Molecular epidemiology used to track viral outbreaks–norovirus and beyond

Viruses in May 2018 - The Carrington Hotel, Katoomba NSW

18th May 2018

Professor Peter White

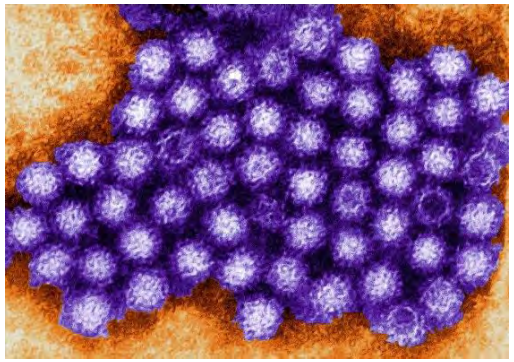
Molecular Microbiology Lab

School of Biotechnology and Biomolecular Sciences

University of New South Wales

Norovirus

- Leading cause of viral gastroenteritis
- Highly infectious and environmentally stable
- Nursing home, hospital wards, cruise ships, childcare centre
- Massive social-economic cost each year



Norovirus transmission

- Person to person, most common via ***faecal – oral route***
- Consumption of contaminated food or drink

- Poor food-handling
- Secondary spread - aerosols, fomites

Classification

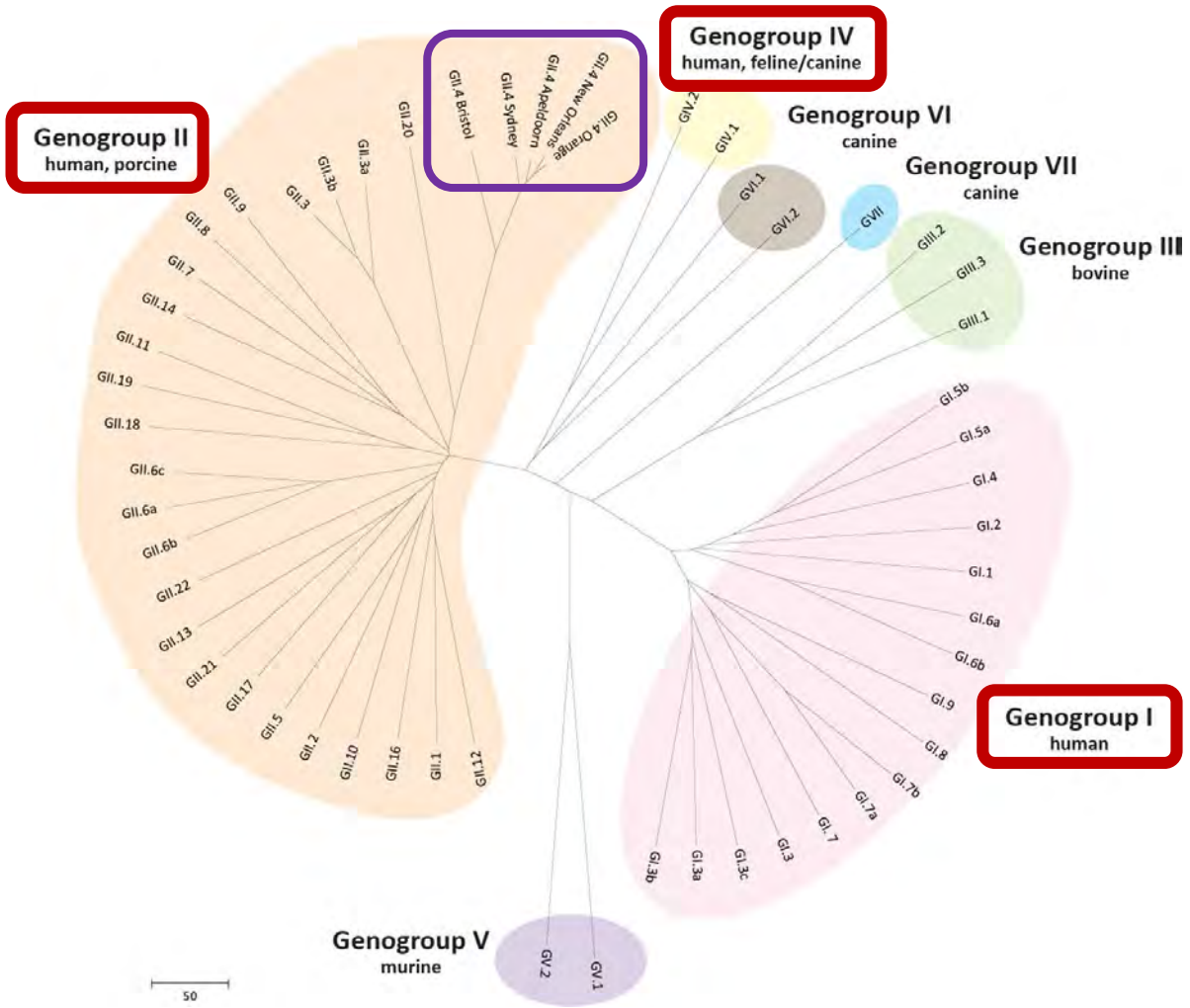
***Caliciviridae* (family)**

- *Lagovirus* [rabbits]
- *Nebovirus* [bovine]
- *Sapovirus* [swine/humans]
- *Vesivirus* [feline/sea lion/swine]
- ***Norovirus* (genus)**

  [swine/humans/mice/feline/dogs]

  - *Norwalk virus* (prototype species)

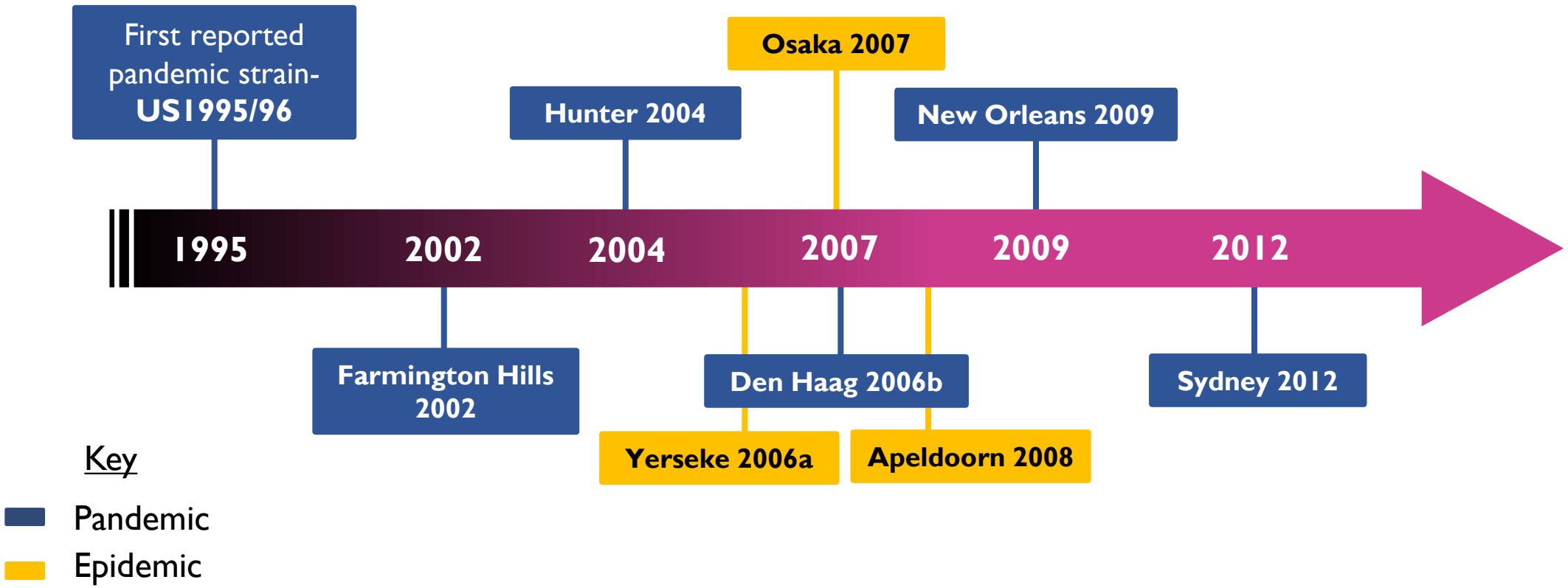
Norovirus classification



- Family: Caliciviridae
- Genus: Norovirus
- Classified into 7 genogroups
- > 40 genotypes

- Genogroup II, genotype 4 (GII.4) is the most prevalent (65-80% of all norovirus infections)

