



# *Viral Gastroenteritis: Outbreaks and Testing*

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# Objectives

- Enteric caliciviruses
- Detection methods
- Epidemiologic observations
- Aspects of molecular phylogeny

# Enteric pathogens group

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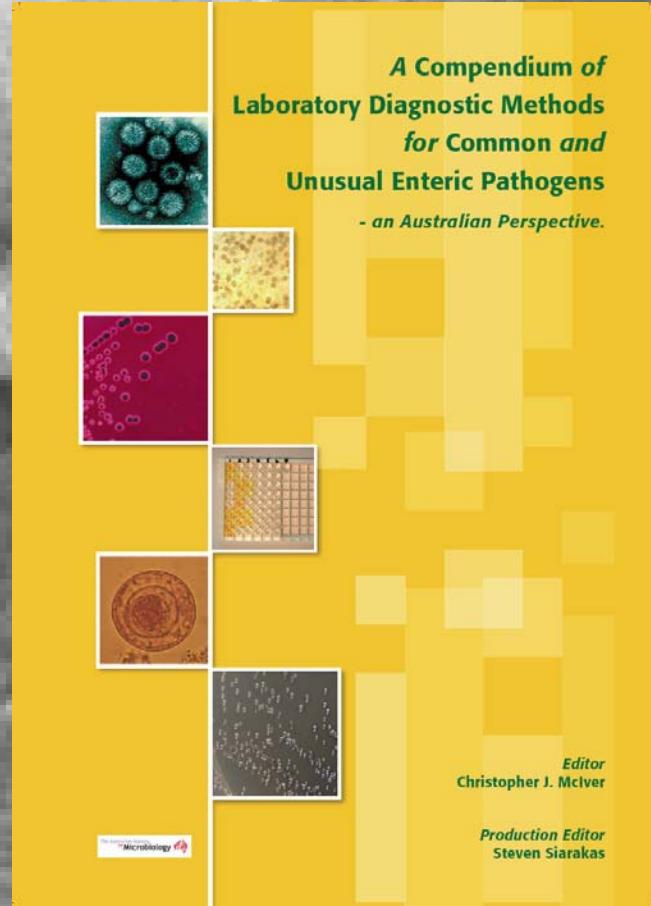
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**A Compendium of  
Laboratory Diagnostic Methods  
for Common and  
Unusual Enteric Pathogens**  
*- an Australian Perspective.*

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The Royal Society for  
Microbiology

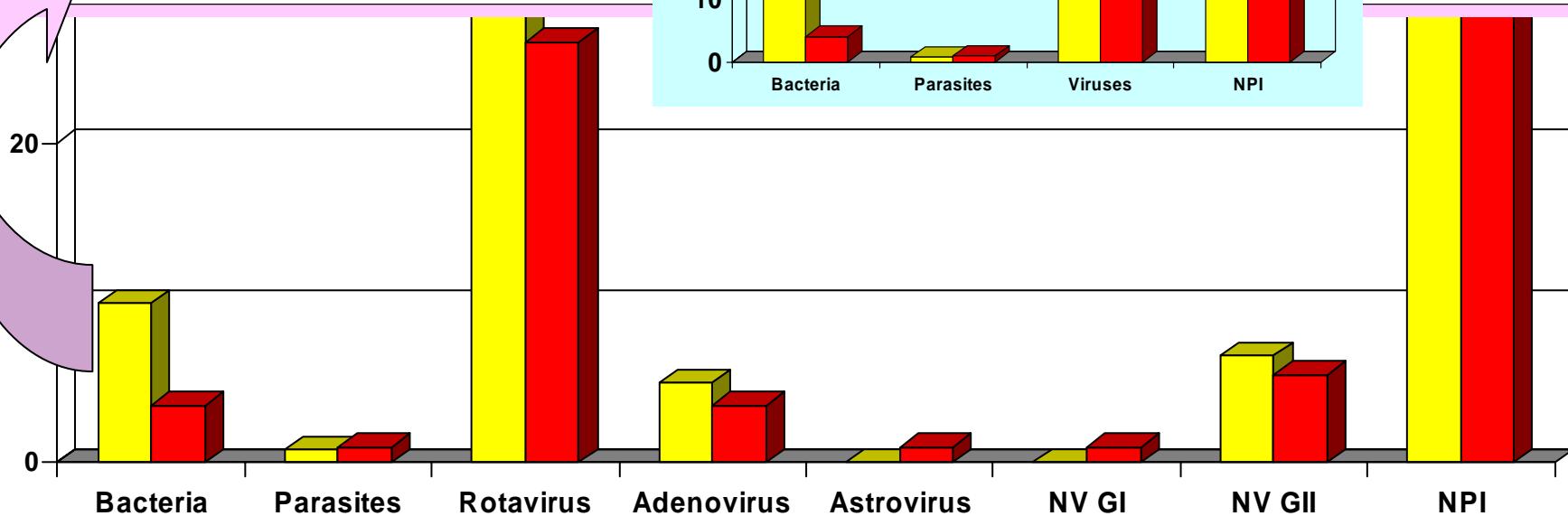
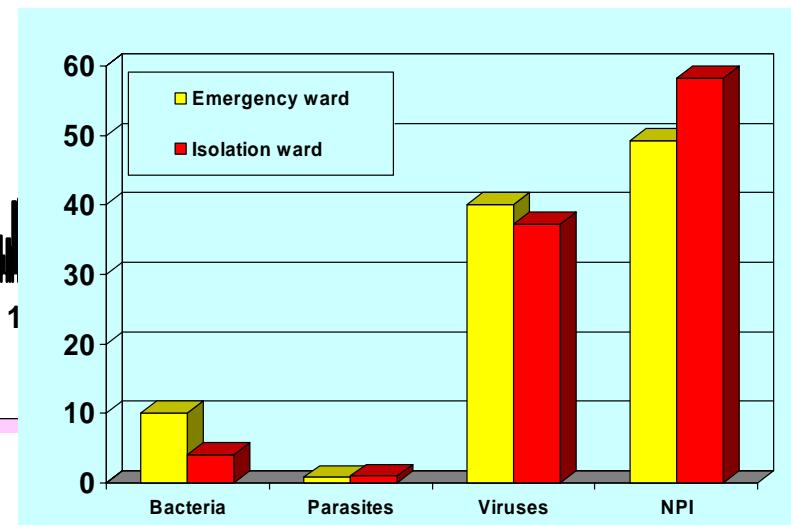
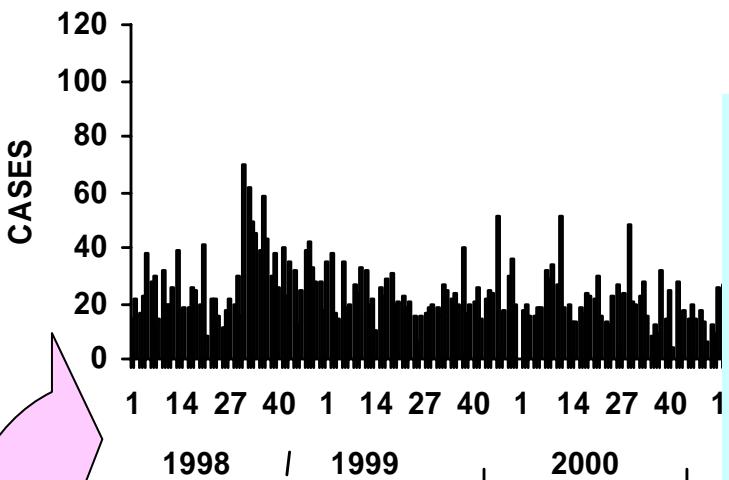
## Chapter 15

