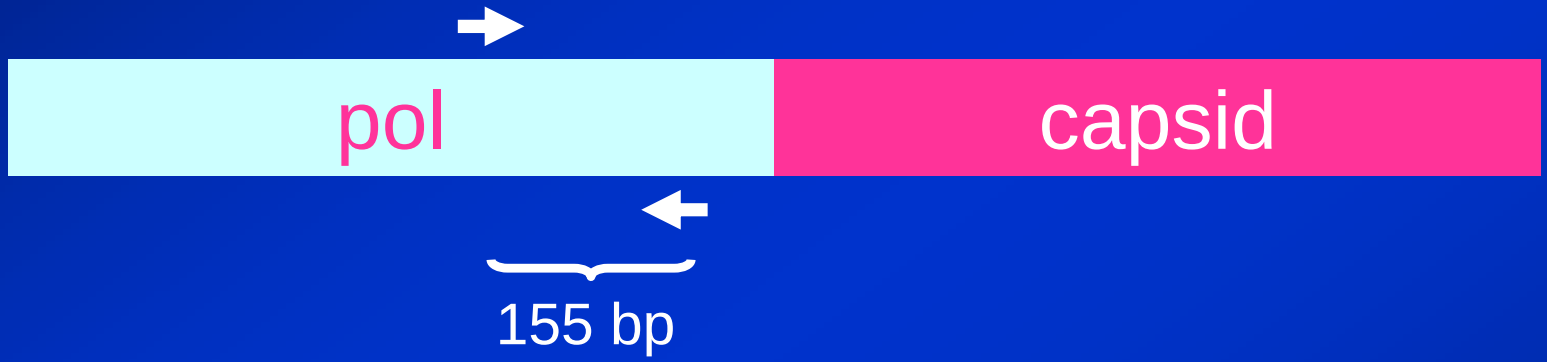
** Primers used for RT-PCR detection are located in the polymerase region.

Norovirus Detection by RT-PCR

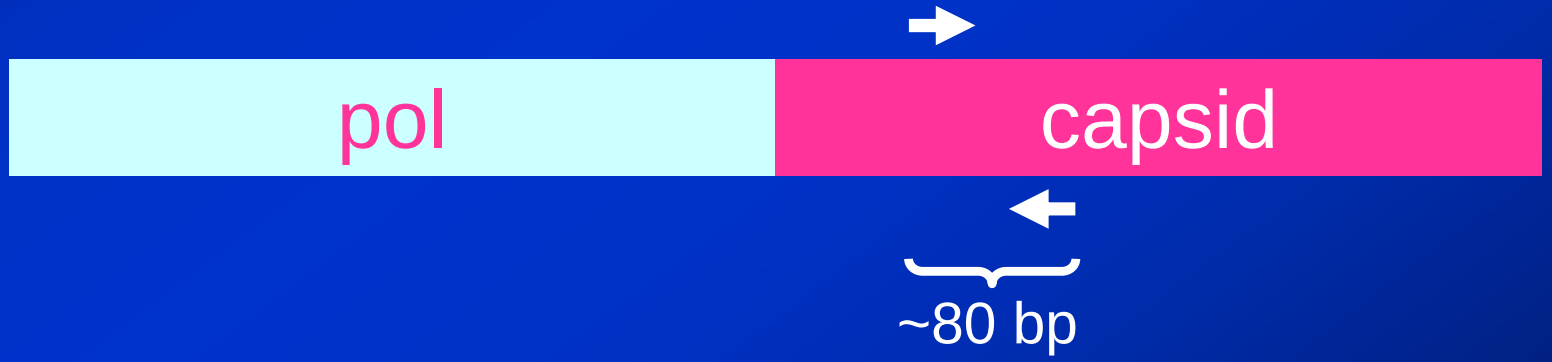


Detection of Norovirus by Real-time PCR

Conventional RT-PCR



Real-time RT-PCR



Sensitivity testing showed that real-time PCR is 20-fold more sensitive than conventional RT-PCR

Comparison Between the Detection of Norovirus by Conventional and Real-time RT-PCR

Real-time PCR	Conventional PCR Positive	Negative
Positive	100	0
Specificity	0	30

Primers targeted at capsid region

Detection Limit Comparison

Test	Dilution*					
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Real-time RT-PCR	+	+	+	+	-	-
Conventional RT-PCR Genogroup I	+	+	-	-	-	-
Conventional RT-PCR Genogroup II	+	+	-	-	-	-

* Serial dilution of clinical samples

Improved Laboratory Method Used at Public Health Laboratory, Hong Kong

- In-house developed multiplex PCR to detect Norovirus and Rotavirus
- Two step real-time RT-PCR system
- Norovirus: Two pairs of primers and probes targeted at the capsid region of Norovirus genogroup I and II
- Rotavirus: Probes and primers targeted at the VP4 region

Nucleotide Sequencing

- Confirmation of standard RT-PCR results
- Limitations to RT-PCR
  - Does not identify genogroup or cluster information
- Clarification of the epidemiology of transmission in outbreaks

Prevalence Rate in Hong Kong(1987-1992)

- Previous study of 27618 stool samples collected from 1987 to 1992 in Hong Kong using EM and ELISA showed rotaviruses accounted for the majority of cases of gastroenteritis
  - Rotavirus 84.4 %
  - Adenovirus 9.0 %
  - Astrovirus 4.3 %
  - Noroviruses 1.9 %
  - Others 0.4 %

Situation in Hong Kong

- 1988-1999 – Approx 10 cases per year diagnosed by direct electron microscopy
- No outbreaks of gastroenteritis was associated with noroviruses
- RT-PCR was routinely performed starting in 2000
- 2000 – noroviruses responsible for 45 outbreaks of gastroenteritis

Acute Diarrhoeal Diseases Surveillance Programme

- Started in July 2001
- Acute watery diarrhoea (passage of 3 or more loose or watery stools in 24 hours) with or without dehydration
- Collection of stool within 72 hours of onset of symptoms
- Patient does not require immediate referral for admission to hospital

Methods

- Electron microscopy
- RT-PCR
- Nucleotide sequencing

Acute Diarrhoeal Diseases Surveillance

Year	No. of specimens	No. of Bacteria	No. of specimens	No. of viruses	
				NV	Rota
2001 (J-D)	565	73	600	52	7
2002	729	125	711	74	22
2003	311	60	358	35	8
2004	163	40	189	30	5
2005	93	11	123	21	3
2006	81	5	123	50	0

Results – Acute Diarrhoeal Diseases Surveillance

	No.	NV	%		No.	NV	%
<5	87	21	24.1	Jul – Sep	377	28	7.43
5 – 14	71	9	12.7	Oct – Dec	223	31	13.9
14 – 24	84	4	4.76	Jan – Mar	178	23	12.9
25 – 59	496	38	7.66	Apr - Jun	209	16	7.66
>60	249	26	10.4		987	98	9.93
Total	987	98	9.93				

Rotavirus was seen in 29 (2.9%) of specimens of which 2 were also positive for norovirus RNA. There were no significant differences between sexes.

Gastroenteritis in Communities and Sentinel General Practices in the UK

- 1993-96
- Community cohort and GP based incidence studies
- 70 general practices
- Surveillance of 9776 randomly selected patients
- 1 in 5 persons had AGE
- 1 in 6 with AGE sought care
- Bacteria 23.8%
- Viruses 22% --Rotavirus 7.9%
  --SRSV 8%
- Case to reporting ratio – Campylobacter 1:7.6
  -- Salmonella 1:3.2
  -- Rotavirus 1:35
  -- SRSV 1:1562

Gastroenteritis in Sentinel General Practices in the Netherlands

- 1996-9
- 34 General practice
- 1 in 125 persons per year sought care for AGE
- Higher incidence in those aged less than 5
- Bacteria 15.73%
- Viruses 15.4% -- Rotavirus 5.3%
  -- SRSV 7 %

Strain Surveillance of Norovirus

- Started in July 2001
- Included NV in specimens from

  Acute Diarrhoeal Diseases Surveillance Programme of the Department of Health.

  Public hospitals in Hong Kong.