GII.4 global pandemic variants



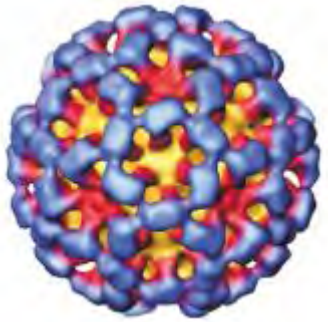
Viral evolution mechanisms

- **ANTIGENIC DRIFT :** point mutations within the capsid region

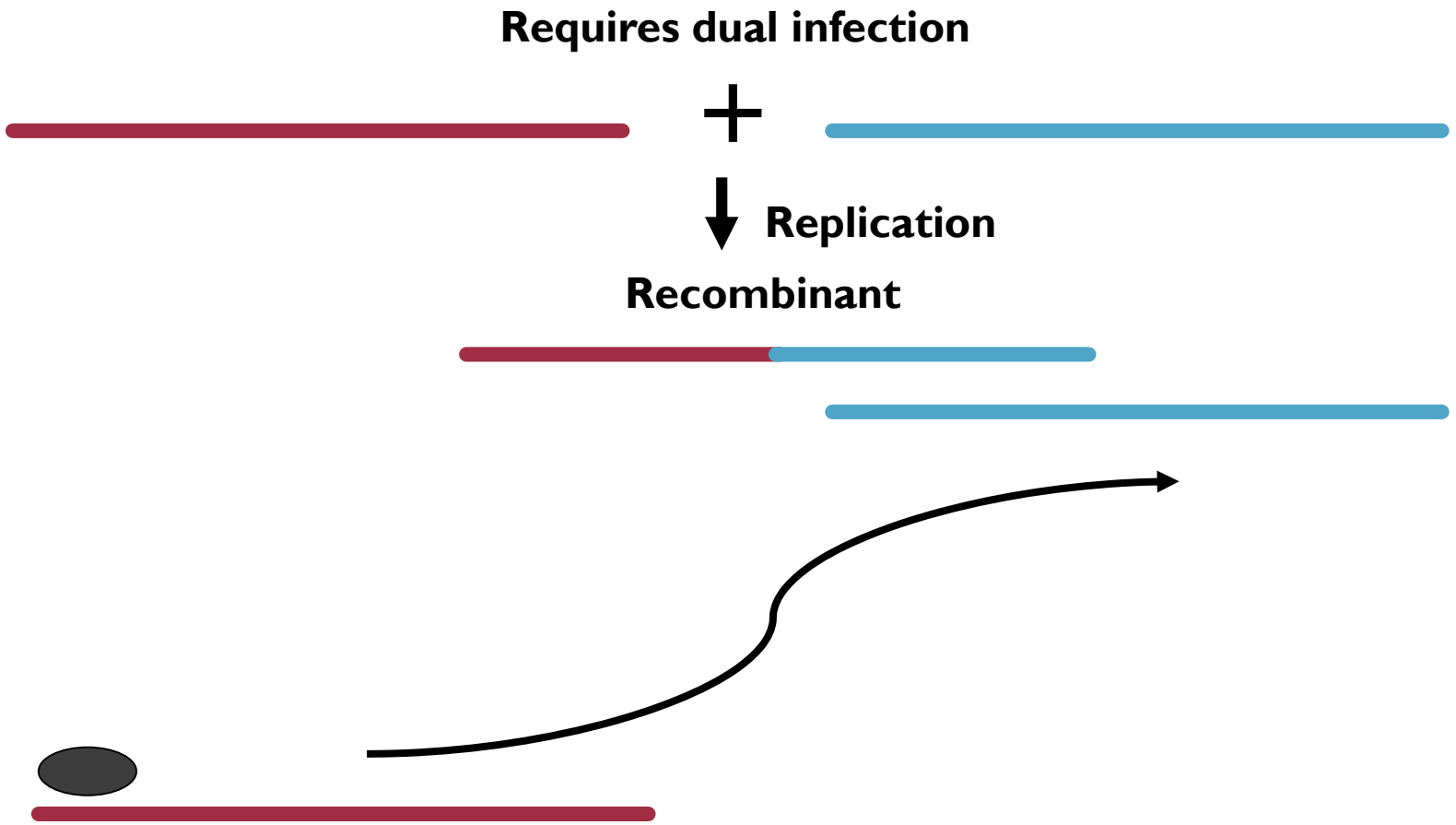
New pandemic variants

- **ANTIGENIC SHIFT :** exchange of genetic material between tw different strains