# *Norovirus and Sapovirus*

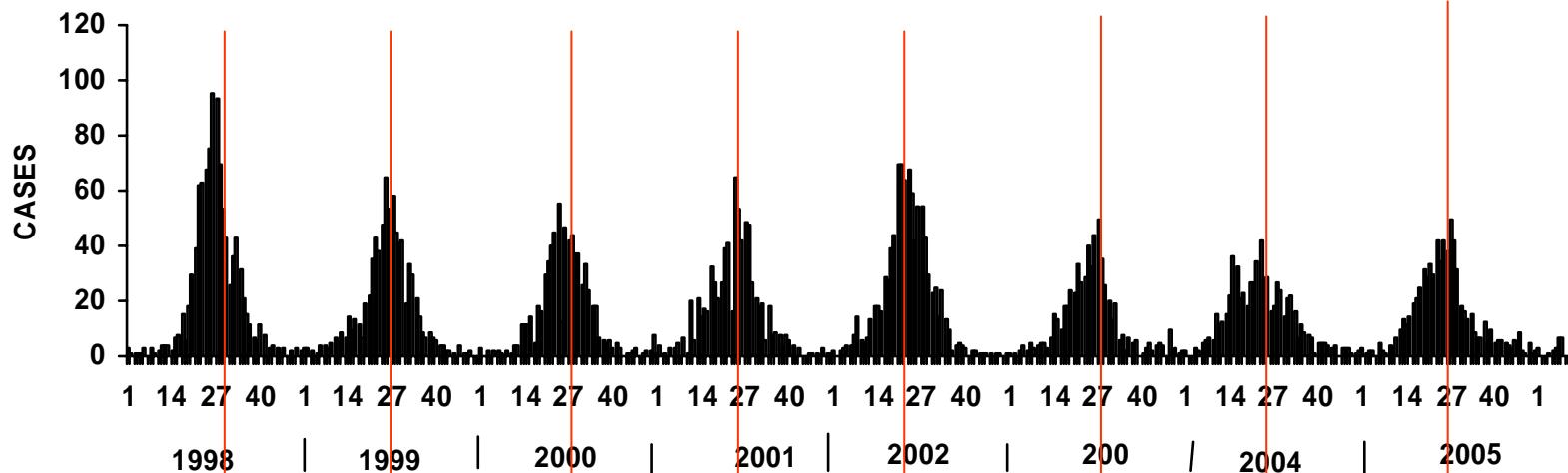
CHRISTOPHER J. McIVER, ROWENA A. BULL, ELISE T. V. TU,  
W. D. RAWLINSON, and PETER A. WHITE

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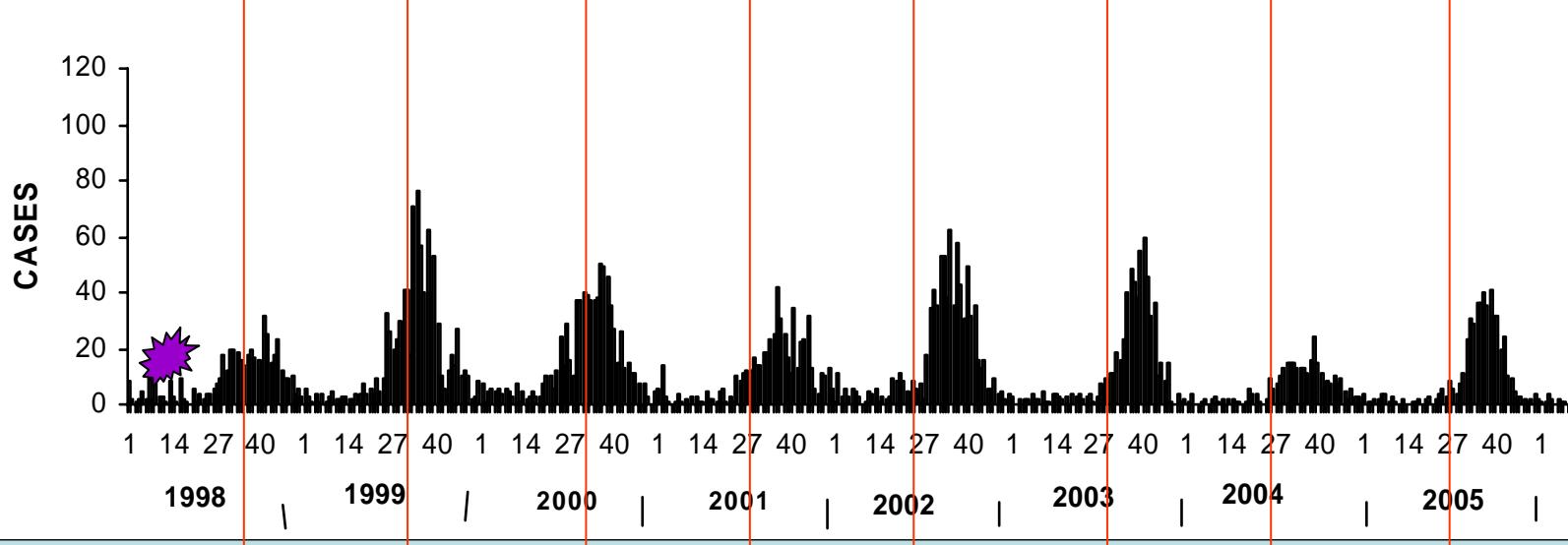
## CAMPYLOBACTER