  Outbreaks reported to the Department of Health, Hong Kong Government.

a. Surveillance Cases (2001-2002)

	Gp I	BV(GII.4)	TV(GII.3)	GWV(GII.7)	Gp II	Gp I+GpII	Total
Jul-Sep	4	15	0	0	1	0	20
Oct-Dec	5	19	1	1	3	0	29
Jan-Mar	7	5	3	2	5	0	22
Apr-Jun	4	3	0	1	3	0	11
Total	20	42	4	4	12	0	82

b. Clinical Cases (2001-2002)

	Gp I	BV(GII.4)	TV(GII.3)	GWV(GII.7)	Gp II	Gp I+GpII	Total
Jul-Sep	1	26	0	1	0	0	28
Oct-Dec	2	63	1	1	0	0	67
Jan-Mar	1	3	3	3	2	0	12
Apr-Jun	1	2	0	0	1	0	4
Total	5	94	4	5	3	0	111

c. Outbreaks (2001-2002)

	Gp I	BV(GII.4)	TV(GII.3)	GWV(GII.7)	Gp II	Gp I+GpII	Total
Jul-Sep	0	10	0	0	2	0	12
Oct-Dec	0	15	0	1	1	0	17
Jan-Mar	3	3	1	1	3	1	12
Apr-Jun	1	1	0	1	0	0	3
Total	4	29	1	3	6	1	44

Genetic Diversity of Noroviruses in Hong Kong

- The distribution of clusters and genogroups was similar between the surveillance and outbreak cases
- For the first six months of the study period, the predominant strain belonged to the Bristol cluster of Genogroup II (GII.4). This strain was associated with pandemic infection from 1995
- In the later six months of the study, Genogroup I and strains belonging to other clusters of Genogroup II were more commonly seen

Surveillance of Norovirus Strains

- Stool specimens from sporadic cases and outbreaks from Jan 2002 to Dec 2004 were studied:
  - 2002 – 1302 specimens, 76 outbreaks
  - 2003 – 2086 specimens, 96 outbreaks
  - 2004 – 4185 specimens, 164 outbreaks
- RT-PCR was performed as in the previous study
- 408 positive specimens were sequenced.

Norovirus Activity in Hong Kong

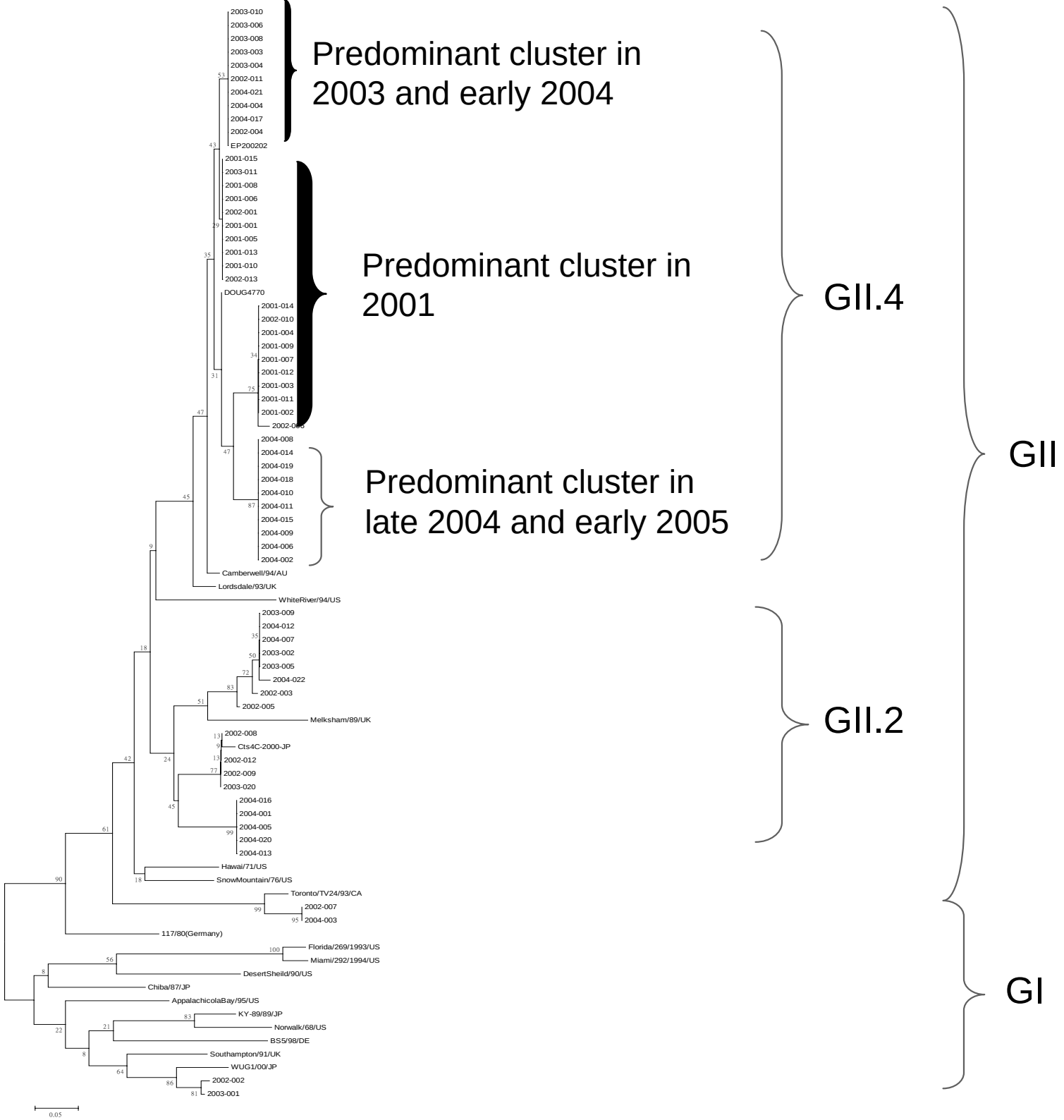
- A new genogroup II.4 cluster, same as that causing epidemic in Europe during 2002, first emerged in 2002, became the predominant cluster in 2003 and first 6 months of 2004
- Another variant of genogroup II.4 was identified in July 2004 and became the predominating cluster in outbreaks and sporadic cases. Similar strain was reported to cause outbreaks in Australia in April-July and Netherlands since August

Norovirus Activity in Hong Kong

- >90% of specimens analyzed belong to genogroup II.
- Most of the identified genogroup II specimens are in genogroup II.2 and II.4.
- Predominant clusters in 2001, 2003 and 2004 are in genogroup II.4.

TABLE II. Predominant NV strains in Hong Kong, 2001-2005.