New recombinants



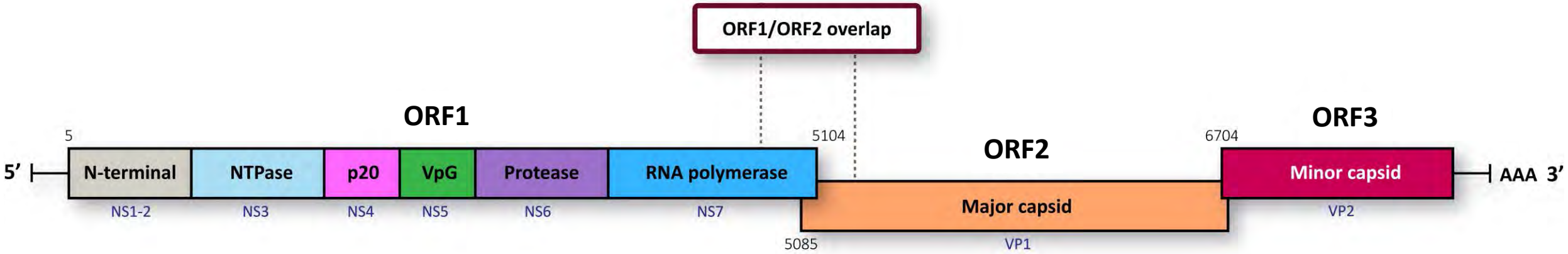
Recombination



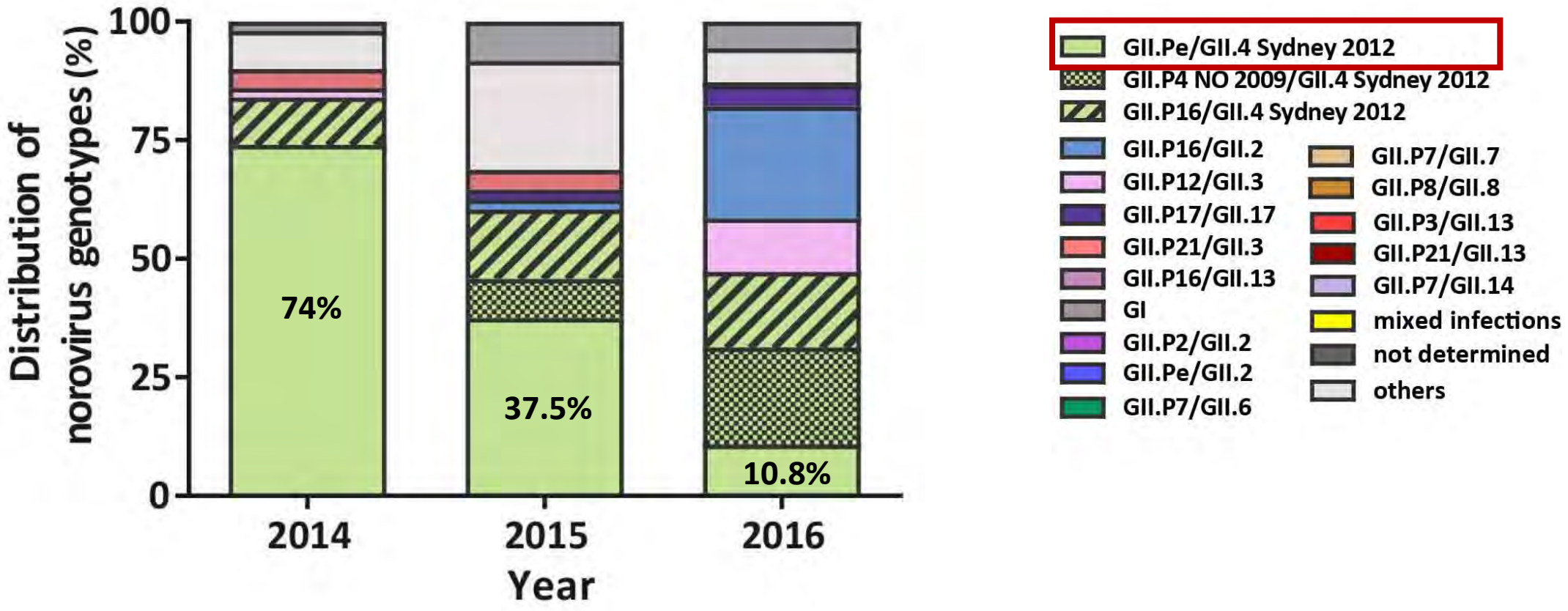
Bull et al., EID, 2005

Norovirus genotyping

GII.Pe/GII.4 Sydney 2012



Emergence of GII.4 recombinants in 2016



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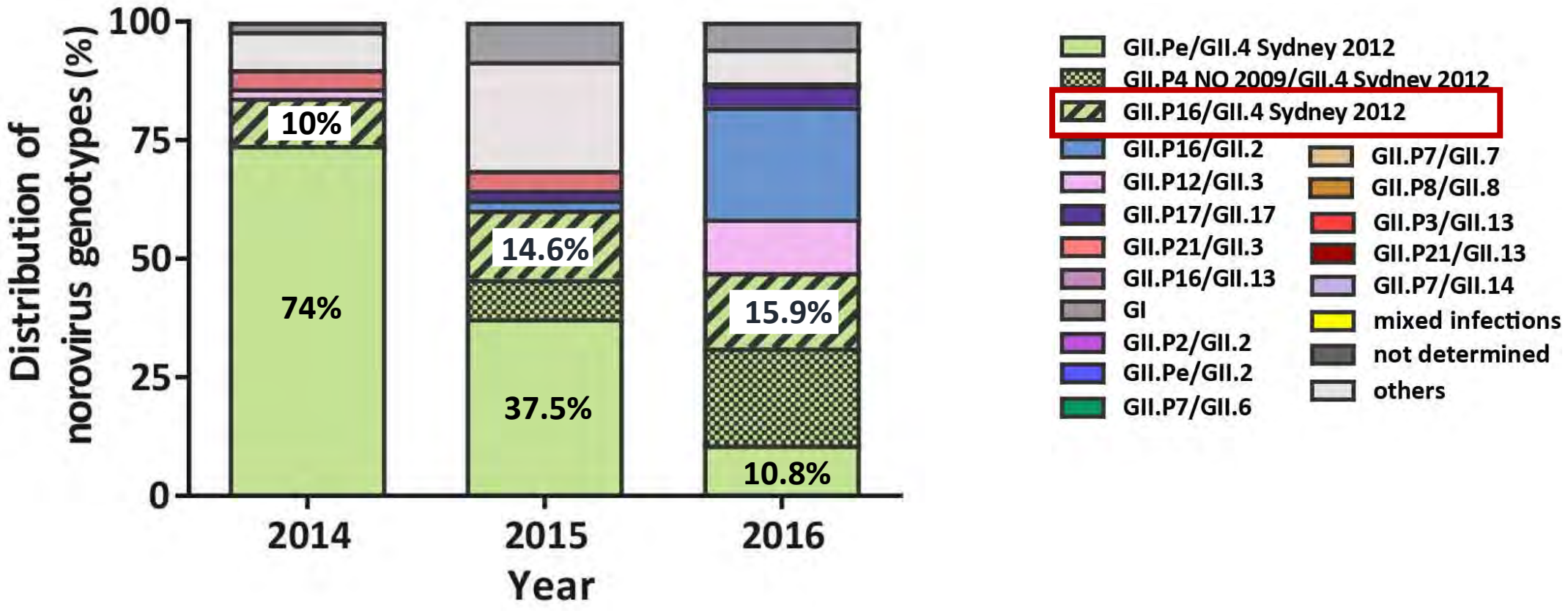

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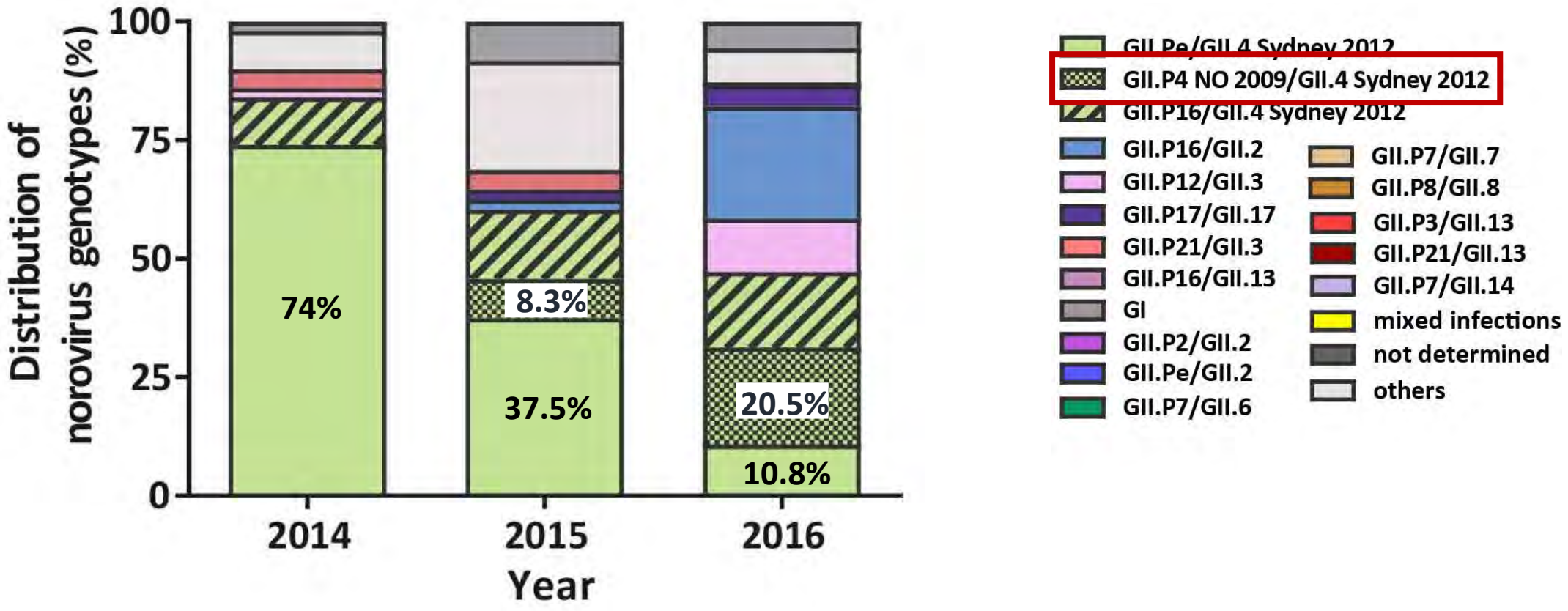

Lun et al. Emerging Microbes & Infections (2018) 7:50

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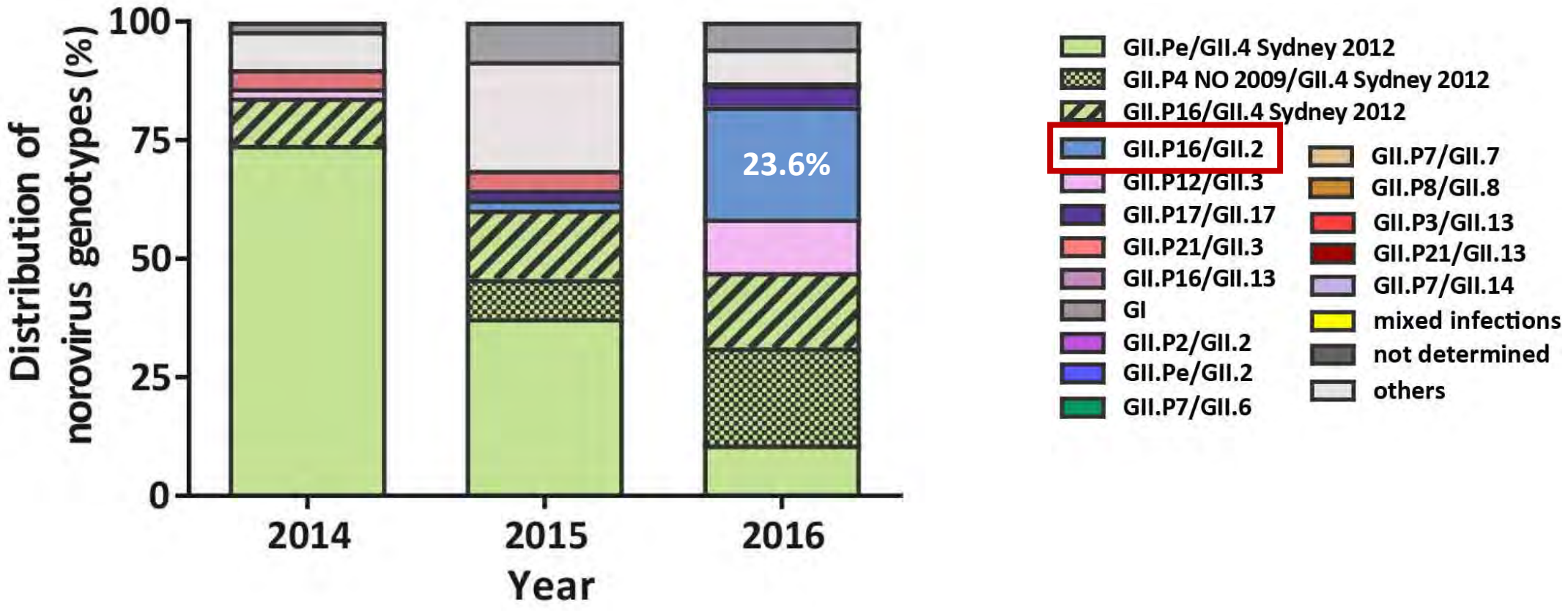