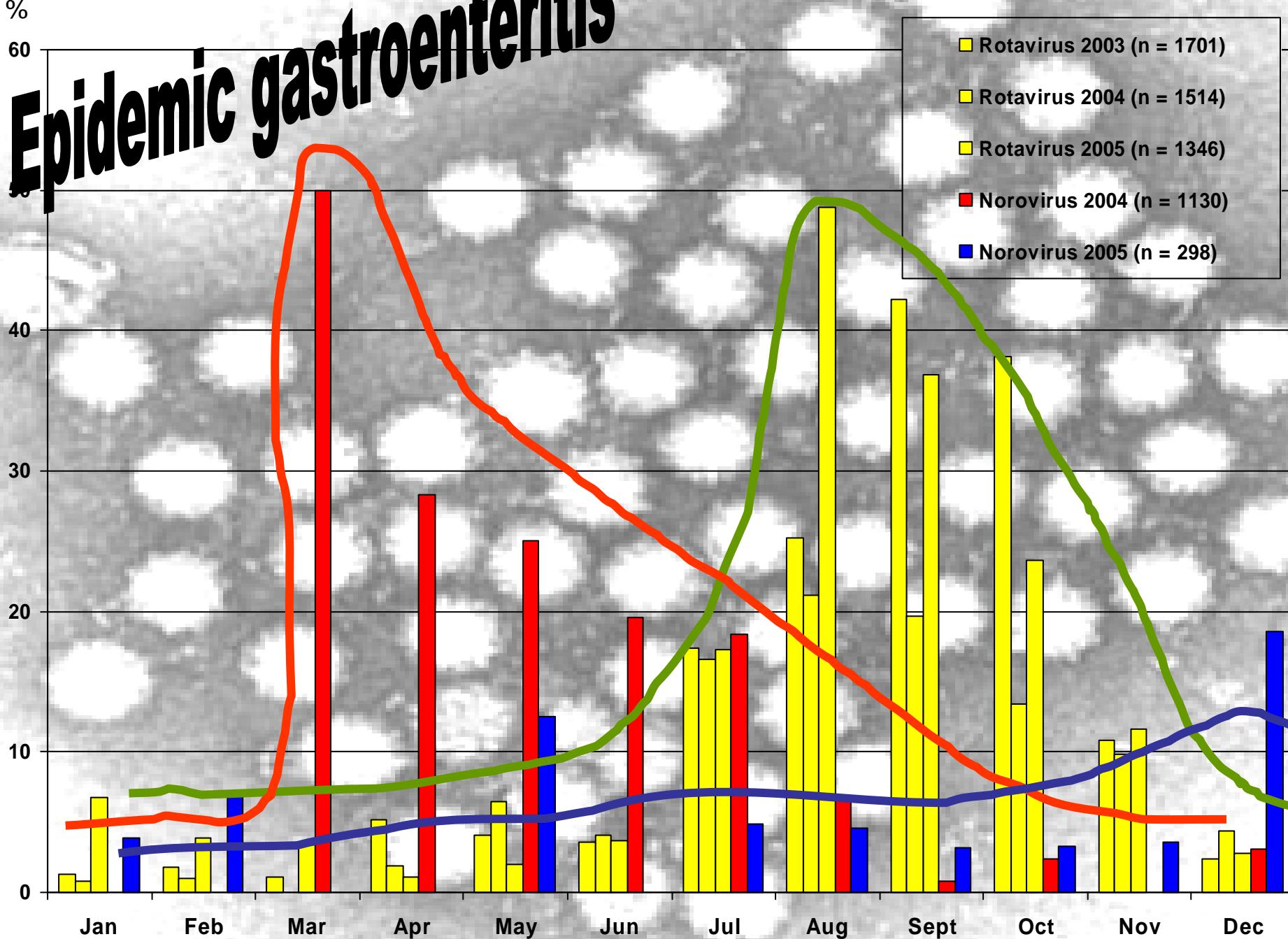
### RSV



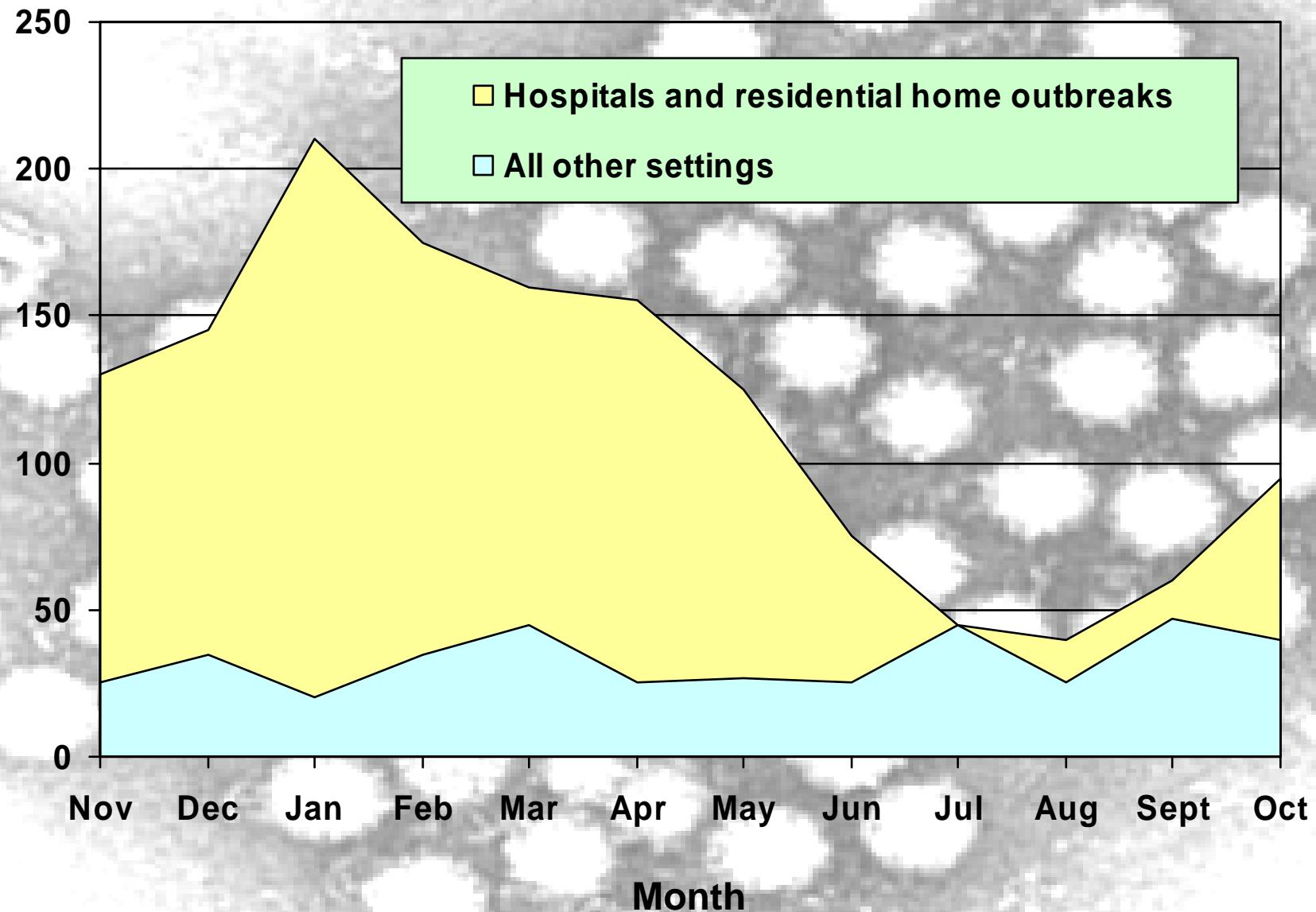
### ROTAVIRUS



# Epidemic gastroenteritis



## Seasonality for Norovirus, England and Wales, 1992 - 2000





## Genus: Norovirus

## Family: *Caliciviridae*

## Genus: Sapovirus

27 - 32 nm

Single stranded

Positive-sense RNA

~ 7,800 nucleotides

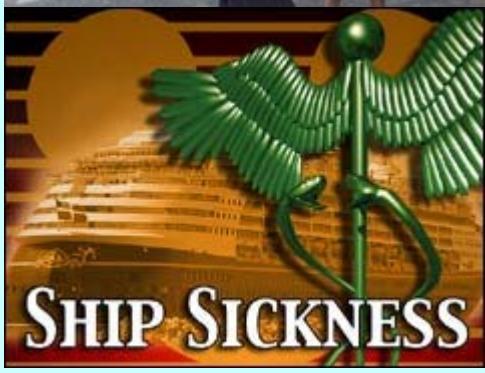
.1  $\mu$ m

Calicivirus	Prevalence	Population at risk	Symptoms
Norovirus	Common	All ages	Mild
Sapovirus	Infrequent	Young children	Less severe

Genogroup	Susceptible	Viruses
GI		Norwalk virus Baltimore virus Southampton virus Desert Shield
GII		Outbreaks world-wide Camberwell virus Frankston virus
GIII		<i>Norovirus</i>
GIV		<i>Norovirus</i>
GV	Mice	

# Symptoms

Symptom	Number	Percentage
Nausea	29	58
Diarrhoea and vomiting	21	42
Vomiting (without diarrhoea)	11	21
Diarrhoea (without vomiting)	11	21
Abdominal pain	20	40
Fever	19	38



# Risk factors

- Contact with persons with gastroenteritis
- Contact with contaminated areas (vomit or faeces)
- Consumption of contaminated food / water
- Age group
- Health care institutions
- Closed environments

# Kaplan criteria for the identification of outbreaks of gastroenteritis due to norovirus

- *Vomiting in more than half of affected persons*
- *Mean (or median) incubation period: 24 – 48h*
- *Mean (or median) duration of illness: 12 – 60h*
- *Bacterial pathogen not detected*



Kaplan et al. 1982. Ann. Intern. Med. 96:756-61

- **Moderately sensitive (68%)**
- **Highly specific (99%)**

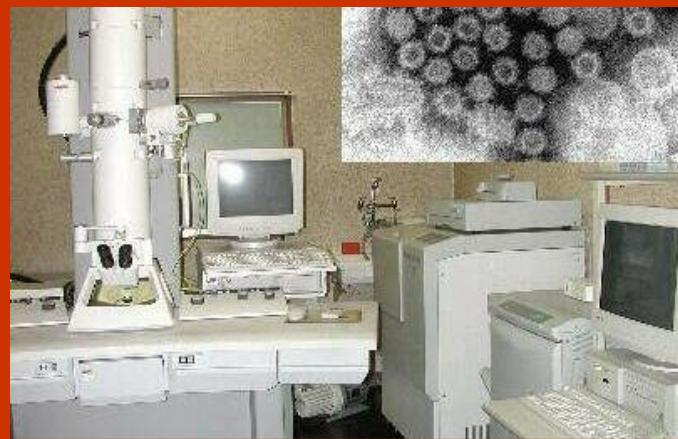
Turcios et al. 2006. CID. 42:964-969

# Why we need to know aetiology ?

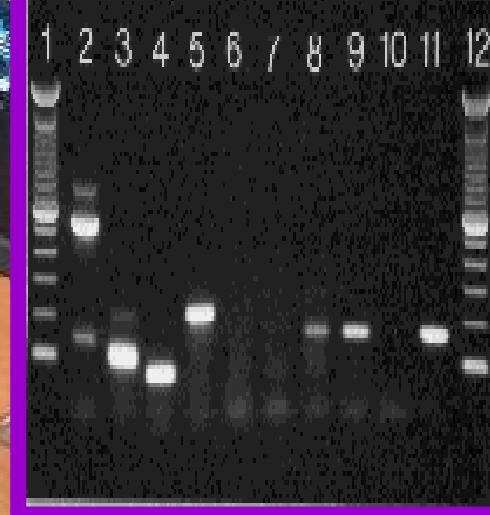
- Implementation of more-specific prevention and control measures
- Surveillance of prevalence (including genotypes)
- True burden

# Detection - Calicivirus

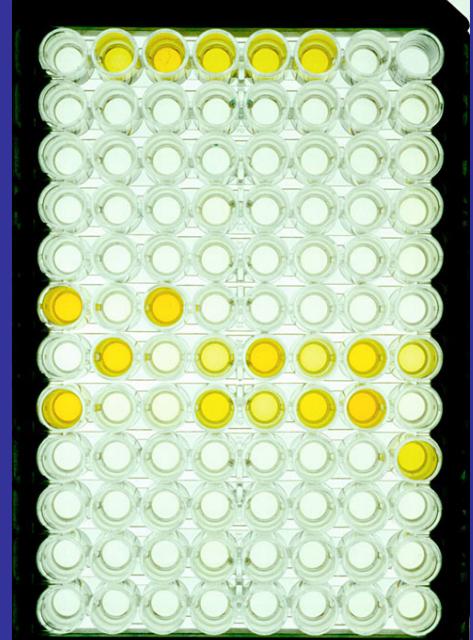
Morphologically intact  
 $> 10^6$  particles / gram



Increased sensitivity  
Lability of viral RNA  
PCR inhibitors  
Genetic diversity



Antigens more stable  
Present in higher concentrations  
Simple and rapid



Broadly reactive  
Non-specific reactions

## **IDEIA™Norwalk-like virus (Dako Cytomation, UK)**

- Genogroup I and II-specific capture monoclonal antibodies (capsid protein)
- 4 monoclonal antibodies – GI
- 10 monoclonal antibodies – GII
- 10% faecal suspension
- 3 h performance time