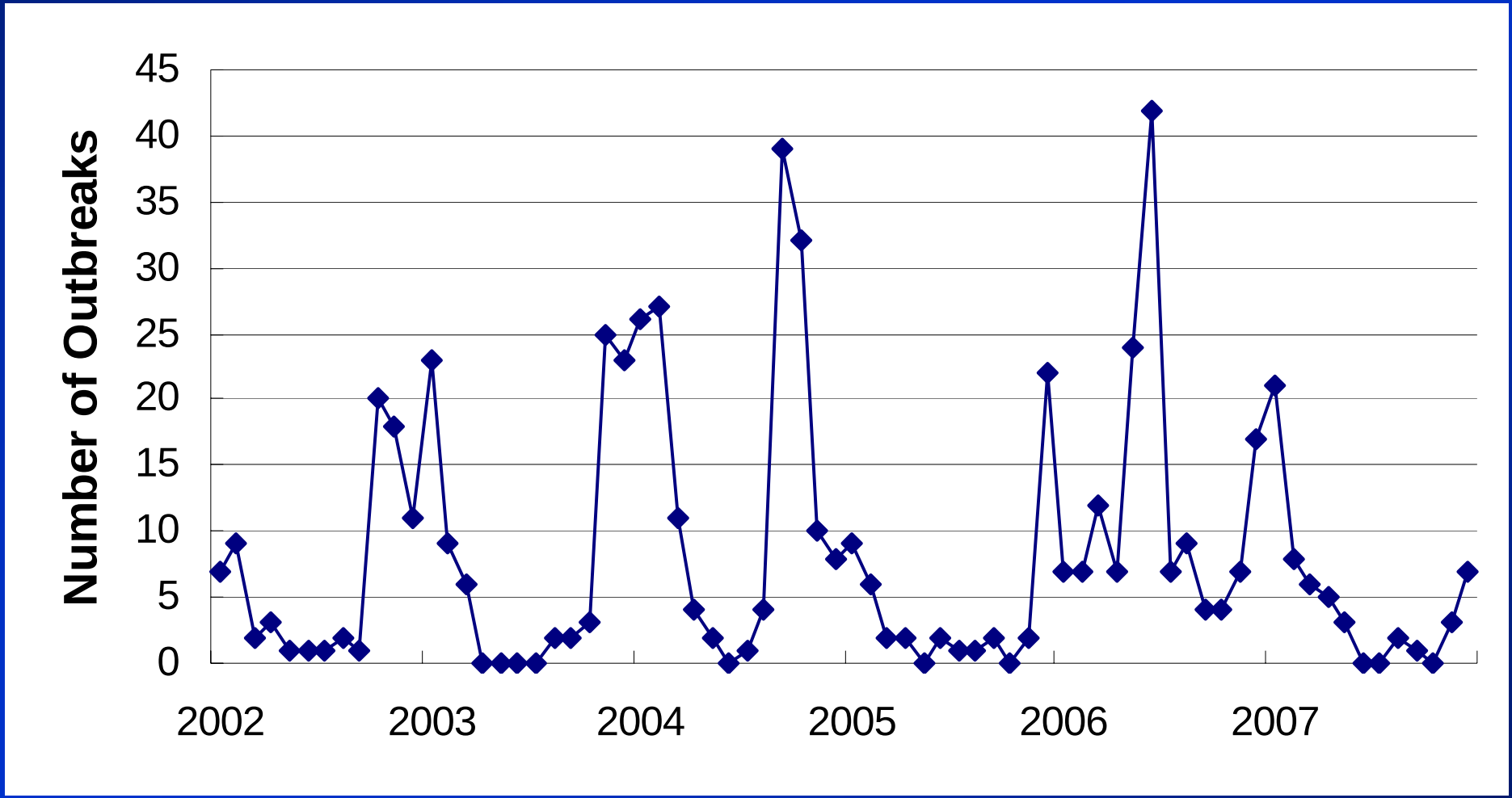
	Genogroup II.4				Genogroup II.2	
	Cluster A	Cluster B	Cluster C	Cluster D	Cluster E	Cluster F
2001 3rd Quarter	22 (35.5%)	30 (48.4%)	---	---	---	---
2001 4th Quarter	61 (53.5%)	36 (31.6%)	---	---	---	---
2002 1st Quarter	1 (3.2%)	4 (12.9%)	---	---	3 (9.7%)	2 (6.5%)
2002 2nd Quarter	---	---	4 (44.4%)	---	1 (11.1%)	---
2002 3rd Quarter	---	---	---	---	---	1 (12.5%)
2002 4th Quarter	5 (15.2%)	---	4 (12.1%)	---	16 (48.5)	6 (18.2%)
2003 1st Quarter	---	---	16 (33.3%)	---	7 (14.6)	1 (2.1%)
2003 2nd Quarter	---	---	1 (100.0%)	---	---	---
2003 3rd Quarter	---	---	---	---	---	---
2003 4th Quarter	---	---	17 (40.5%)	---	---	13 (31.0%)
2004 1st Quarter	---	---	14 (21.5%)	---	15 (23.1)	13 (20.0%)
2004 2nd Quarter	---	---	1 (11.1%)	---	5 (55.6%)	---
2004 3rd Quarter	---	---	1 (1.7%)	48 (80.0%)	10 (16.7%)	---
2004 4th Quarter	---	---	1 (1.6%)	49 (77.8%)	9 (14.3)	---
2005 1st Quarter	---	---		40 (63.5%)	8 (12.7%)	8 (12.7%)
2005 2nd Quarter	---	---	---	5 (41.7%)	1 (8.3%)	---


2003-010
2003-006
2003-008
2003-003
2003-004
2002-011
2004-021
2004-004
2004-017
2002-004
EP200202
2001-015
2003-011
2001-008
2001-006
2002-001
2001-001
2001-005
2001-013
2001-010
2002-013
DOUG4770
2001-014
2002-010
2001-004
2001-009
2001-007
2001-012
2001-003
2001-011
2001-002
2002-006
2004-008
2004-014
2004-019
2004-018
2004-010
2004-011
2004-015
2004-009
2004-006
2004-002
Camberwell/94/AU
Lordsdale/93/UK
WhiteRiver/94/US
2003-009
2004-012
2004-007
2003-002
2003-005
2004-022
2002-003
2002-005
Melksham/89/UK
2002-008
Cts4C-2000-JP
2002-012
2002-009
2003-020
2004-016
2004-001
2004-005
2004-020
2004-013
Hawai/71/US
SnowMountain/76/US
Toronto/TV24/93/CA
2002-007
2004-003
117/80(Germany)
Florida/269/1993/US
Miami/292/1994/US
DesertSheild/90/US
Chiba/87/JP
AppalachicolaBay/95/US
KY-89/89/JP
Norwalk/68/US
BS5/98/DE
Southampton/91/UK
WUG1/00/JP
2002-002
2003-001
0.05
Predominant cluster in 2003 and early 2004
Predominant cluster in 2001
Predominant cluster in late 2004 and early 2005
GII.4
GII.2
GII
GI

Outbreaks of Norovirus in 2006

- Atypical high norovirus activity in summer
- Mostly affected elderly homes(78%) and hospitals(16.6%)
- Person-to-person transmission
- New variant of GII.4 norovirus identified by sequencing

Norovirus Activity in Hong Kong



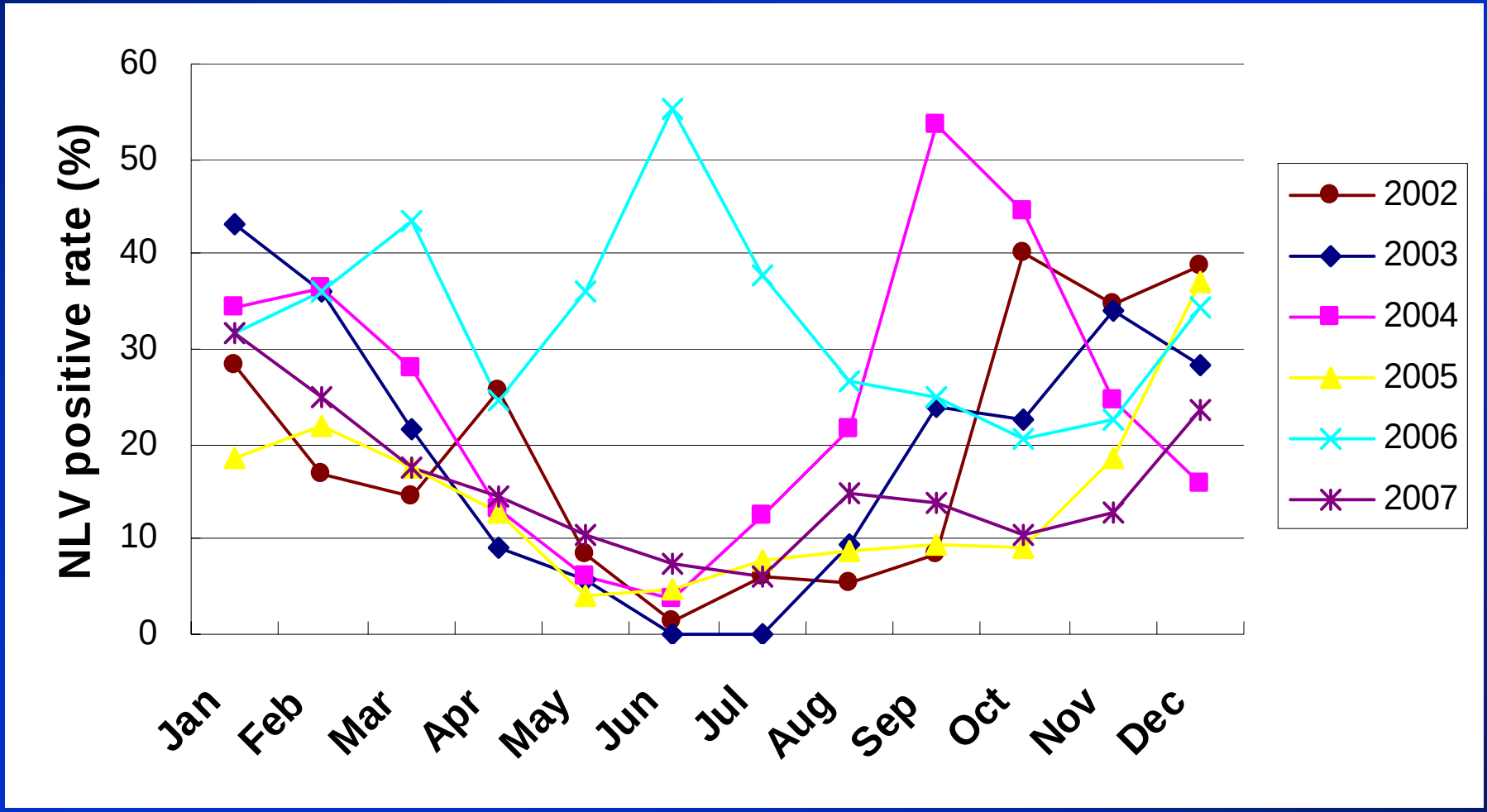
Detection of Norovirus in PHLC (2002-2007)