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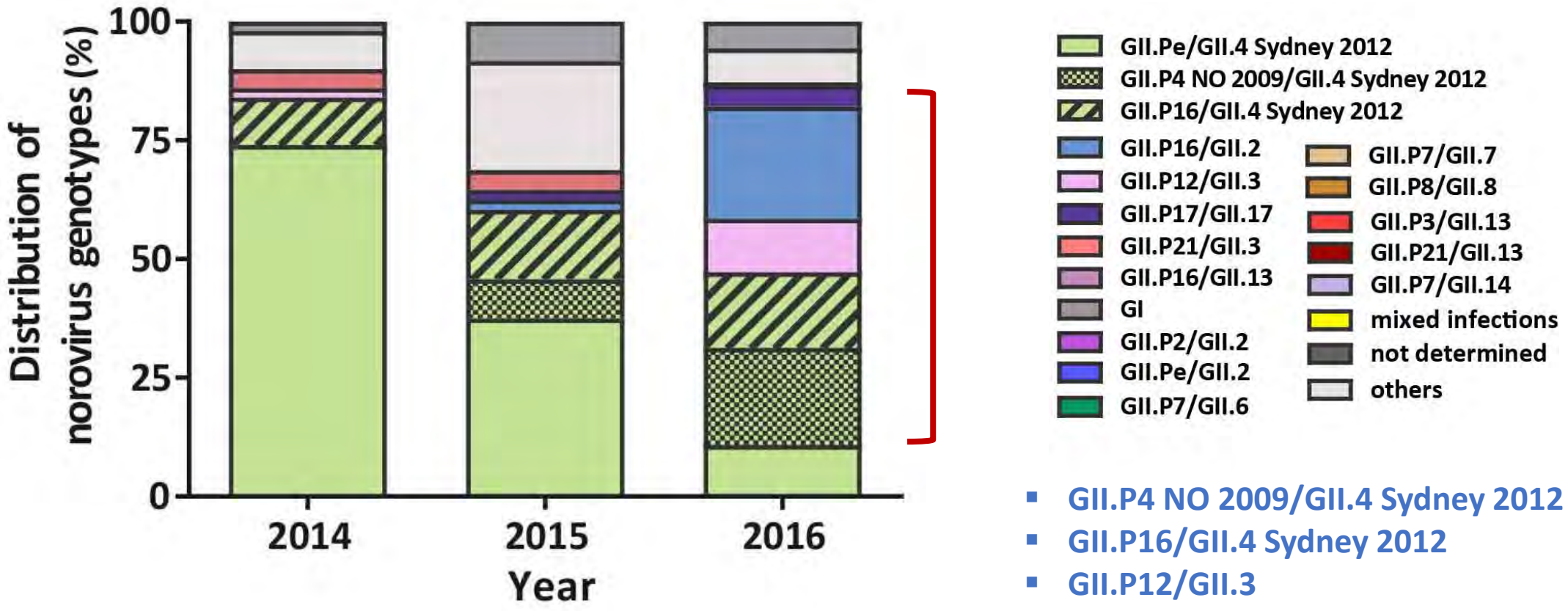
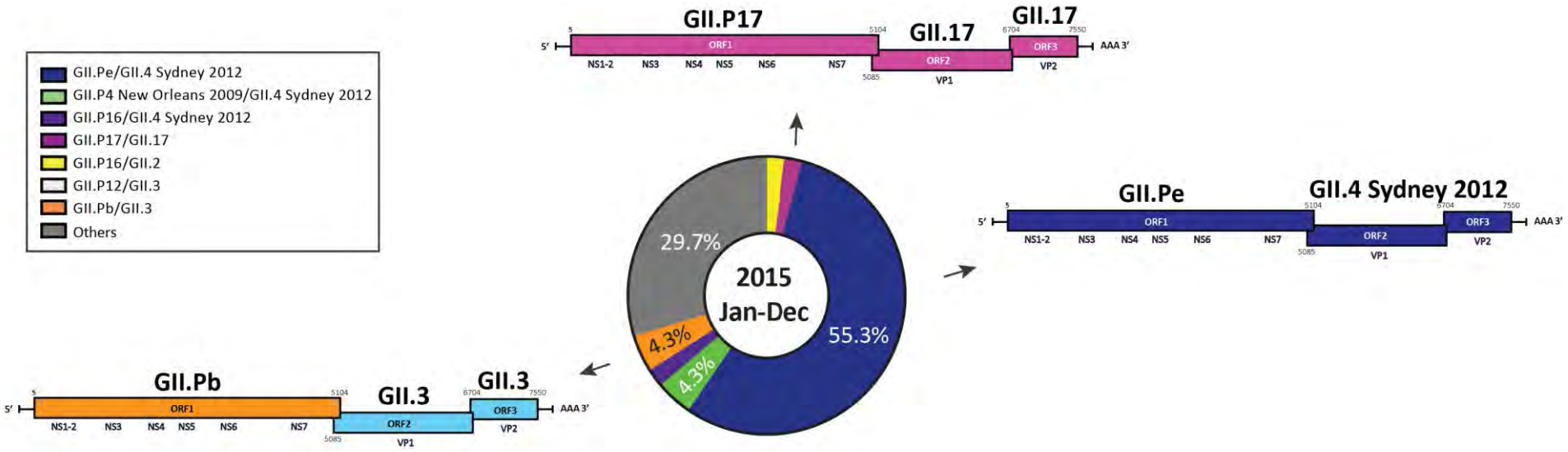