# Evaluation IDEIA™Norwalk-like virus (Dako Cytomation, UK)

Parameter	In-house PCR	OligoDetect, Light Diagnostics	Richards et al., 2003. J Clin Virol 26:109-115
Sensitivity	64.3	71.4	55.5
Specificity	100	96.9	98.3
PPV	100	93.8	95.0
NPV	76.2	83.8	76.9
Total	30	53	479

# Norovirus

27 – 35 nm  
Single-stranded / positive sense RNA genome

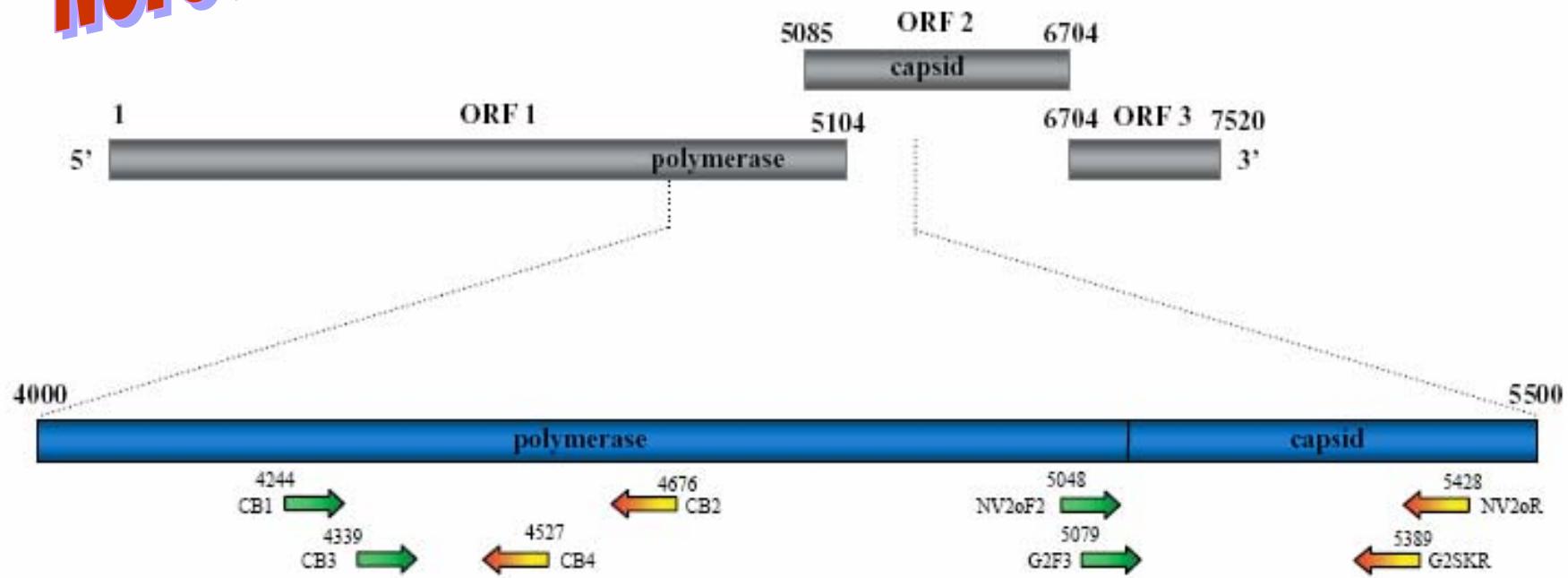


FIG. 1 Genomic organisation of norovirus and primer target sites for genogroup II strains (Table 1).



TABLE I List of primers for the detection of norovirus and sapovirus.

Primer	Function <sup>a</sup>	Sequence (5' to 3')	Size (bp)	Reference
<b>Norovirus genogroup I</b>				
<i>Capsid gene region (ORF 2)</i>				
COG1F	A	CGY TGG ATG CGN TTY CAT GA	381	Kageyama et al., 2003 (30)
G1SKR	B	CCA ACC CAR CCA TTR TAC A		Kojima et al., 2002 (36)
<b>Norovirus genogroup II</b>				
<i>RNA polymerase region (ORF 1)</i>				
CB1	A	GGC CCC ATC ATC TTC GAG AG	433	White et al., 2002 (65)
CB2	B	GTT TYA RCC CGT ATT CCT TG		White et al., 2002 (65)
CB3	C	AGC AGC CCT AGA AAT CAT GG	189	White et al., 2002 (65)
CB4	D	CAG AGA GTG AGG AGC CAG TG		White et al., 2002 (65)
<i>3' end of the polymerase region and the 5' end of the capsid gene region (ORF 2)</i>				
NV2oF2	A	GGG AGG GCG ATC GCA ATC		Bull, 2003 (5)
NV2oR	B	GTR AAC GCR TTY CCM GC	380	Bull, 2003 (5)
G2F3	C	TTC TGA ATG AAG ATG GCG TCG A		Hansman et al. 2004b (24)
G2SKR	D	CCR CCN GCA TRH CCR TTR TAC AT	311	Kojima et al. 2002 (36)
<b>Sapovirus</b>				
<i>Capsid gene region</i>				
SV5317	A	CTC GCC ACC TAC RAW GCB TGG TT		Hansman et al. 2004b (24)
SV5749	B	CGG RCY TCA AAV STA CCB CCC CA	437	Hansman et al. 2004b (24)

B = C, G, or T; H = A, C or T; M = A or C; N = A, C, G or T; R = A or G; S = C or G; V = A, C, or G; W = A or T; Y = C or T.

<sup>a</sup>A = Outer sense primer; B = Outer anti-sense primer; C = Inner sense primer; D = Inner anti-sense primer.



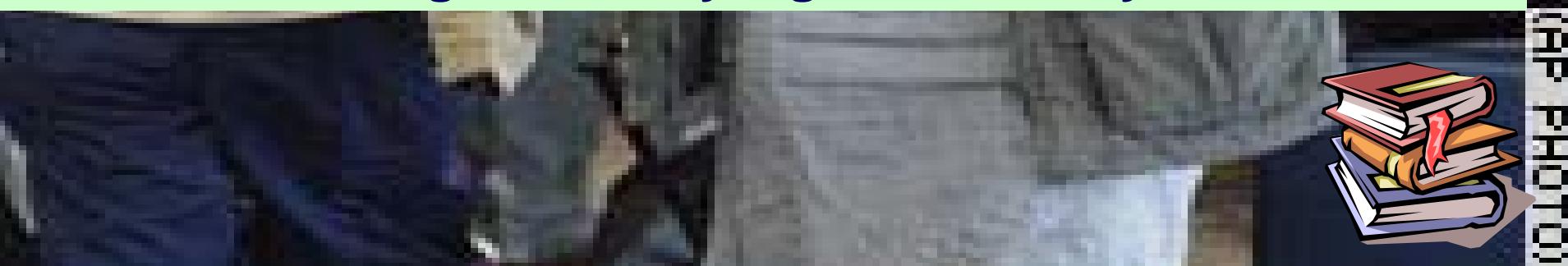
## Norovirus outbreaks July, 1997– September, 2000.

Outbreak	Setting	Strain	Genotype
1	Nursing home	US95/96 ★	GII/4
2	Day care	US95/96 ★	GII/4
3	Hospital-geriatric	US95/96 ★	GII/4
4	Hospital-pediatric	US95/96 ★	GII/4
5	Day care	Sydney cluster	GII/3
6	Day care	Sydney cluster	GII/3
7	Nursing home	US95/96 ★	GII/4
8	Wedding	US95/96 ★	GII/4
9	Hospital	US95/96 ★	GII/4
10	Prison	Hawaii-like	GII/1
11	Community	US95/96 ★	GII/4

Noel et al. 1999. Identification of a distinct common strain of “Norwalk-like viruses” having a global distribution. *J Infect. Dis.* 179:1334-144.



- ✓ “95/96-US” strain predominated in 1995-96 winter season.
- ✓ How does the virus spread to geographically distant locations in US and abroad.
- ✓ What mode(s) of transmission or selective factor(s) allowed the sudden emergence and rapid spread of strain.
- ✓ “95/96-US” predominated in 1995-96 was replaced in the following season by a greater variety of strains.