	2002	2003	2004	2005	2006	2007
No. of positive samples	306	562	1335	496	5423	3017
No. of samples tested	1680	2011	4358	3102	15956	16362
Positive rate (%)	18.21	27.94	30.63	15.99	33.99	18.44

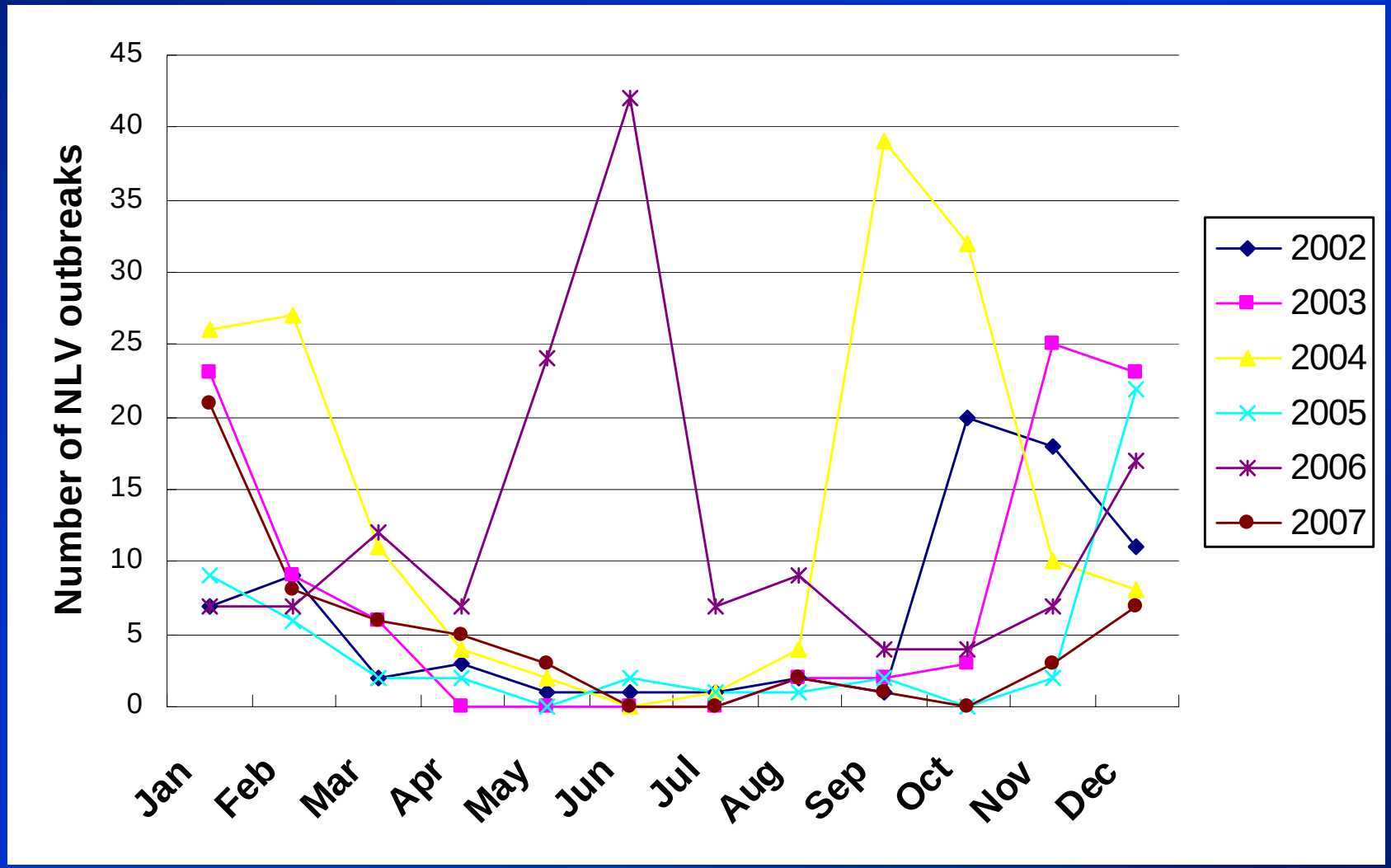
Norovirus Detected in PHLC

2002	Age					Total
	0-4	5-14	15-60	>60	Unknown	
Case tested	556	234	602	260	28	1680
Positive cases (%)	80 **(14.39%)**	63 **(26.92%)**	107 **(17.77%)**	52 **(20.00%)**	4 **(14.29%)**	306 **(18.21%)**
2004						
Case tested	2190	493	689	941	45	4358
Positive cases (%)	591 **(26.99%)**	134 **(27.18%)**	205 **(29.75%)**	391 **(41.55%)**	14 **(31.11%)**	1335 **(30.63%)**
2006						
Case tested	4300	841	2983	7813	19	15956
Positive cases (%)	1595 **(37.09%)**	218 **(25.92%)**	866 **(29.03%)**	2732 **(34.97%)**	12 **(63.16%)**	5423 **(33.99%)**
2007						
Case tested	4771	972	3469	7143	7	16362
Positive cases (%)	1061 **(22.24%)**	175 **(18.00%)**	625 **(18.02%)**	1152 **(16.13%)**	4 **(57.14%)**	3017 **(18.44%)**