- GII.P4 NO 2009/GII.4 Sydney 2012
- GII.P16/GII.4 Sydney 2012
- GII.P12/GII.3
- GII.P16/GII.2
- GII.P17/GII17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GII.Pb/GII.3
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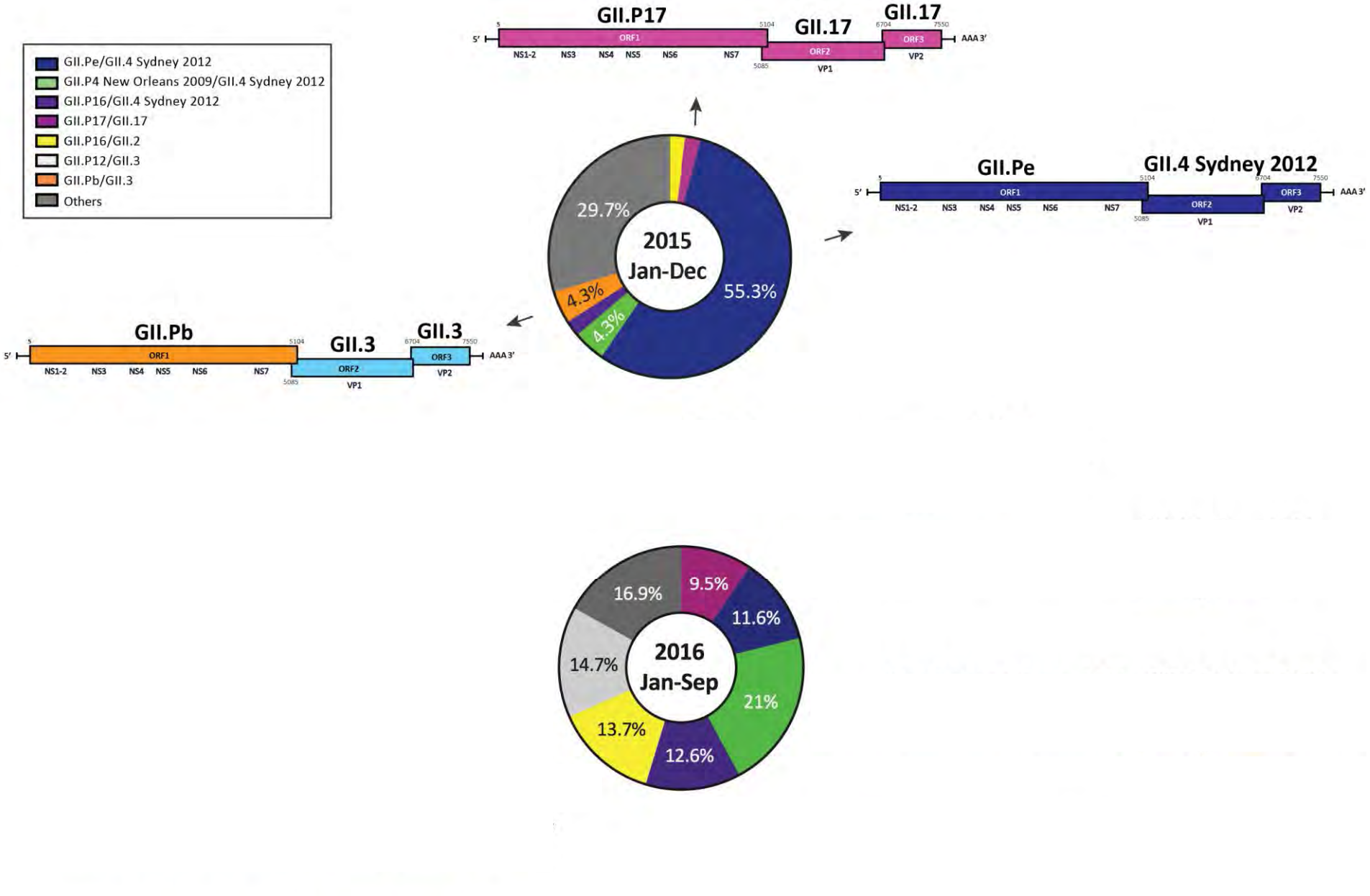
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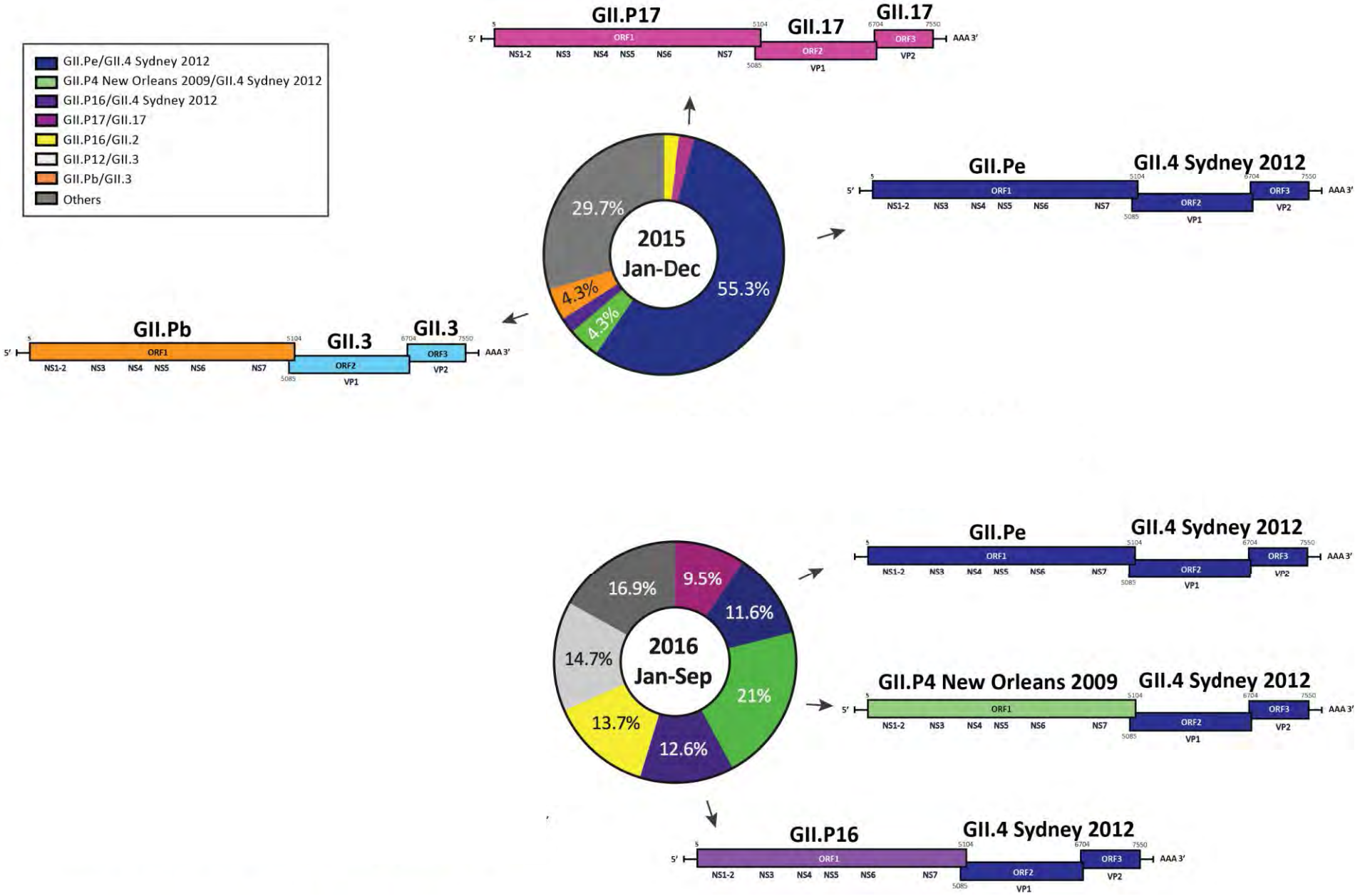
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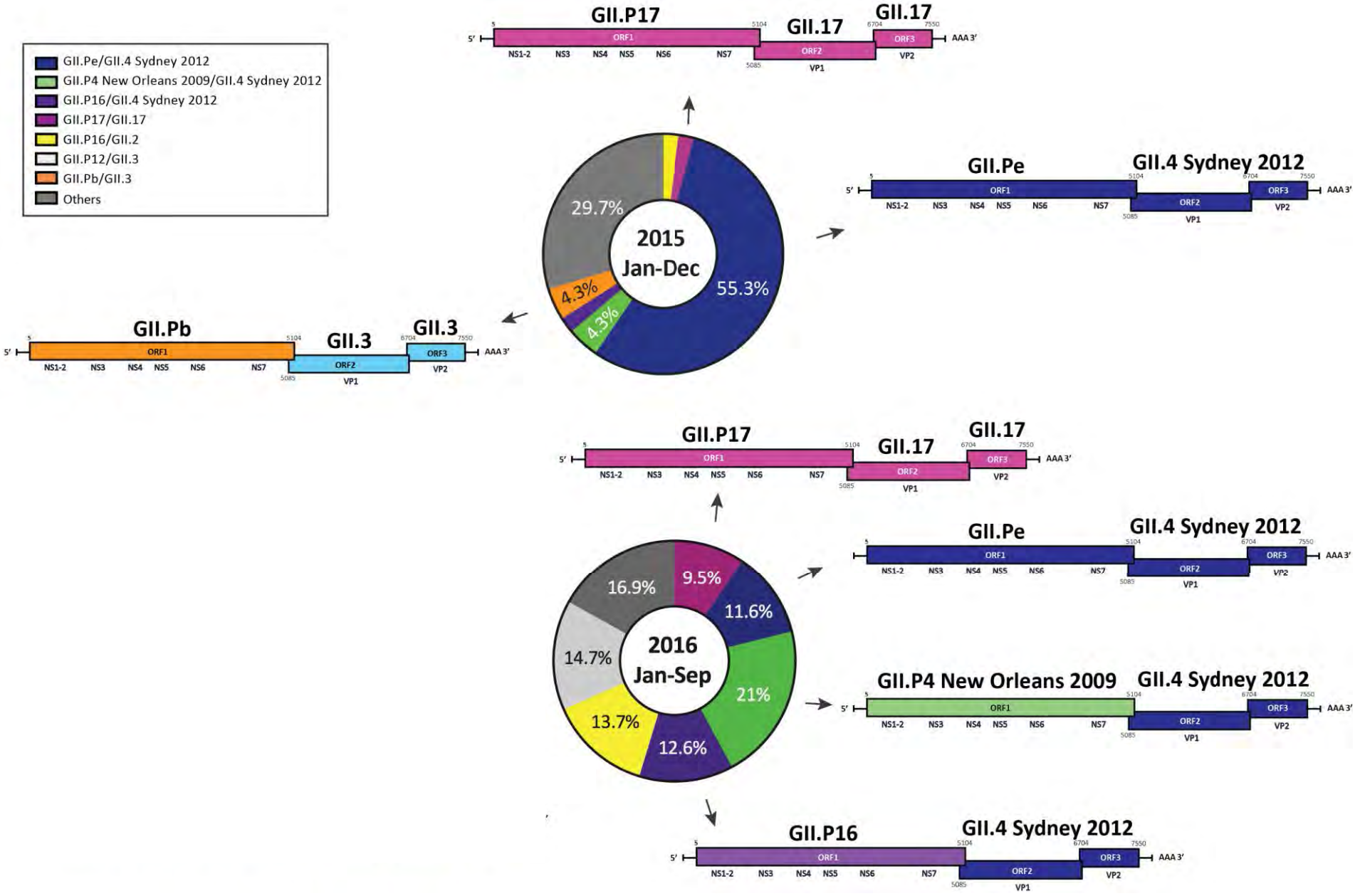
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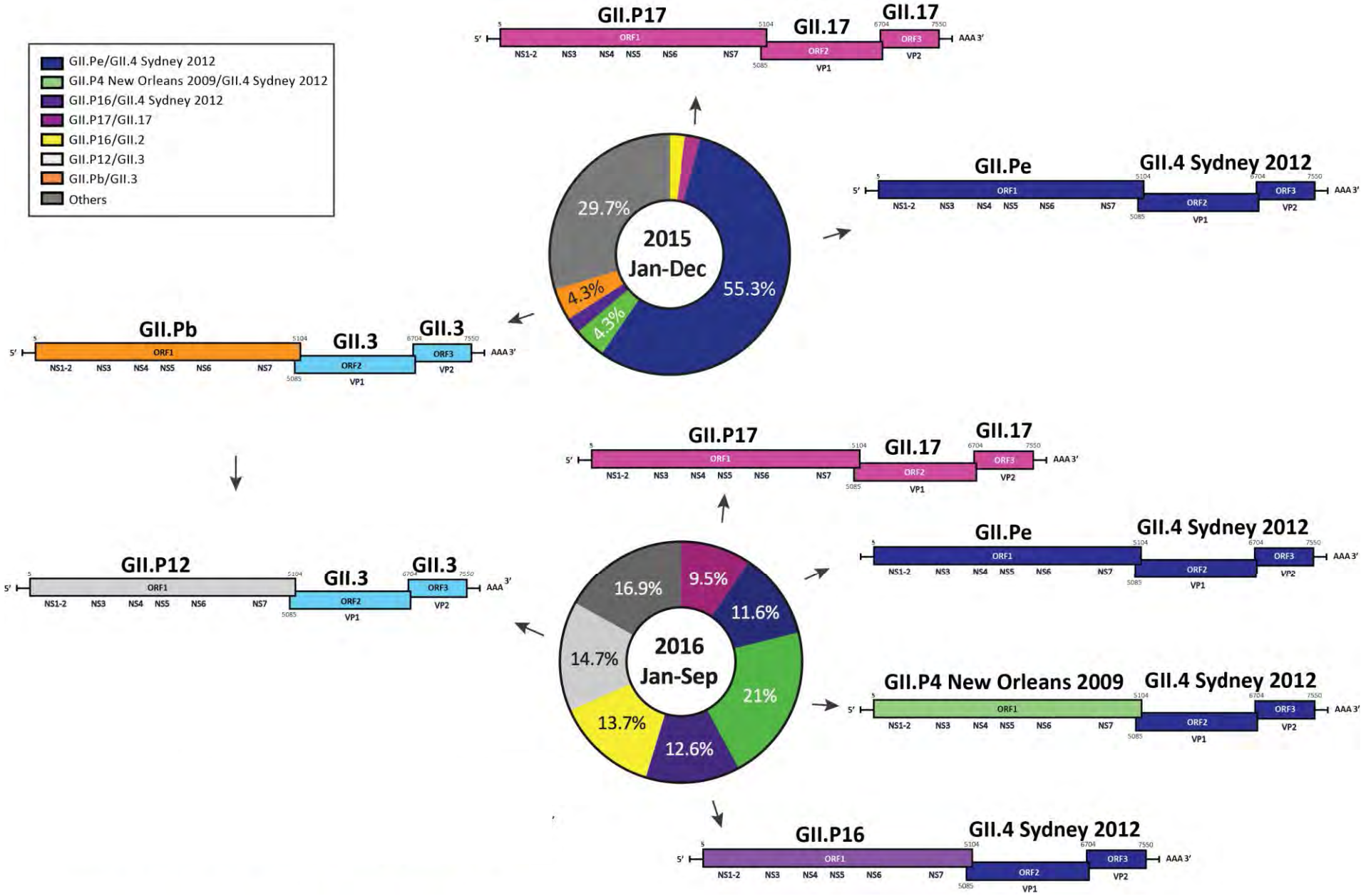
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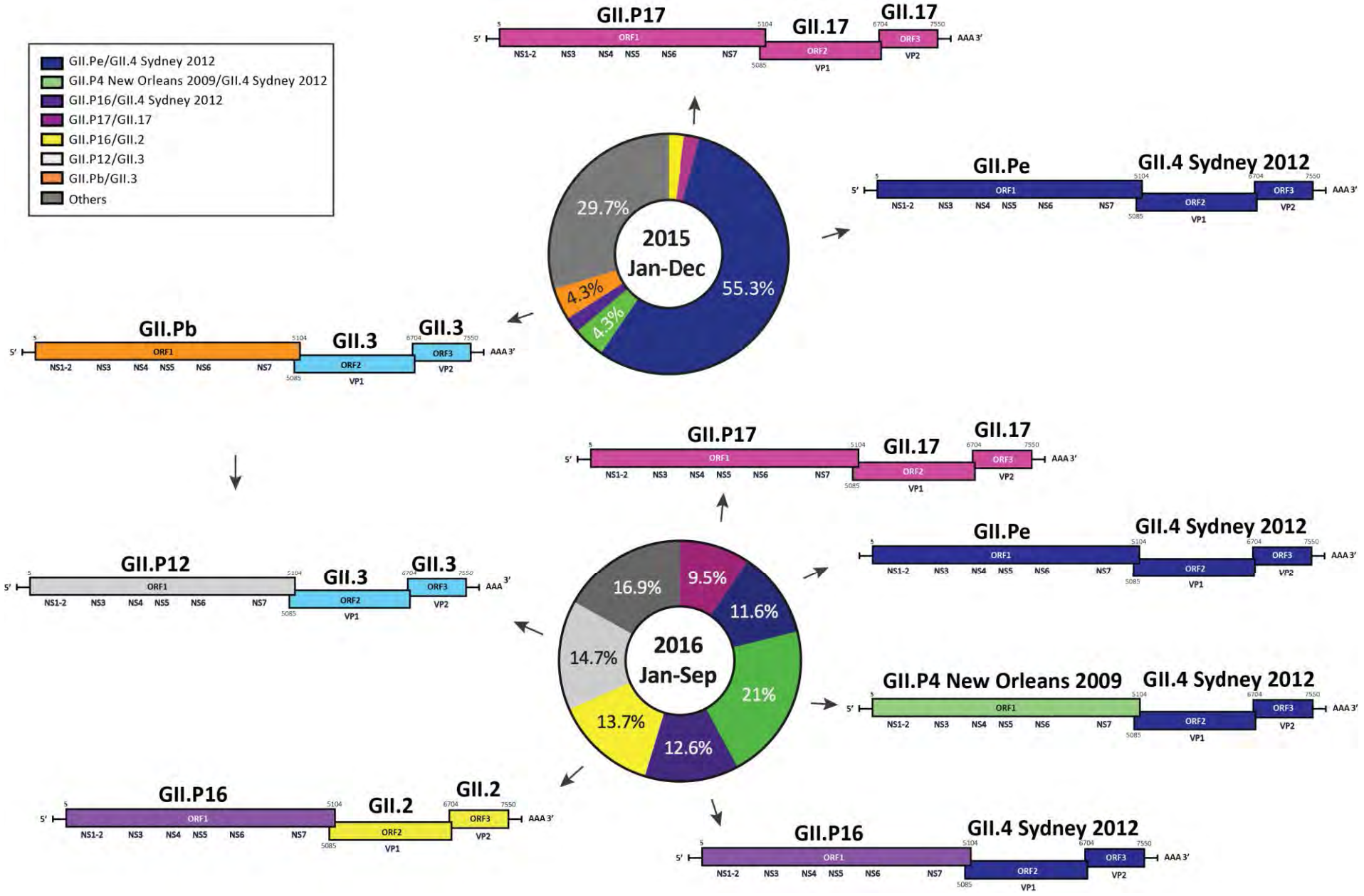
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Aim