**FIGURE 1****REPORTS OF SELECTED COMMUNICABLE DISEASES, NSW, JAN 1999 TO AUG 2004, BY MONTH OF ONSET**

Preliminary data: case counts in recent months may increase because of reporting delays.  
 Laboratory-confirmed cases only, except for measles, meningococcal disease and pertussis  
**BFV** = Barham Forest virus infections, **RRV** = Ross River virus infections  
**lab+** = laboratory confirmed

**Men Gp C and Gp B** = meningococcal disease due to serogroup C and serogroup B infection,  
 other/unk = other or unknown serogroups.  
**NB:** multiple series in graphs are stacked,  
 except gastroenteritis outbreaks.  
**NB:** Outbreaks are more likely to be reported by nursing homes & hospitals than from other institutions

NSW population	
Male	50%
<5	7%
5-24	28%
25-64	52%
65+	13%
Rural*	42%



cases

cases

➤ Late autumn early winter (mainly nursing homes and some hospitals)

➤ May, 100 outbreaks (3,294)

➤ August, 17 outbreaks (184)

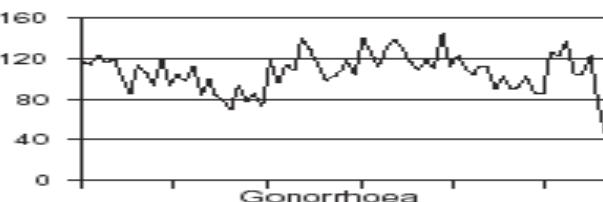
July 04–Aug 04
Male 85%
<5 0%
5–24 26%
25–64 74%
65+ 0%
Rural 20%

July 04–Aug 04
Male 61%
<5 0%
5–24 22%
25–64 68%
65+ 10%
Rural 19%

July 04–Aug 04
All outbreaks 188
Nursing homes 139
Hospitals 33
Child care 12
Schools 1
Other 3

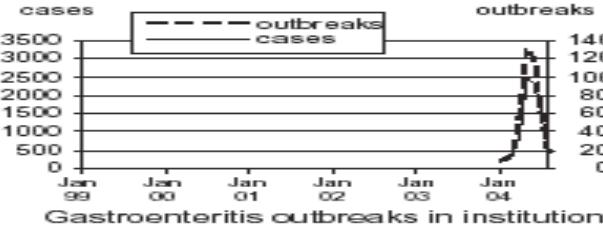
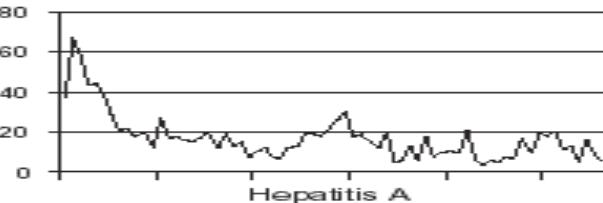


Cryptosporidiosis



July 04–Aug 04
Male 61%
<5 0%
5–24 22%
25–64 68%
65+ 10%
Rural 19%

July 04–Aug 04
All outbreaks 188
Nursing homes 139
Hospitals 33
Child care 12
Schools 1
Other 3



Measles

July 04–Aug 04	188
All outbreaks	188
Nursing homes	139
Hospitals	33
Child care	12
Schools	1
Other	3

July 04–Aug 04
Male 48%
<5 29%
5–24 43%
25–64 21%
65+ 7%
Rural 43%

July 04–Aug 04
Male 43%
<5 7%
5–24 31%
25–64 51%
65+ 11%
Rural 41%

July 04–Aug 04
Male 51%
<5 31%
5–24 27%
25–64 33%
65+ 9%
Rural 45%

## Norovirus outbreaks March – July, 2004.

Outbreak	Setting	Strain	Genotype
1	Hostel	Hunter	GII/4
2	Nursing home	Germanton	GII/4
3	Farmington Hills strain		GII/4
4			GII/4
5			GII/4
6			GII/4
7			GI/1
8		Hunter Germanton	GII/4 GII/4
9	Hostel	Hunter	GII/4
10	Nursing home	Hunter	GII/4
11	Lodge	Hunter	GII/4
12	Nursing home	Hunter	GII/4

*Marc-Alain et al. 2004. Outbreaks of acute gastroenteritis on cruise ships and on land: Identification of a predominant circulating strain of norovirus – United states, 2002. J Infect. Dis. 190:27-36.*

- ✓ Sharp increase in outbreaks - 2002 (cruise ships and on land)
- ✓ Clinical manifestations (increased vomiting, environmental persistence) facilitated the spread of this strain in closed settings
- ✓ Cruise ship outbreaks featured higher attack rates and persisted longer on successive cruises than did outbreaks caused by other Norovirus strains.
- ✓ Herd immunity that would be expected to develop to some degree after exposure of a population to an emergent GII/4 strain (i.e. 95/96-US) does not seem to have prevented the emergence of a related GII/4 strain.
- ✓ Consistent with very early observations that strain-specific protective immunity may last only 6 months.