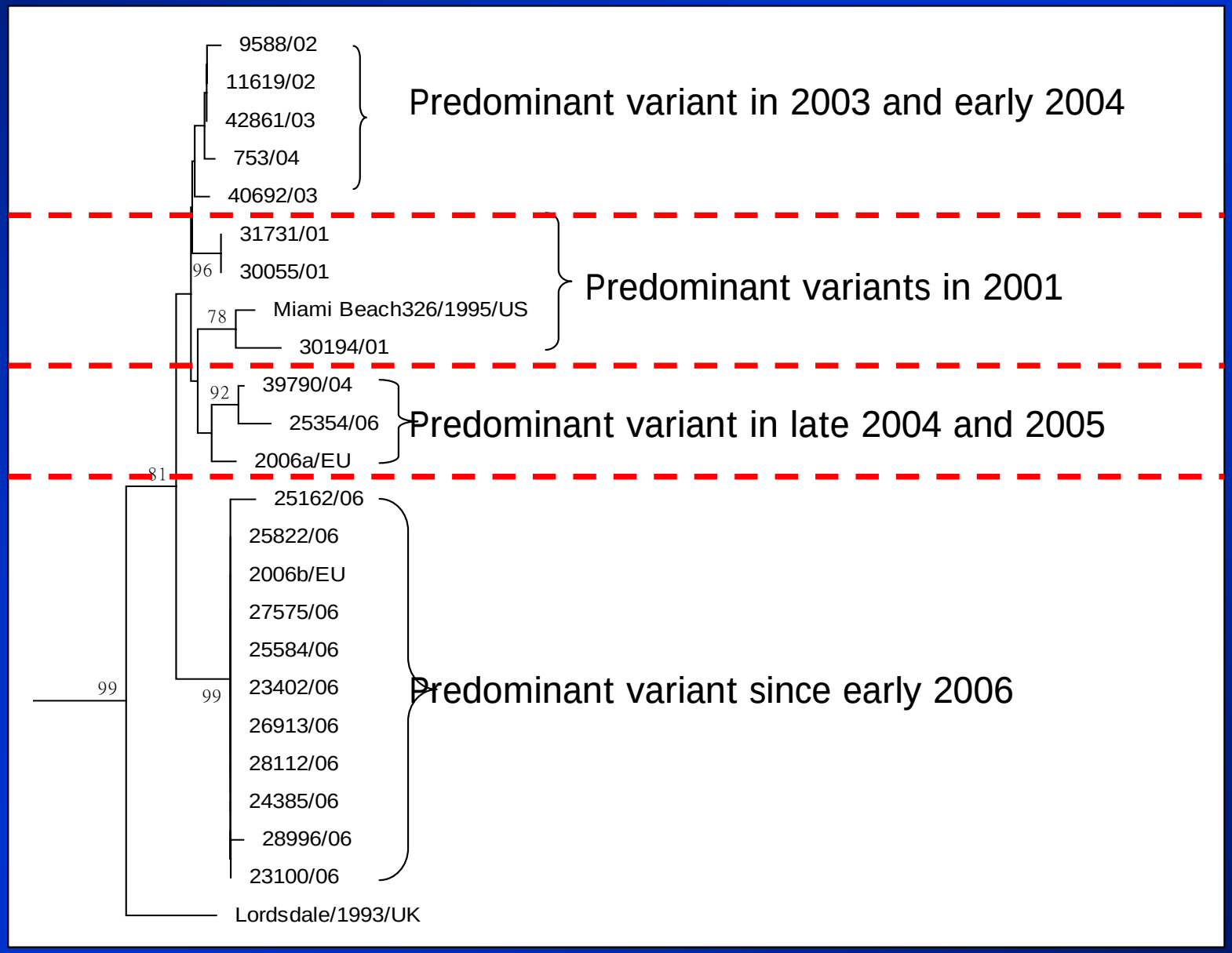
Norovirus Activity in Hong Kong



Norovirus Activity in Hong Kong

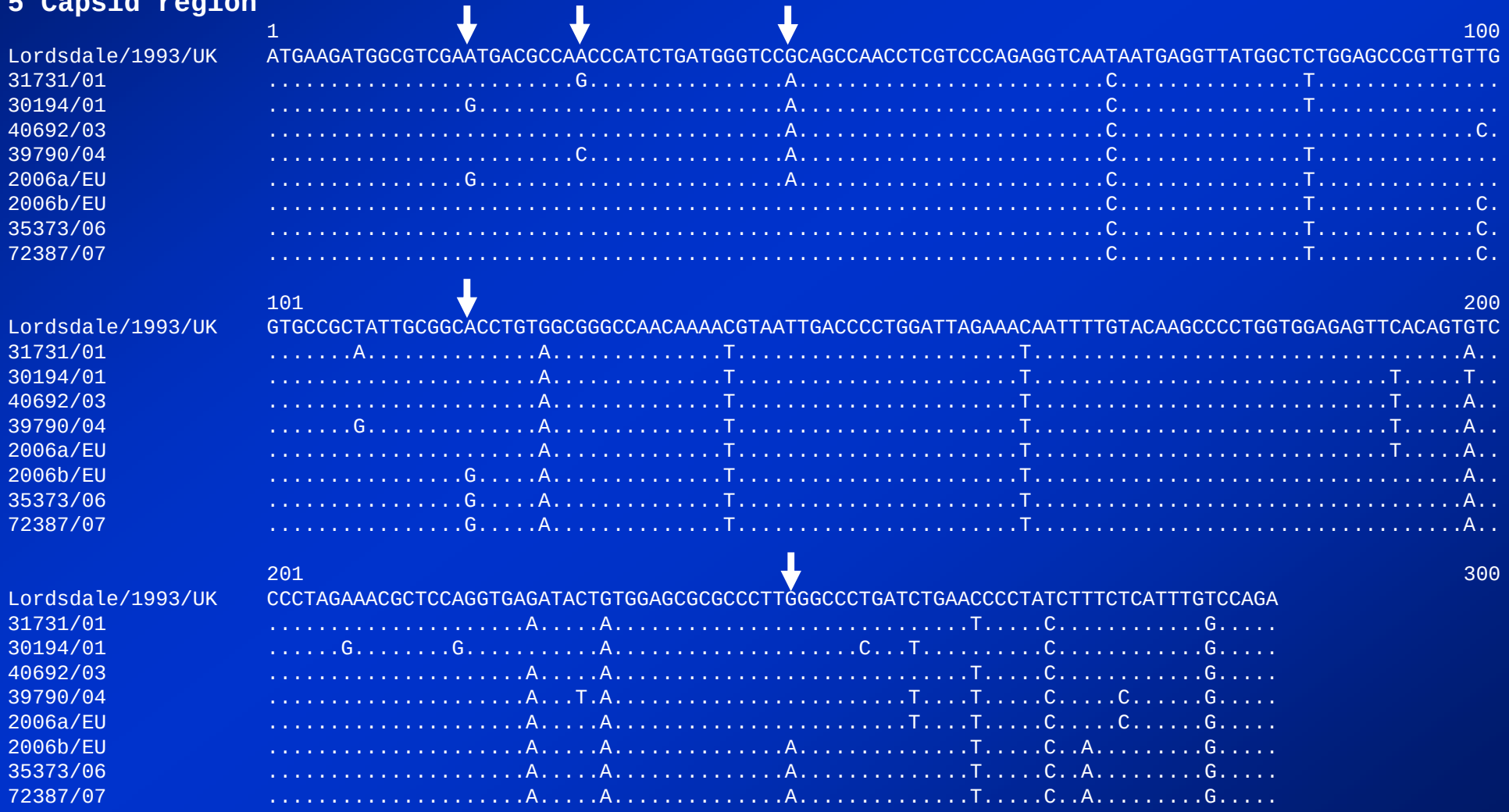


Predominant Norovirus Variants in Hong Kong



Comparison of the Predominant Norovirus Strains in Hong Kong and Europe

5’Capsid region



TABLE

Norovirus GII.4 lineages and their nomenclature

NoV season	GII.4 variant, nomenclature per country/group				
	FBVE [a]	USA[b]	Japan[c]	UK[d]	Australia[e]
<1990	Camberwell				
<1995	Bristol				
1995–6	1996		GII/4 g	Grimsby, v1	
2002–3	2002	Farmington Hills	GII/4 e	v2	
	2003/Asia			v5	
2004–5	2004		GII/4 f	v3	Hunter
2006–7	2006a	Laurens		v4	2006a
2006–7	2006b	Minerva	Kobe034	v6	2006b

[a] See: http://www.rivm.nl/bnwww

[b] Farmington Hills: Promedmail Archive Number 20030123.0206 23-JAN-2003 Norovirus activity 2002 – USA
Minerva: Promedmail Archive Number 20070310.0849 10-MAR-2007 Norovirus - USA (multistate): new strain
Laurens: Promedmail Archive Number 20070824.2779 24-AUG-2007 Norovirus activity, 2006-2007 – USA

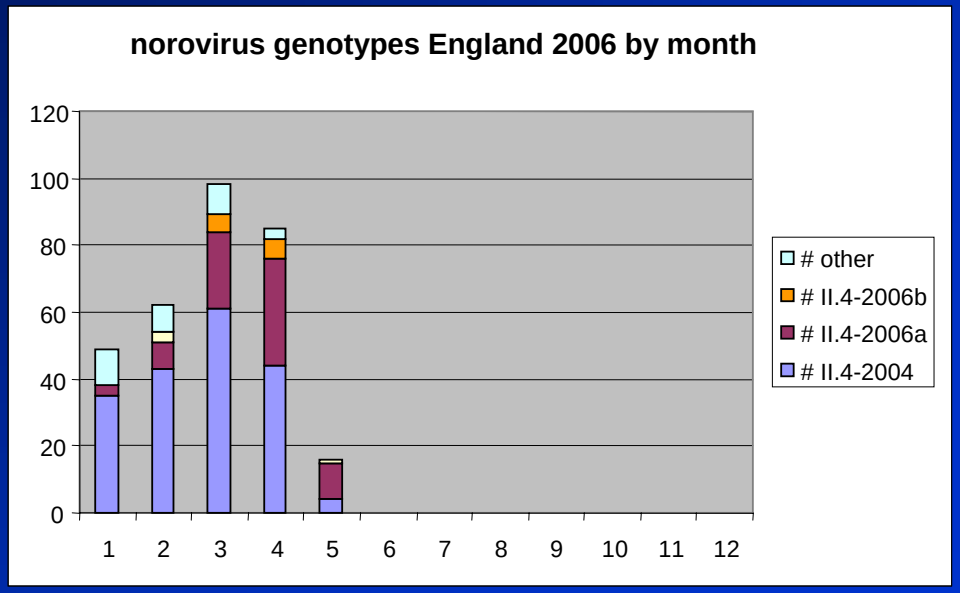
[c] Kobe :NCBI database AB291542 Norovirus Hu/GII.4/Kobe034/2006/JP genes for nonstructural polyprotein (RNA-dependent RNA polymerase region), capsid protein, partial and complete cds.
Japanese nomenclature: Phan TG, Kaneshi K, Ueda Y, Nakaya S, Nishimura S, Yamamoto A, Sugita K, Takanashi S, Okitsu S, Ushijima H. Genetic heterogeneity, evolution, and recombination in noroviruses. J Med Virol. 2007 Sep;79(9):1388-400.