Examine the molecular epidemiology of circulating *clinical* norovirus in NSW from January to October 2017

Gastro outbreak at Calvary Hospital as NSW cases spike

Gastro warning: 49 outbreaks in NSW childcare and nursing homes

Sick passengers on board Diamond Princess cruise ship in Sydney

Triple-dose of gastro has lawyers circling cruise ship Sun Princess

Primary school closed over suspected Norovirus outbreak affecting 1/5 of pupils

Quarter of passengers on British cruise ship fall sick with norovirus

Cruise ship hit by norovirus gastroenteritis docks in Sydney

Norovirus outbreak reported at Victoria General Hospital

Virus Hunters Follow One Gastro Bug's Rise And Demise To Make Way For A New Strain

Norovirus outbreak closes school and leaves 150 pupils sick

Nottingham hospitals urge public to help stop spread of Norovirus

Seattle elementary school stays closed after norovirus outbreak

Cruise Ships in Sydney

Table 1. Cruise ship arrivals in Sydney and number/cause of reported acute gastroenteritis (AGE) outbreaks 2007–2016 (source: Cruise Ship Health Surveillance Program, Public Health Unit, SESLHD).

		Norovirus	AGE other cause	AGE unknown cause	Total AGE outbreaks	Total no. of arrivals
Arrival year	2007	3 (3.0%)	0	6 (6.0%)	9 (9.0%)	99
	2008	6 (5.9%)	0	0	6 (5.9%)	102
	2009	1 (0.9%)	0	4 (3.7%)	5 (4.6%)	109
	2010	3 (2.2%)	0	4 (2.9%)	7 (5.1%)	136
	2011	2 (1.0%)	0	5 (2.6%)	7 (3.7%)	191
	2012	6 (2.8%)	0	8 (3.7%)	14 (6.5%)	214
	2013	4 (1.6%)	1 (0.4%)	12 (4.8%)	17 (6.8%)	250
	2014	7 (2.6%)	1 (0.4%)	6 (2.2%)	14 (5.2%)	268
	2015	4 (1.4%)	0	5 (1.8%)	9 (3.2%)	278
	2016	4 (1.3%)	1 (0.3%)	5 (1.6%)	10 (3.1%)	320
Total		40	3	55	98	1967

Norovirus and cruise ships

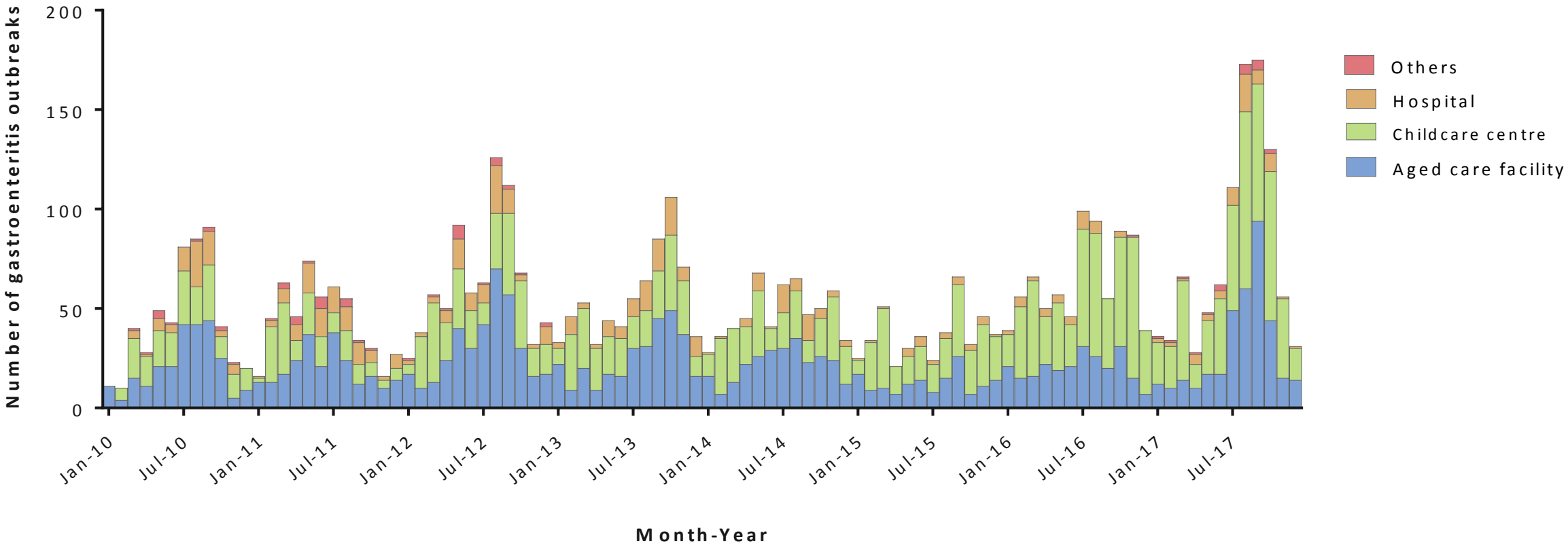
Jennifer H Lun[A,D], *Kelly-Anne Ressler*[B,E], *Mark J Ferson*[B,C,F] *and Peter A White*[A,G]