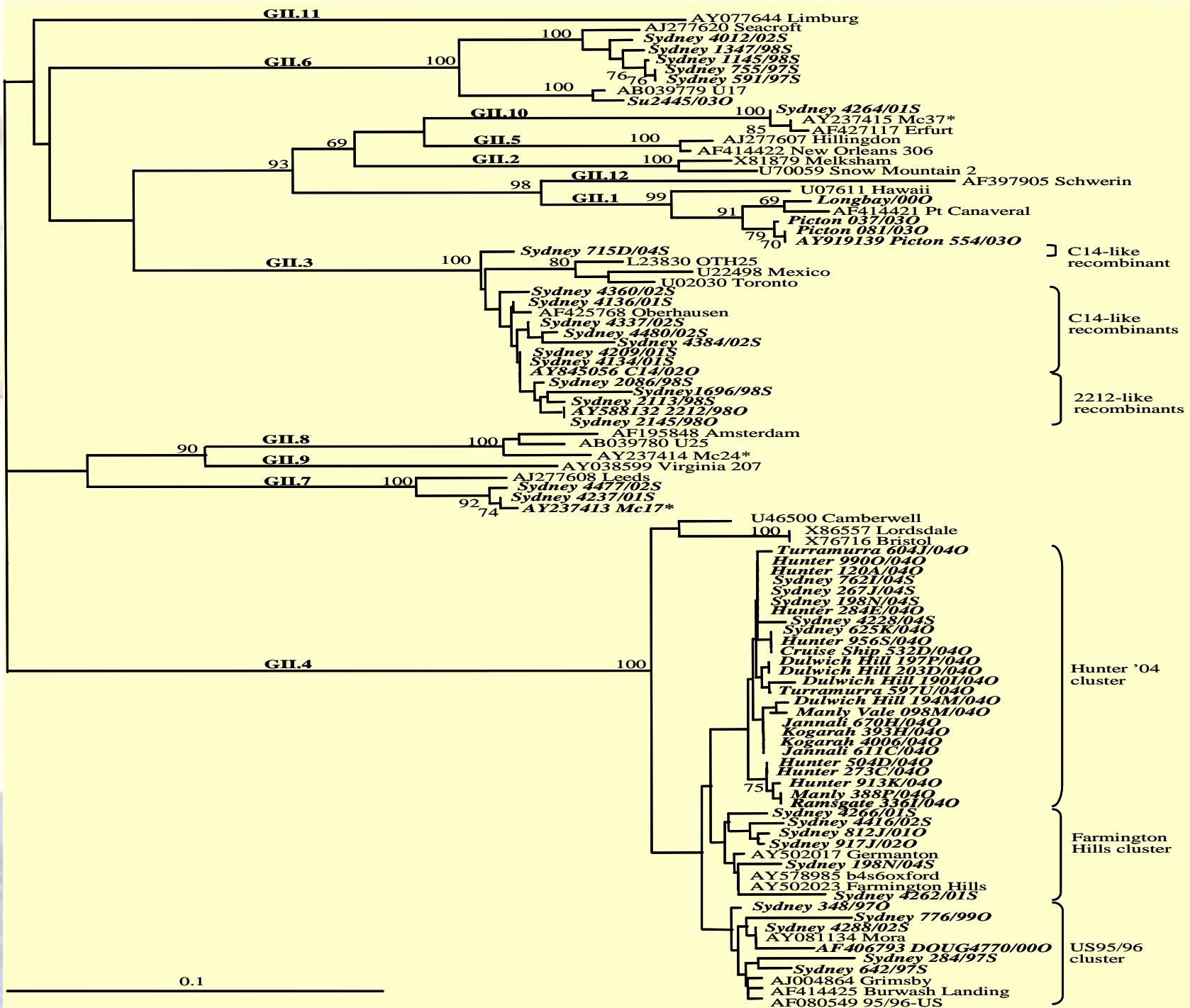


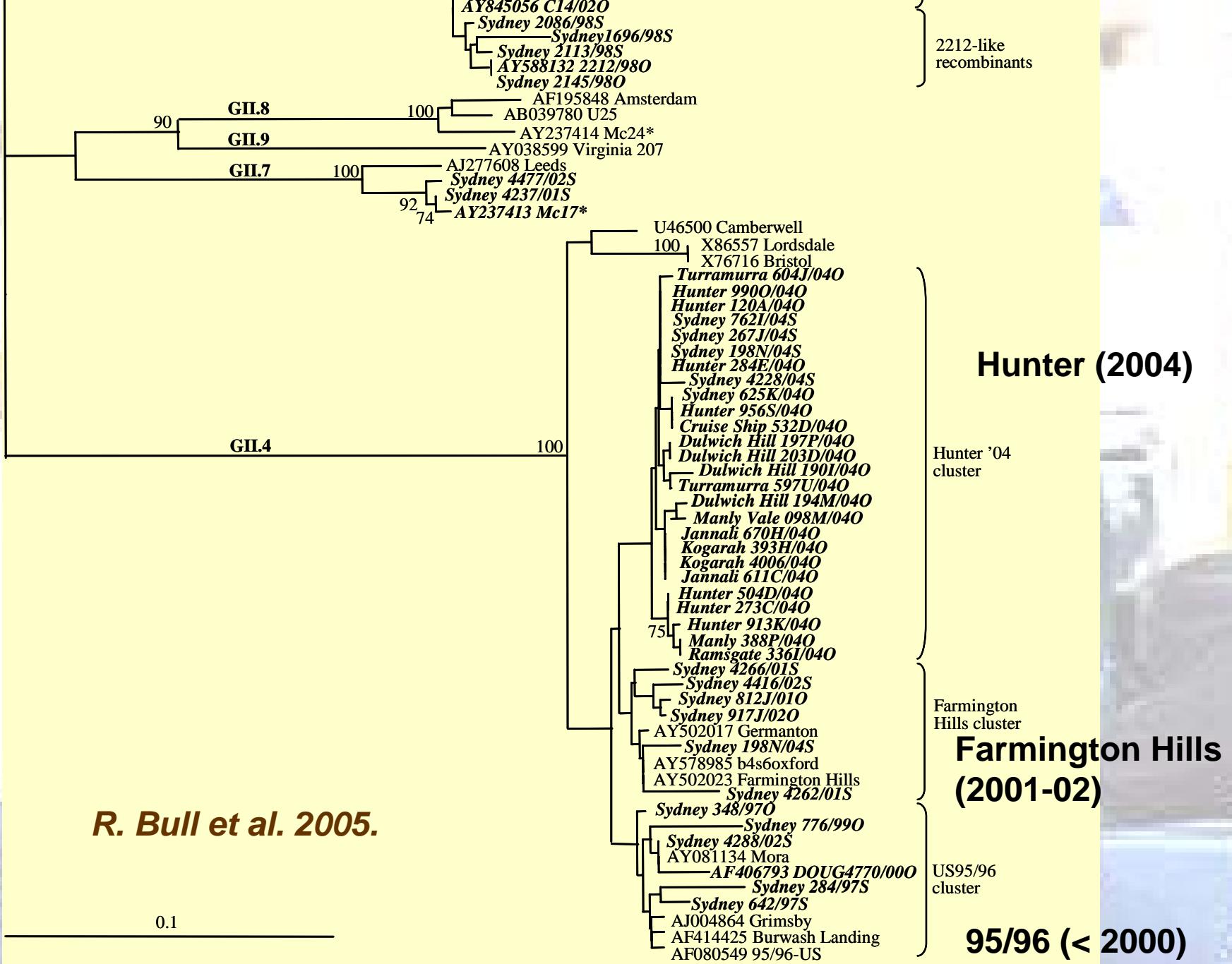
## Number of norovirus outbreaks reported in the Netherlands in the winter seasons from 2000/2001 to 2004/2005

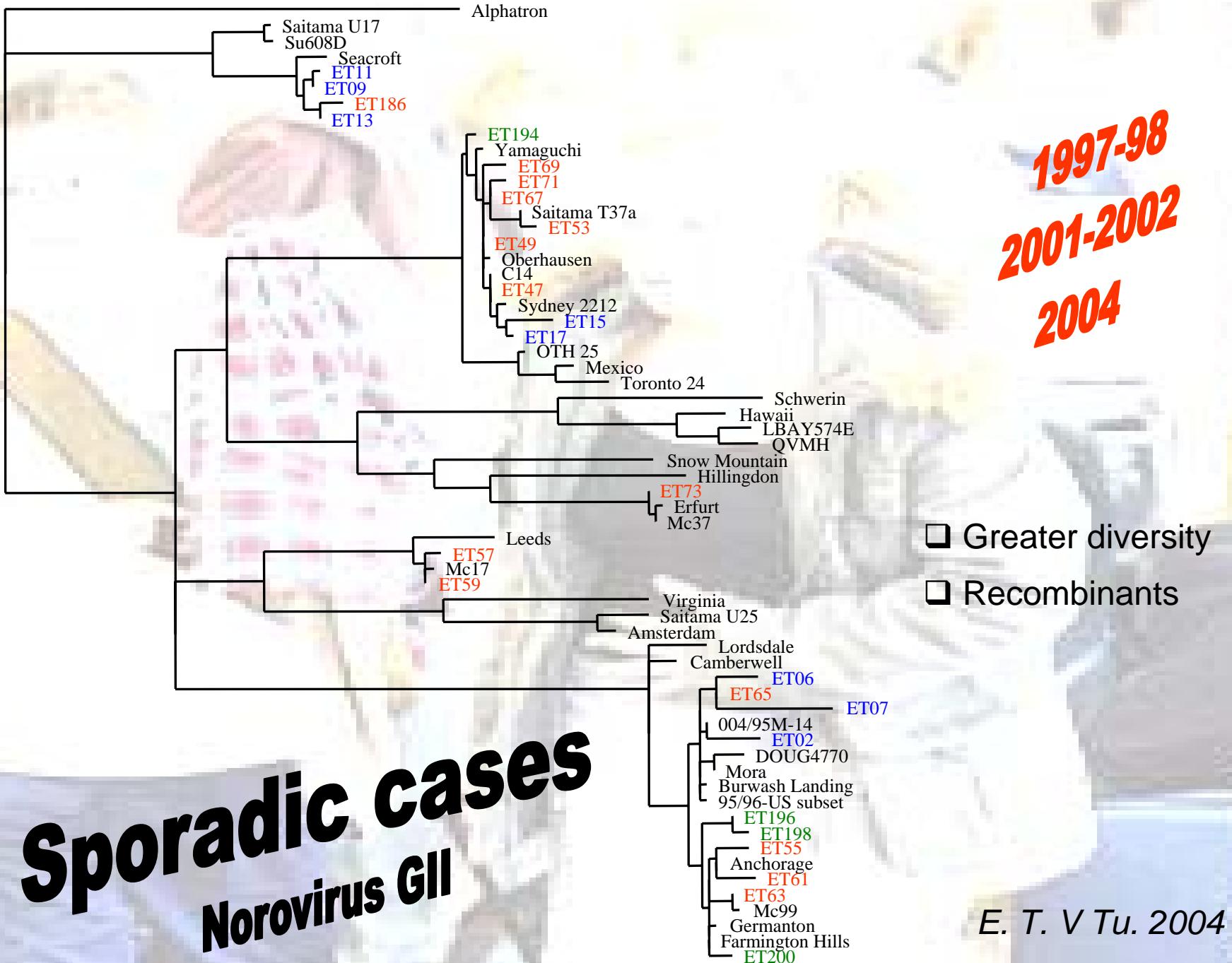
	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
2000/ 2001	3	1	4	3	13	11	8
2001/ 2002	2	6	8	14	18	12	8
2002/ 2003	7	11	33	52	26	12	2
2003/ 2004	1	1	1	2	9	4	3
2004/ 2005	9	18	31	13	-	-	-

Hunter-like

(PPV)  
(NPV)

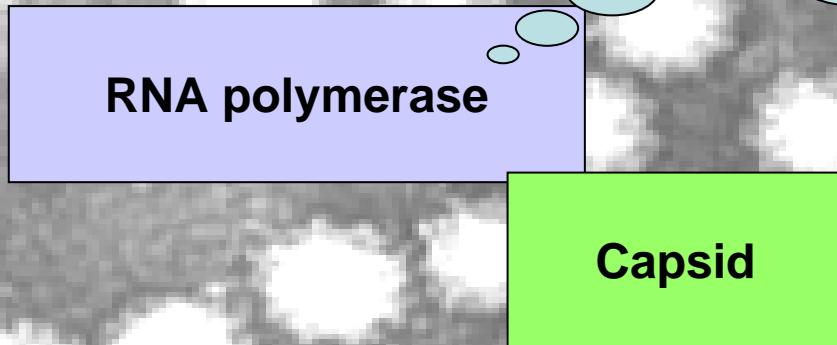






E. T. V Tu. 2004

# Recombinants



- Phylogenetic grouping**
- Molecular epidemiologic studies**
- Immunity does not protect against variant strains**
- Impact vaccine design**

# Infection Control Strategies

## Patients

- Isolate/ cohort symptomatic patients
- Place on contact and airborne precautions
- Emphasis on hand washing/ cleansing after patient contact or environment
- Duck bill masks (patient's with explosive diarrhoea or vomiting)
- Advice to relatives
- Terminal clean room/ ward
- ? Stop new admissions via A&E for 24 h