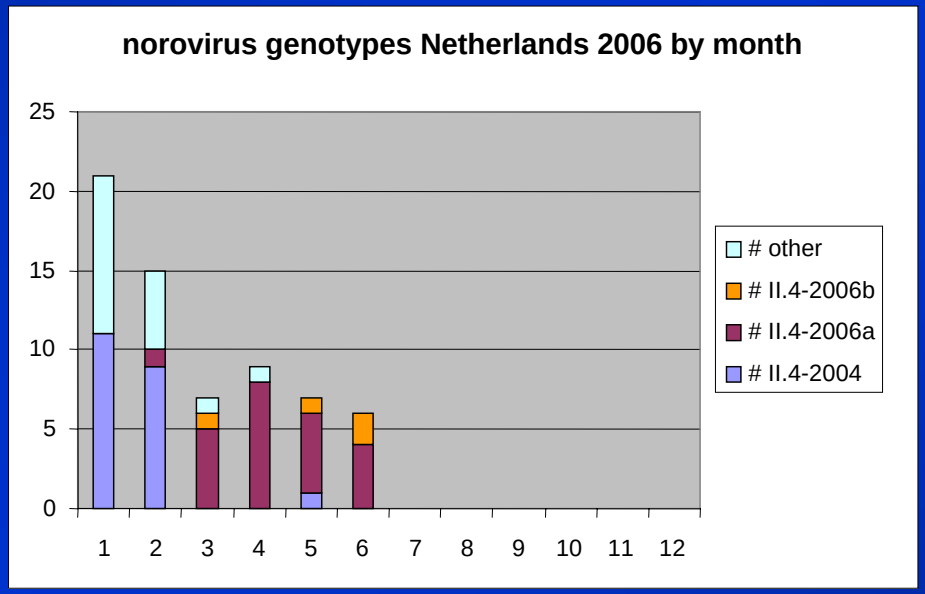
[d] Grimsby: Maguire AJ, Green J, Brown DW, Desselberger U, Gray JJ. Molecular epidemiology of outbreaks of gastroenteritis associated with small round-structured viruses in East Anglia, United Kingdom, during the 1996-1997 season. J Clin Microbiol. 1999 Jan;37(1):81-9.
UK variants: Gallimore CI, Iturriza-Gomara M, Xerry J, Adigwe J, Gray JJ. Inter-seasonal diversity of norovirus genotypes: emergence and selection of virus variants. Arch Virol. 2007;152(7):1295-303.

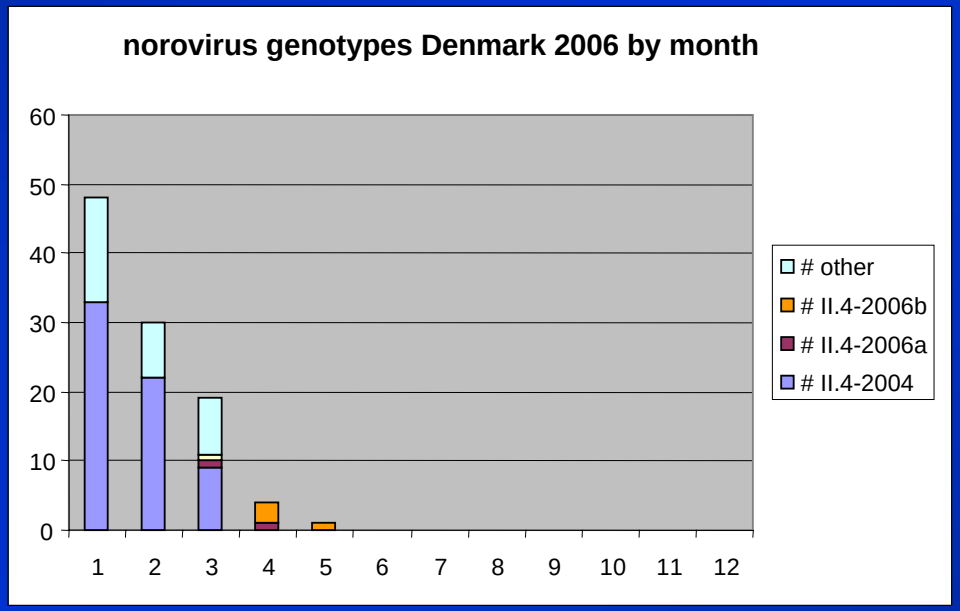
[e] Hunter : Bull RA, Tu ET, McIver CJ, Rawlinson WD, White PA. Emergence of a new norovirus genotype II.4 variant associated with global outbreaks of gastroenteritis.. J Clin Microbiol. 2006 Feb;44(2):327-33

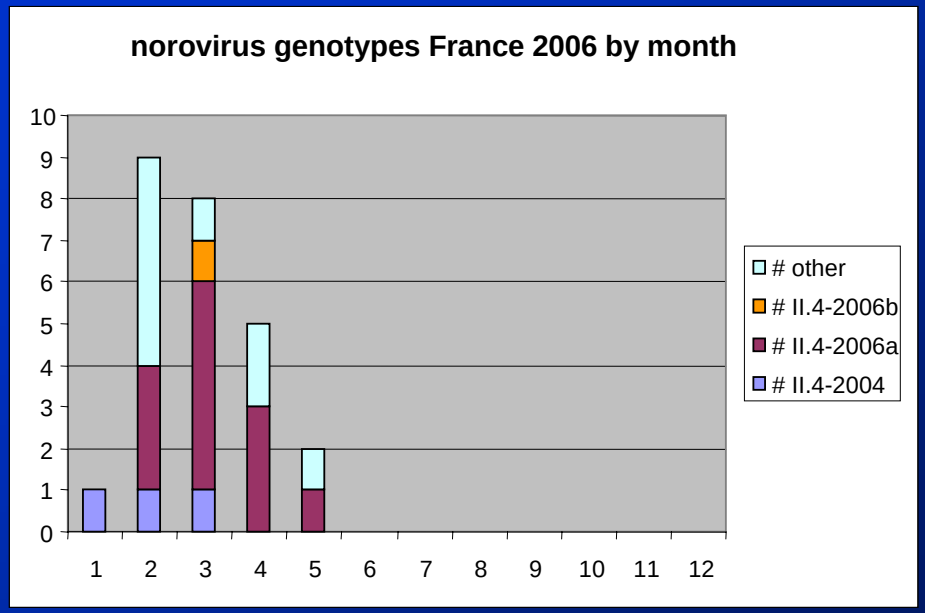
Eurosurveillance 2008; 13(2)