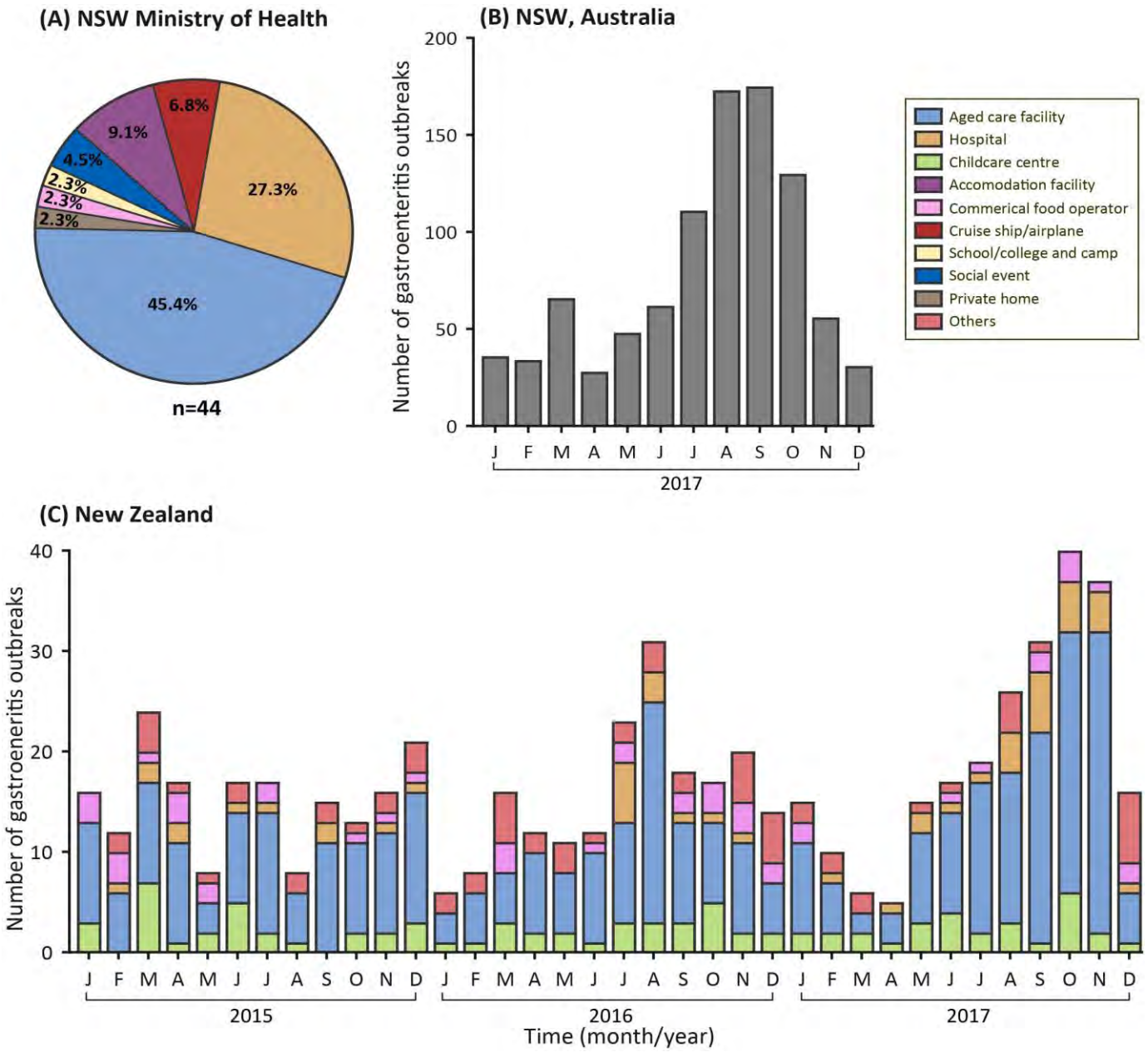
[A]School of Biotechnology and Biomolecular Sciences, Faculty of Science, University of New South Wales, Sydney, NSW, Australia

[B]Public Health Unit, South Eastern Sydney Local Health District, Randwick, NSW, Australia

[C]School of Public Health and Community Medicine, University of New South Wales, Kensington, NSW, Australia

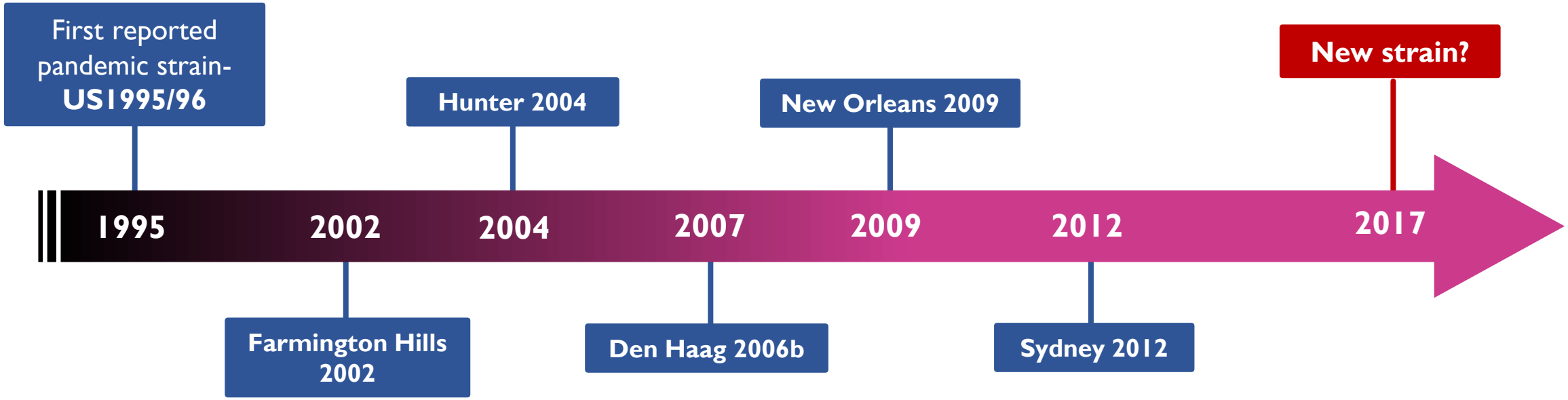
An increase in outbreaks of gastroenteritis, 2017



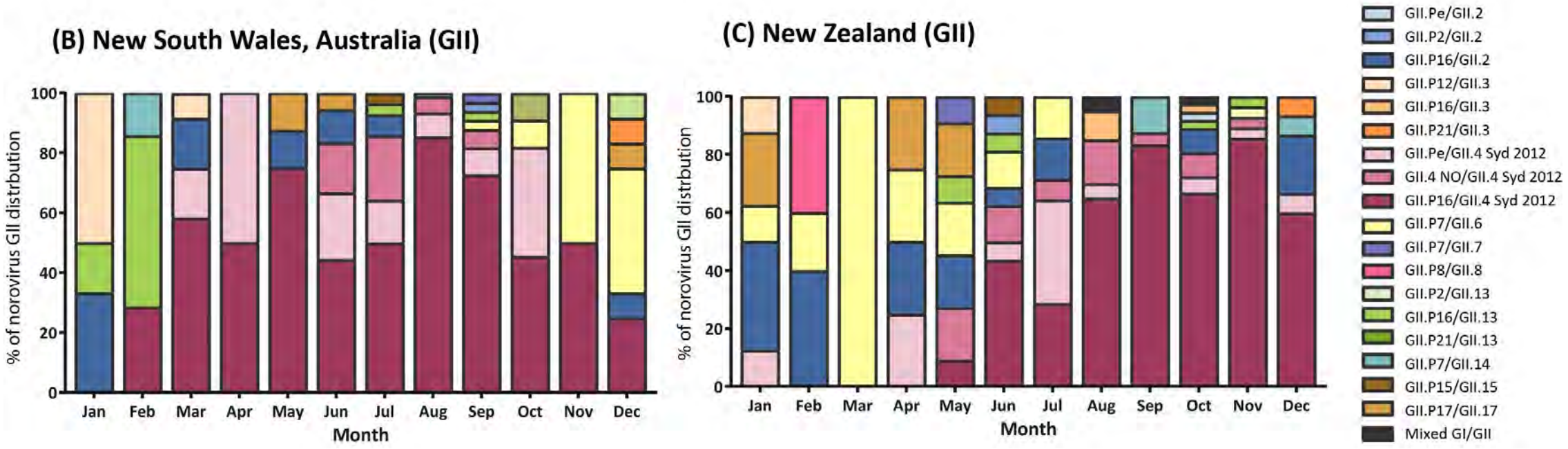


The number of gastroenteritis and norovirus outbreaks reported in NSW, Australia and New Zealand, 2017.