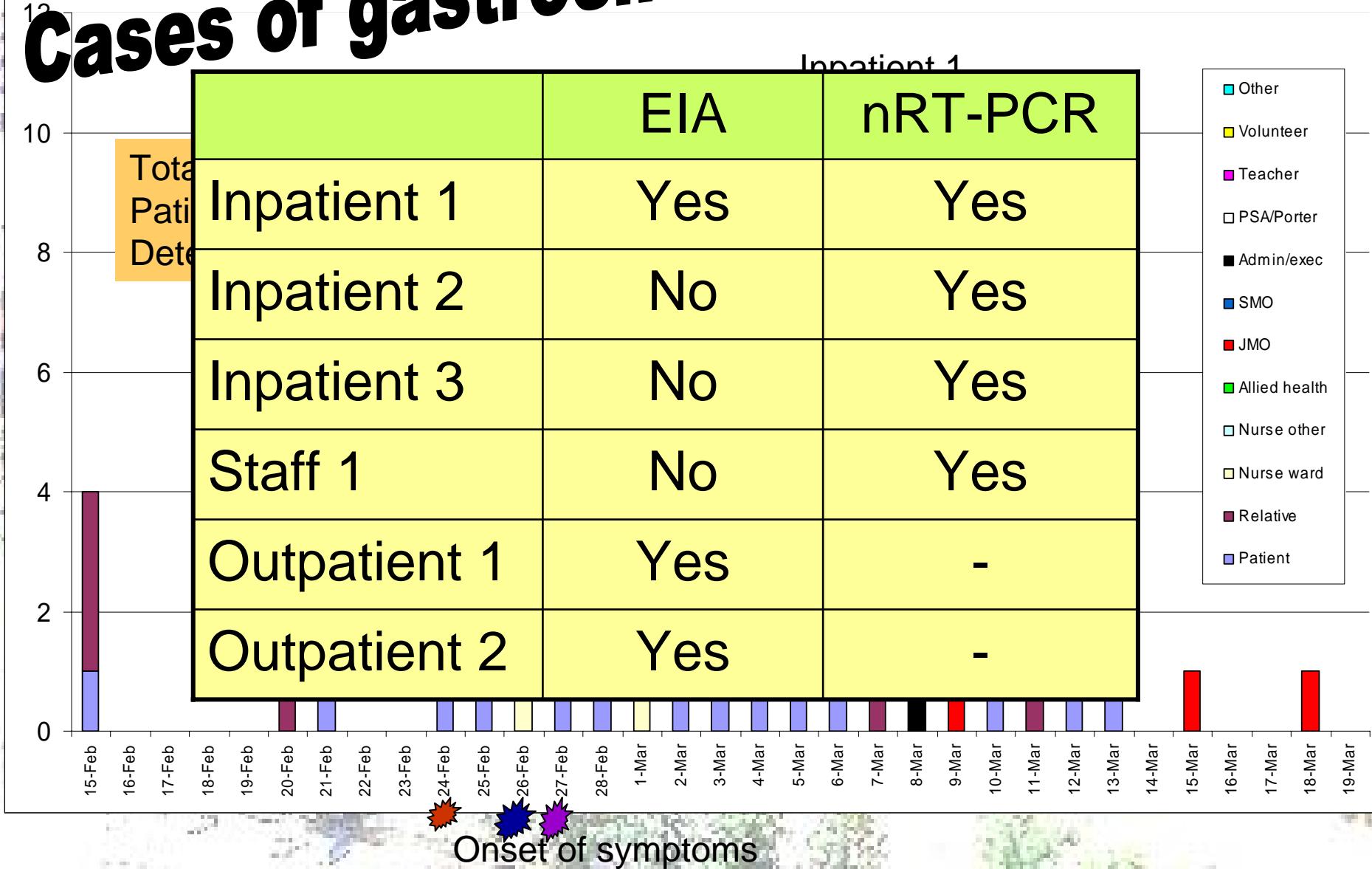
# Infection Control Strategies

## Staff

- Symptomatic staff (nausea/ vomiting/ diarrhoea) excluded from duty
- Excluded infected staff from duty until symptom free from diarrhoea or vomiting for 48hrs
- Agency staff to work in wards not affected
- Daily meeting to review outbreak



# Cases of gastroenteritis



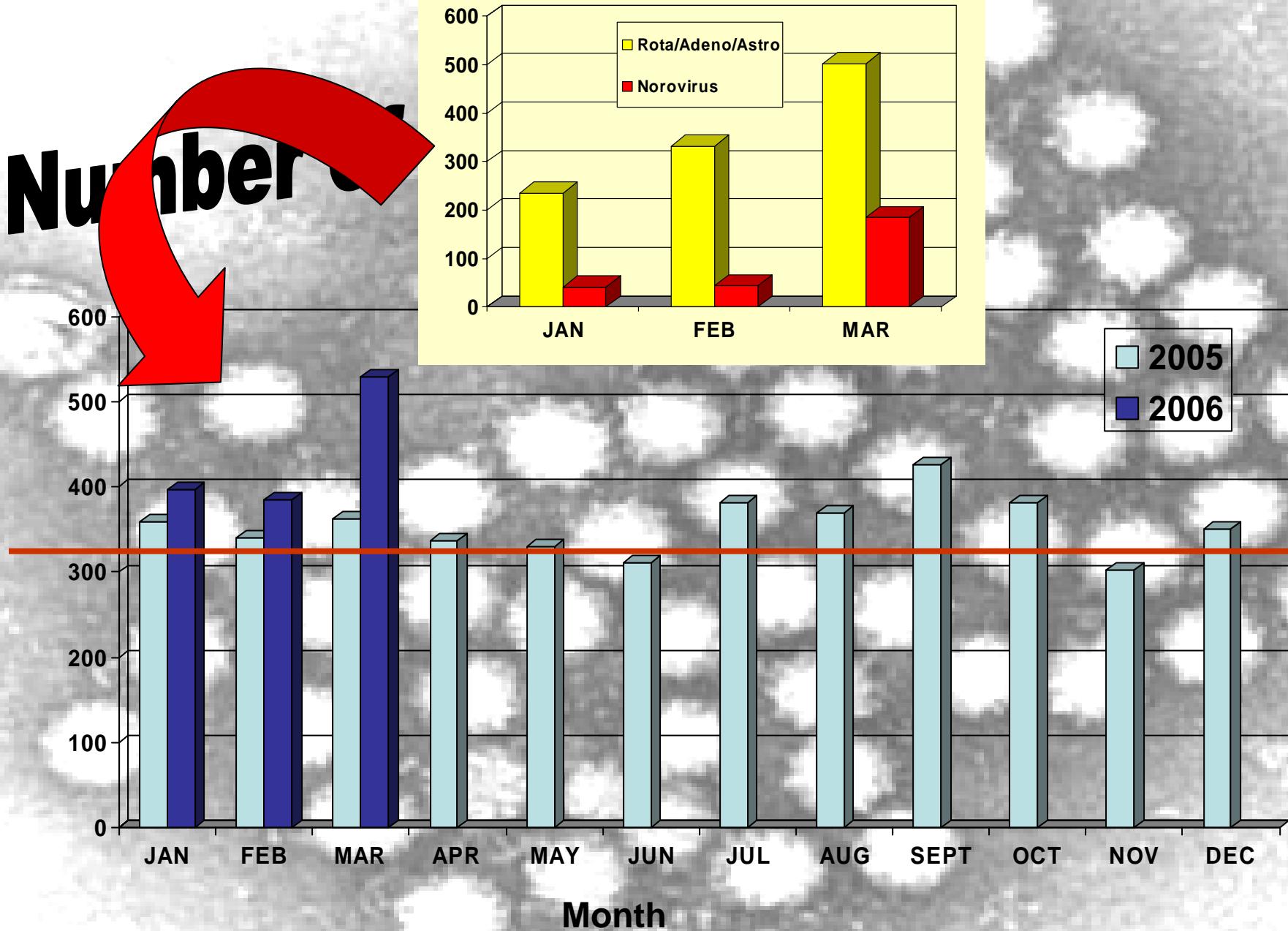




## Physician-based study The Netherlands from May 1996 – May 1999

	Overall		< 1 y		1-4 yrs		5-14 yrs		15-29 yrs		30-59 yrs		> 60 yrs	
	Case	Cont	Case	Cont	Case	Cont	Case	Cont	Case	Cont	Case	Cont	Case	Cont
N	857	574	32	17	136	69	96	58	170	72	313	244	102	102
NLV	5.1	1.1	15.2	0	8.7	1.5	3.1	1.7	5.9	1.4	3.9	0.8	1.0	1.0
SLV	2.4	1.5	4.2	8.3	4.9	5.0	6.1	0	2.8	0	0.6	0.7	0	0

# Number



# Investigations

	Rate
1	30.8
2	83.3
3	18.2
4	53.3
5	50.0
6	50.0
7	100
8	100
9	100
10	47.0
11	100

Timing of collection

Preservation of samples (RNase activity)