Norovirus genotypes 2006 by month



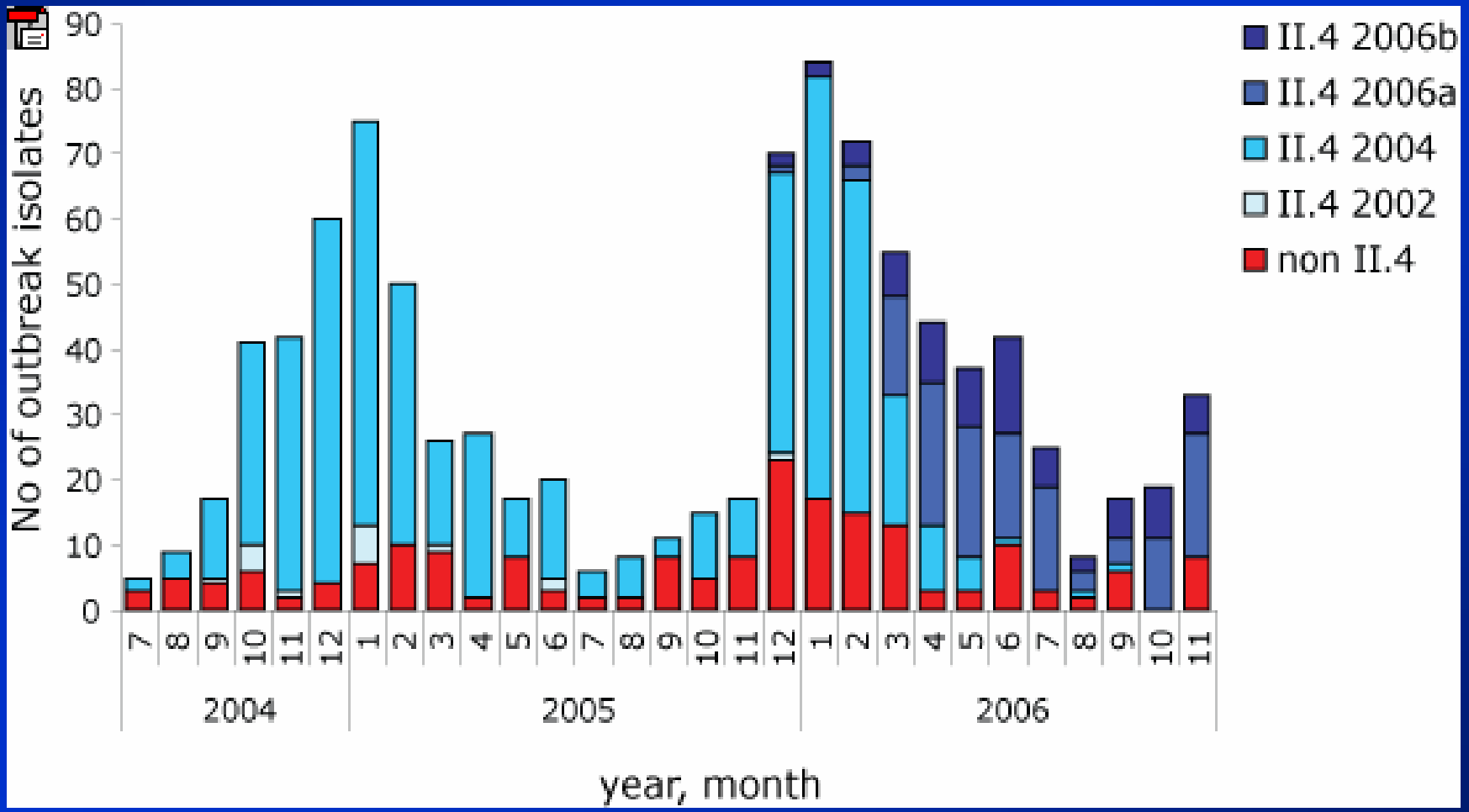




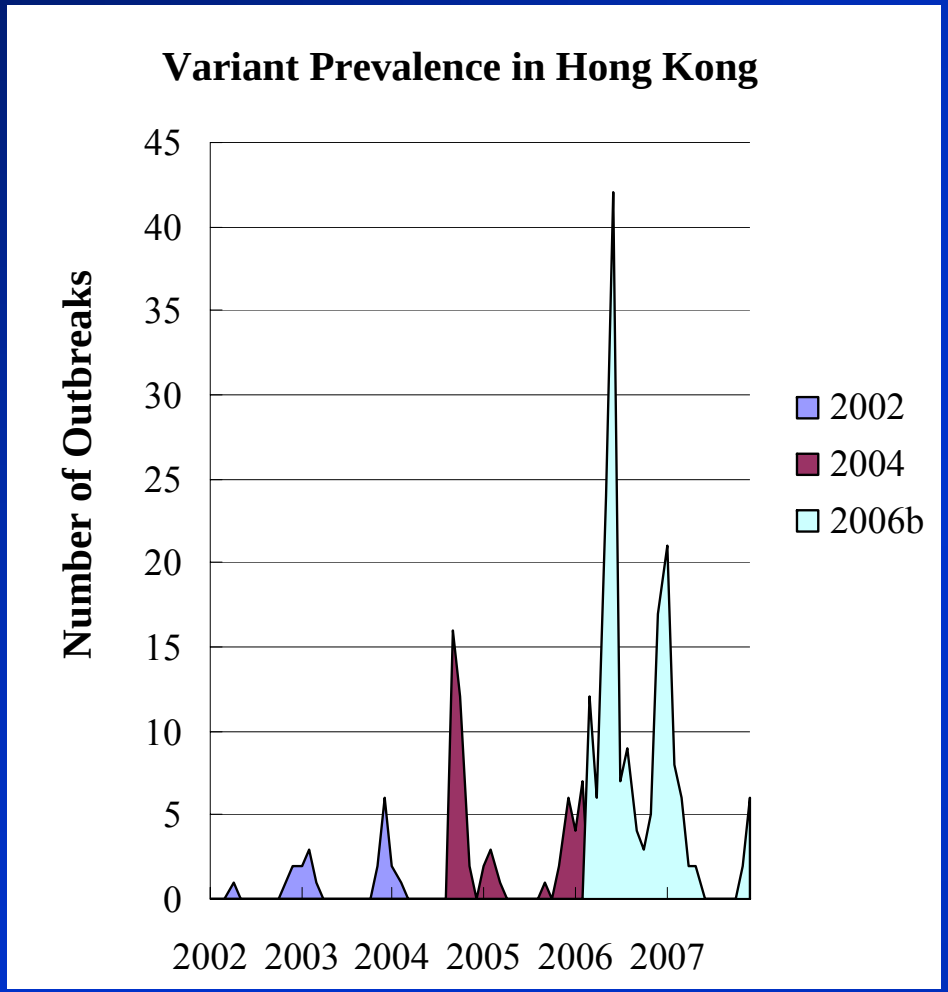


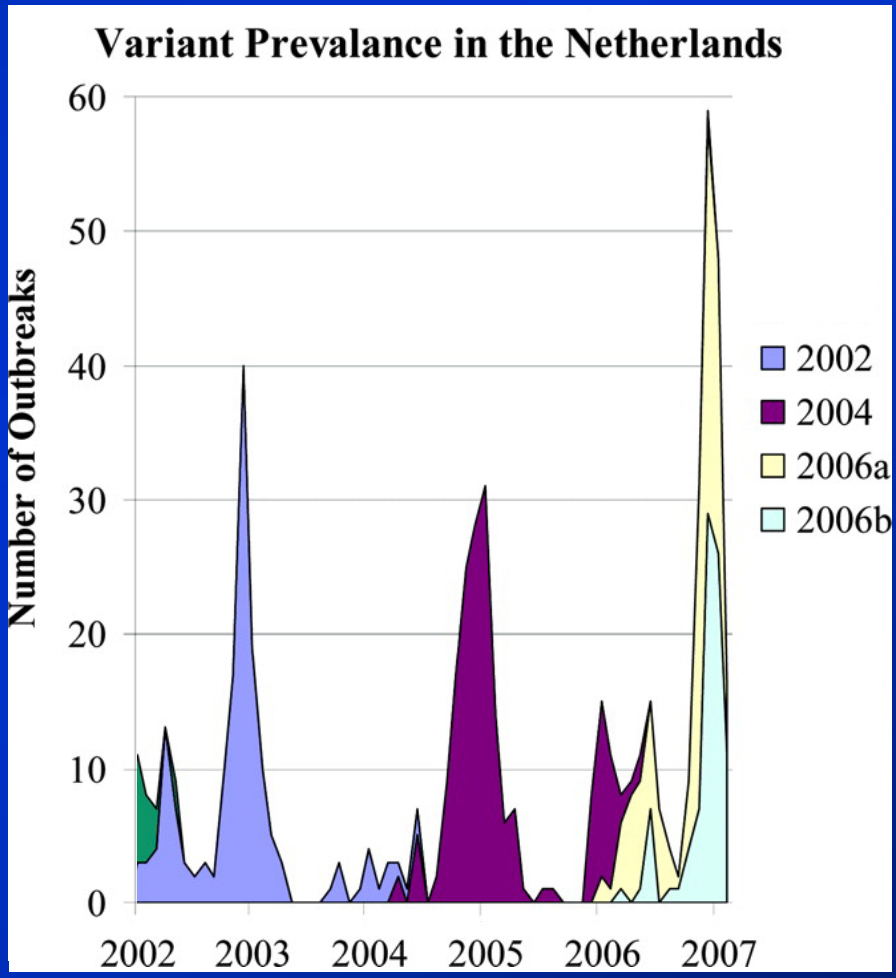
FBVE data provided by Marion Koopmans, RIVM