Limit of detection

Internal controls



# Detection of norovirus in nasopharyngeal aspirates

Condition	Positive	Negative	Total
Asthma	12	32	44
Non-asthma	3	17	20

Rhinovirus = 9/12

Diarrhoea = 0

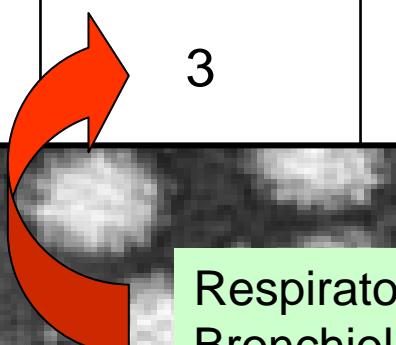
Vomiting = 3/12

P = 0.3536  
(considered not significant)

Relative risk = 1.818  
CI = 0.5759 – 5.741

Respiratory illness  
Bronchiolitis  
Cough

Rhinovirus = 0  
Diarrhoea = 0  
Vomiting = 1



# Sapovirus

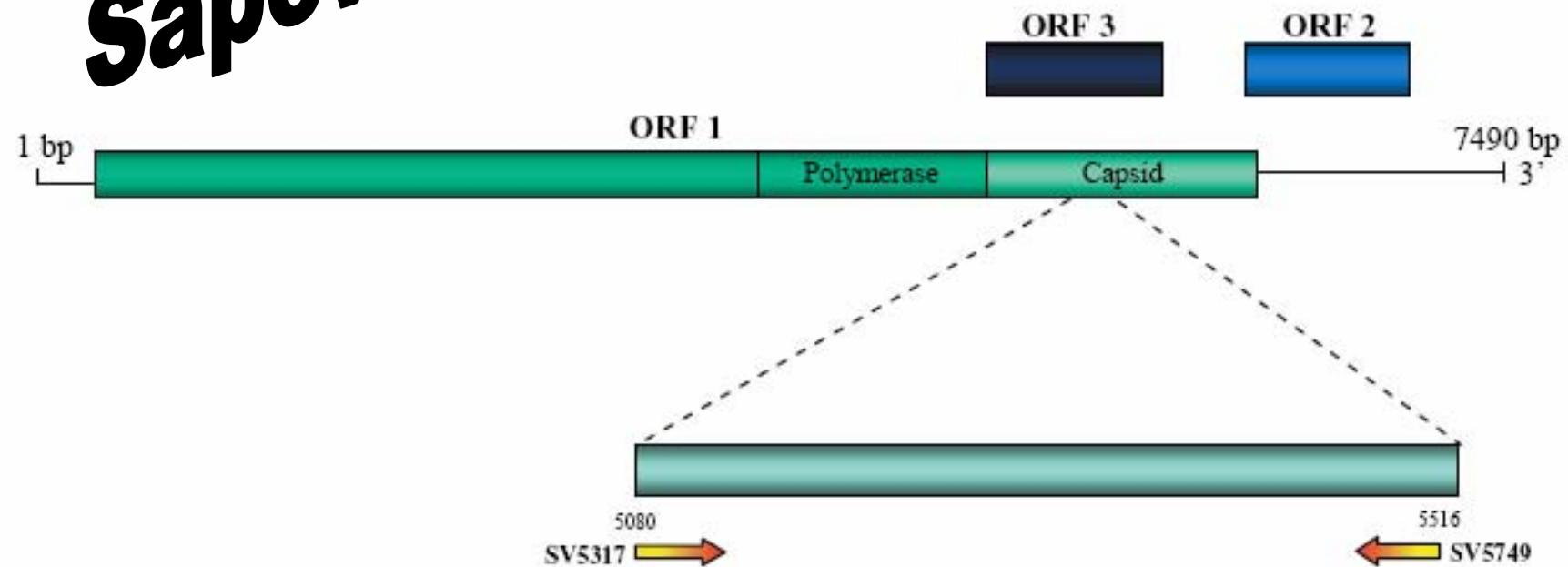


FIG. 2 Genomic organisation of sapovirus and primer target sites (Table 1).



# **Sapovirus**

**170 patients (< 18 y), gastroenteritis**

**Treated at Sydney Children's Hospital**

**Timeframe: 2001 – 2002; 2004**

**Negative (94/170): Salmonella, Shigella, Campylobacter.  
Rotavirus, Adenovirus, Astrovirus and Norovirus)**

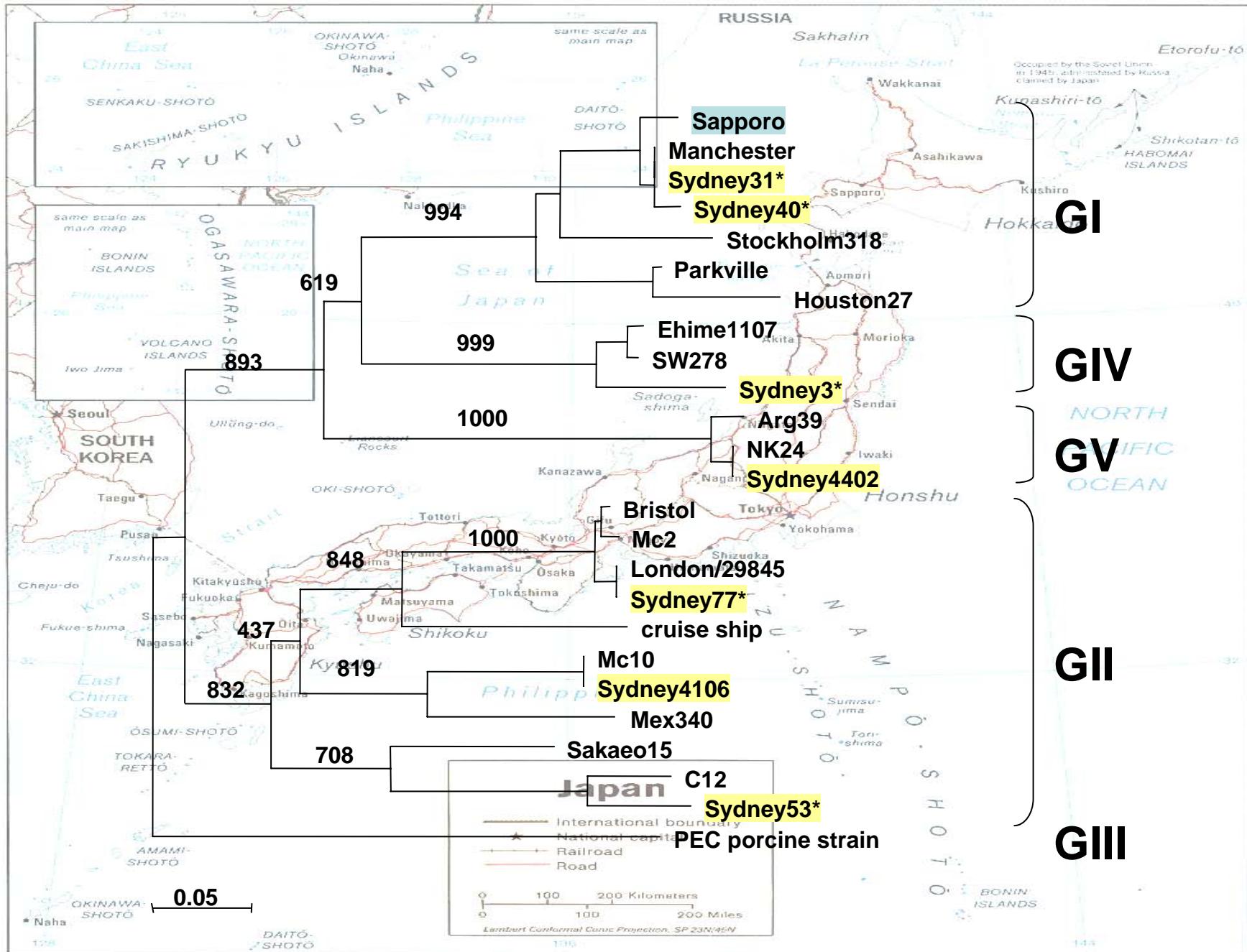
**7/94 (7.4%) positive for Sapovirus**

**7/170 (4.1%) “minimal” prevalence**

*Hansman et al. 2006. EID*

# Sapovirus

Research group	Setting	Prevalence %
Kirkwood & Bishop (2001)	Melbourne	0.56
Wright et al. (1998)	SE Australia (1980-96)	0.32
Grohman et al. (1991)	Sydney	0.46
Hansman et al. (2006)	Sydney	4.1



# Conclusions

- ❖ Common cause - gastroenteritis
- ❖ Immunogenic detection - under scrutiny
- ❖ Molecular detection methods favoured
- ❖ Emergence of GII/4
- ❖ Recombinant strains
- ❖ Diversity of Sapovirus in Sydney
- ❖ Improvements needed