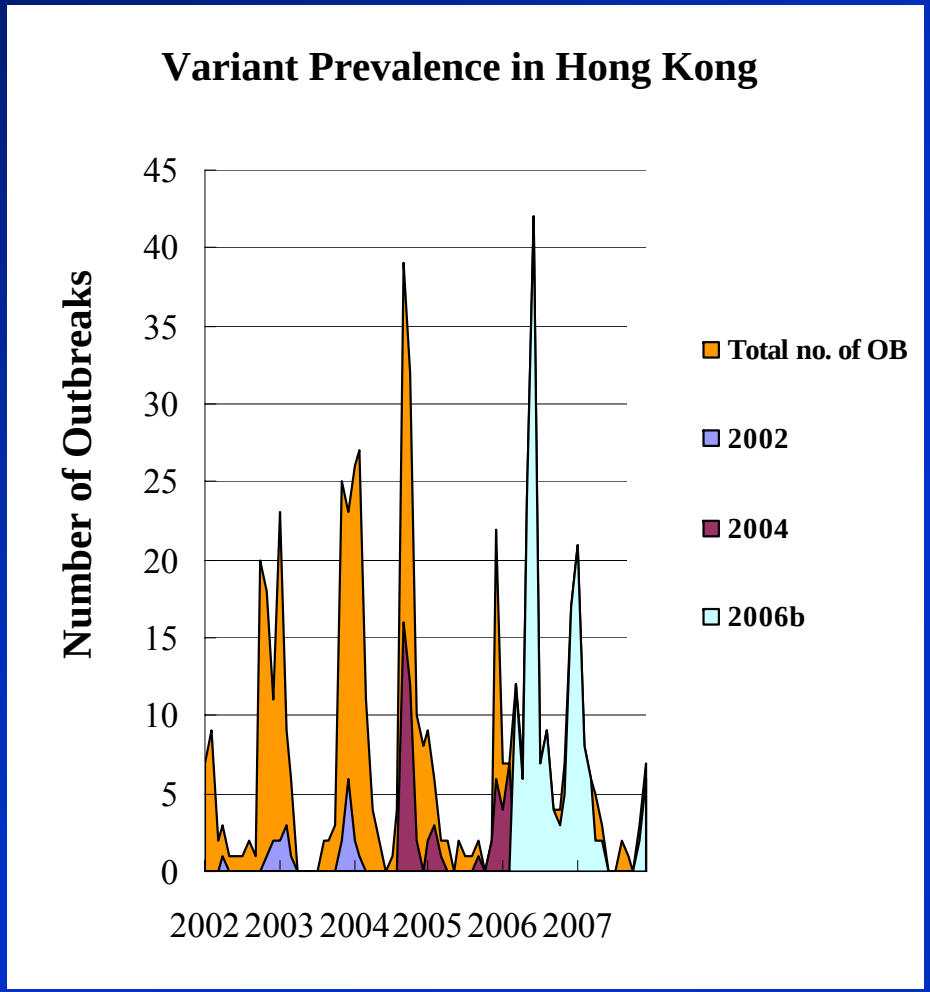
Norovirus activity in Europe

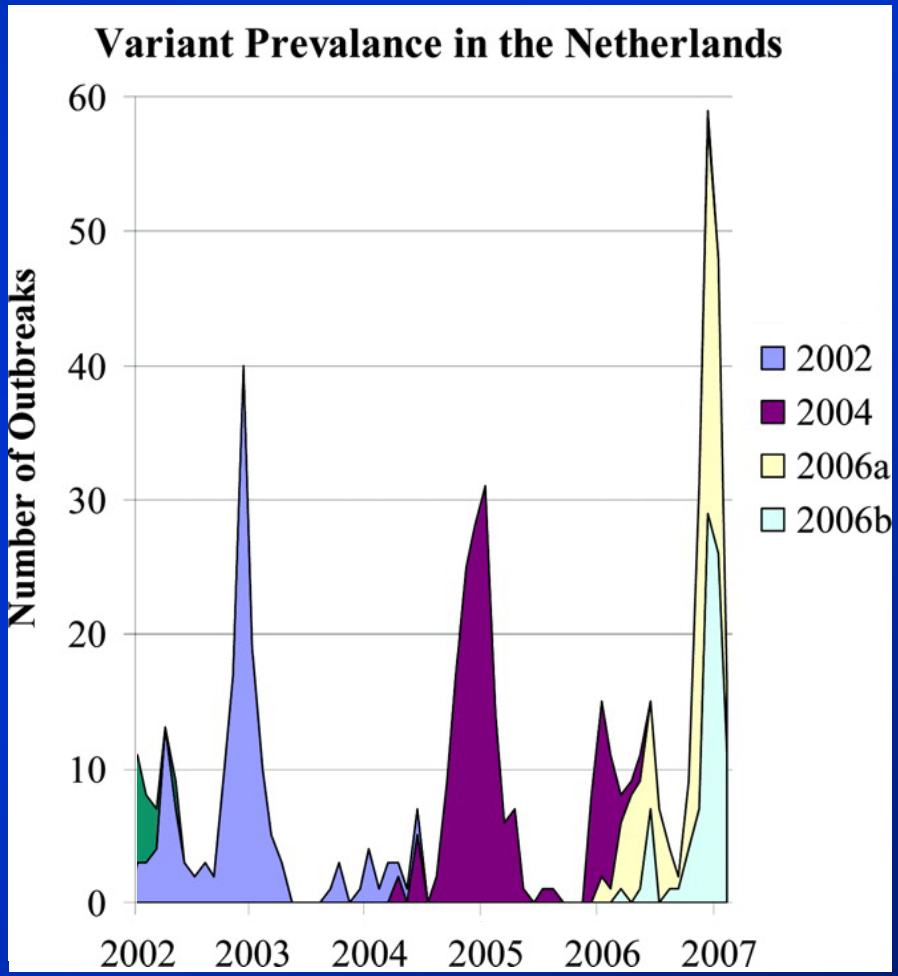


From Eurosurveillance 2006; 11(12):E061214.1


Variant Prevalence in Hong Kong
Number of Outbreaks
45
40
35
30
25
20
15
10
5
0
2002 2003 2004 2005 2006 2007
2002
2004
2006b


Variant Prevalance in the Netherlands
Number of Outbreaks
60
50
40
30
20
10
0
2002 2003 2004 2005 2006 2007
2002
2004
2006a
2006b





Oct 07 2006a 11.5% 2006b 46%
Nov 07 2006a 10% 2006b 73%

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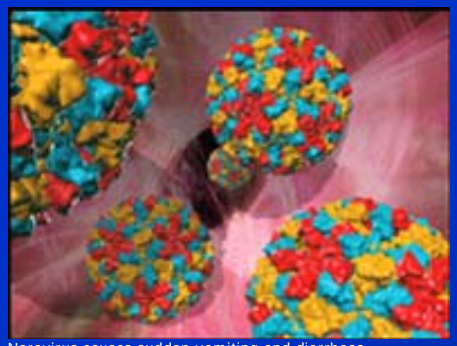
BBC(3.1.08) Stomach bug sweeping the country

People struck down by a stomach bug sweeping the UK have been urged not to go back to work.



Norovirus causes sudden vomiting and diarrhoea

Doctors estimate more than **100,000** people a week are catching norovirus, which causes diarrhoea and vomiting.

Norovirus Lab Reports in the UK

Year	Week 36 – 48
2000	288
2001	311
2002	1845
2003	313
2004	803
2005	281
2006	682
2007	1325

Variants of GII.4 Causing Epidemics Worldwide

--95/96 variant (mainly circulating before 2002)

– 2002 variant (emerged in 2002)

– 2004 variant (emerged in 2004)

– 2006 variant (2006a emerged in Dec 2005 in UK
2006b in Dec 2005 in Spain)

Summary of Norovirus Sequence Analysis (2002-2007

	2002	2003	2004	2005	2006	2007
No. of positive cases	306	562	1335	496	5422	2987
No. of cases typed (%)	81 **(26.5%)**	94 **(16.7%)**	197 **(14.8%)**	73 **(14.7%)**	267 **(4.9%)**	134 **(4.5%)**
GII.4 (%)	18 **(22.2%)**	34 **(36.2%)**	114 **(57.9%)**	60 **(82.2%)**	238 **(89.1%)**	126 **(94.0%)**
2002 variant (%)	8 **(44.4%)**	34 **(100%)**	17 **(14.9%)**	0 **(0.0%)**	0 **(0.0%)**	1 **(0.7%)**
2004 variant (%)	0 **(0.0%)**	0 **(0.0%)**	97 **(85.1%)**	50 **(83.3%)**	19 **(8.0%)**	1 **(0.7%)**
2006 variant (%)	0 **(0.0%)**	0 **(0.0%)**	0 **(0.0%)**	0 **(0.0%)**	213 **(89.5%)**	124 **(98.4%)**

Conclusions

- Norovirus is the leading cause of sporadic cases and outbreaks of AGE in people of all ages
- Studies on norovirus had been hampered by the lack of a culture system and the high genetic variability of the virus
- Multiple strains co-circulate; genetic characterization of strains is essential for distinguishing and linking cases
- New variants emerged in 95/96, 2002, 2004, 2006
- Continuous monitoring is essential to detect newly emergent strains
- Increased strain surveillance with international collaboration should be our goal
- There is clearly a need for international standards to monitor norovirus in food and water

Thank You