GII.4 global pandemic variants

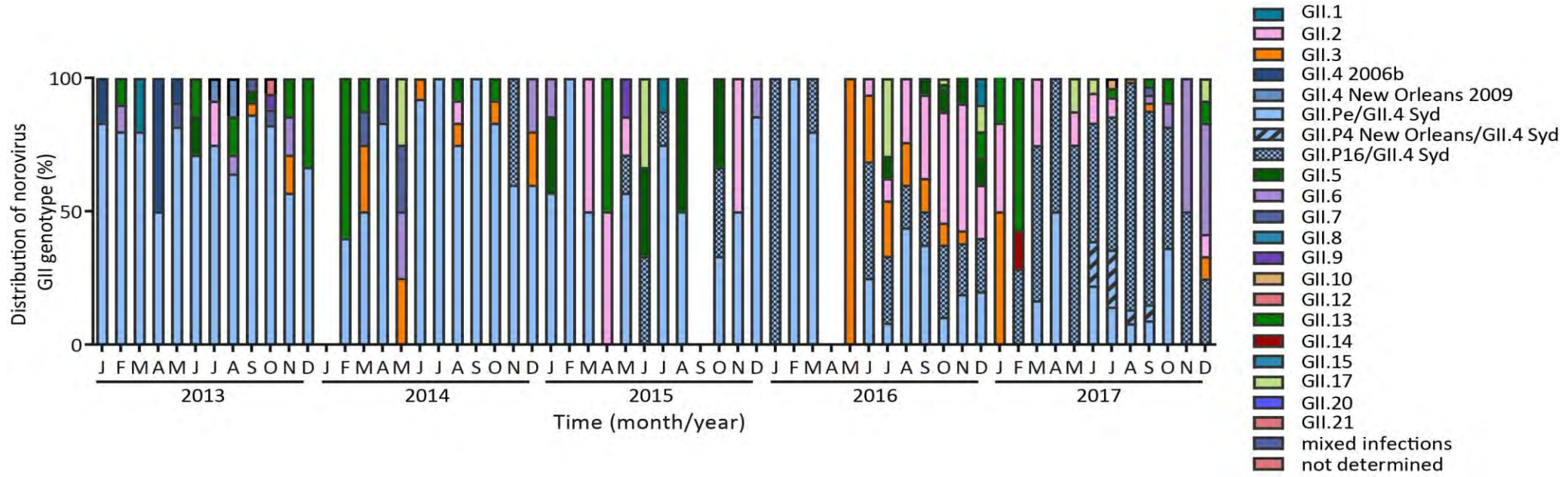


Monthly distribution of norovirus genotypes identified in Australia and New Zealand, 2017.

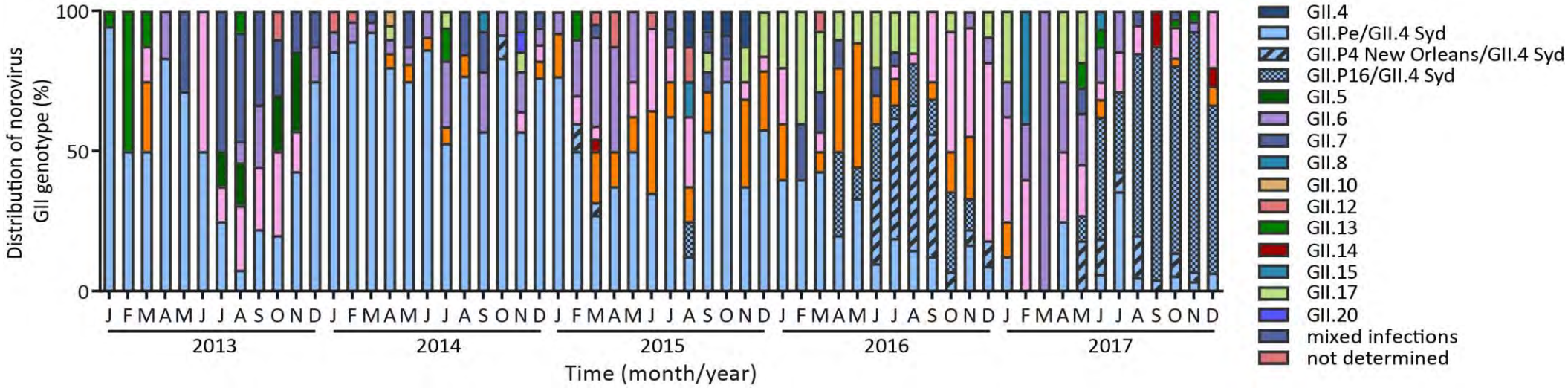


Norovirus GII distribution in clinical samples from Australian and New Zealand, 2013-2017

NSW



New Zealand



Problems with current screening methods

- We only use clinical samples
- Symptomatic cases only
- Norovirus-related gastroenteritis is generally ***self-limiting***
- Over-representation of childhood infection
- A system to screen for norovirus at a population level

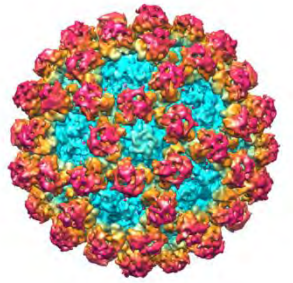
Where can we find norovirus at population level?

Wastewater treatment plants

- Collection of waste from within a population
- Prolong and high viral load shedding
- Abundance of enteric viruses

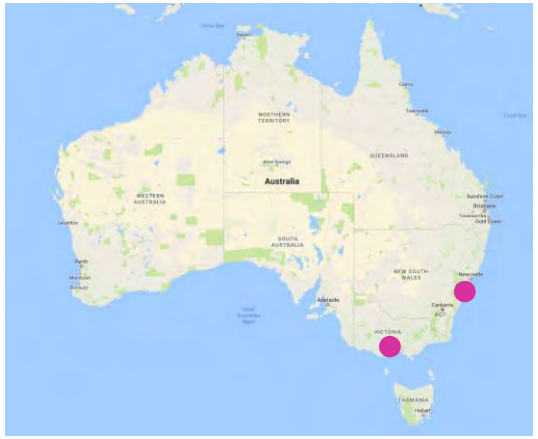
Study aims

- To detect and quantify norovirus RNA levels in wastewater samples
- Examine the norovirus genetic diversity in wastewater
- Compare norovirus diversity in clinical and wastewater samples



Wastewater sample collection

- Monthly collections throughout 2016 and 2017, from 3 sites;
- Sydney – Bondi and Malabar
- Melbourne – Western Treatment Plant

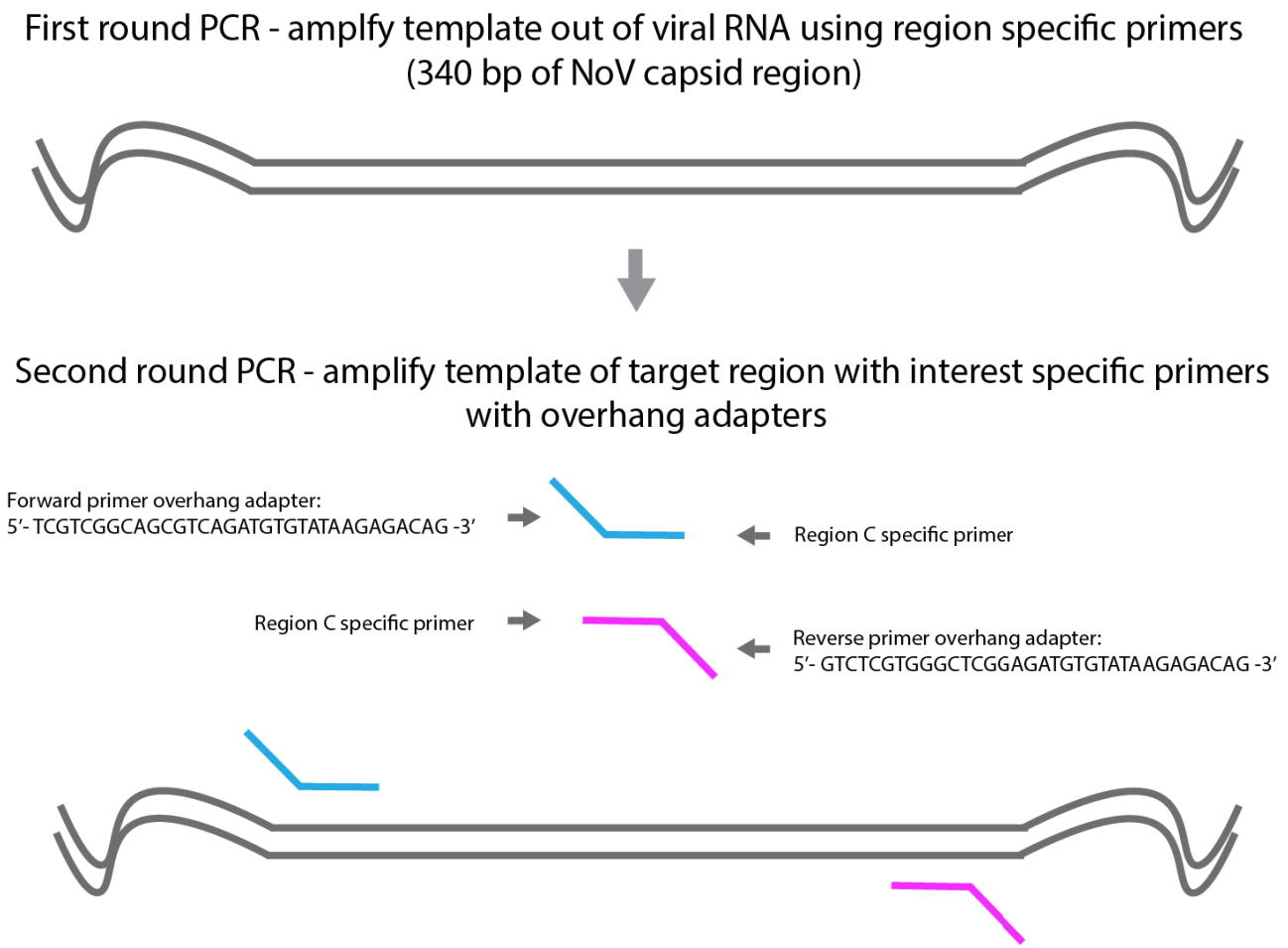


Bondi
(population 296,350)

Malabar
(population 1,667,460)

Melbourne WTP
(population 2,400,000)

Sample preparation for NGS



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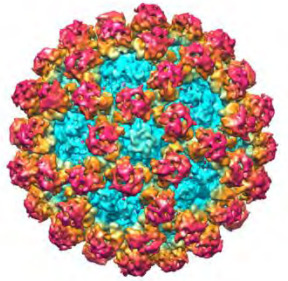
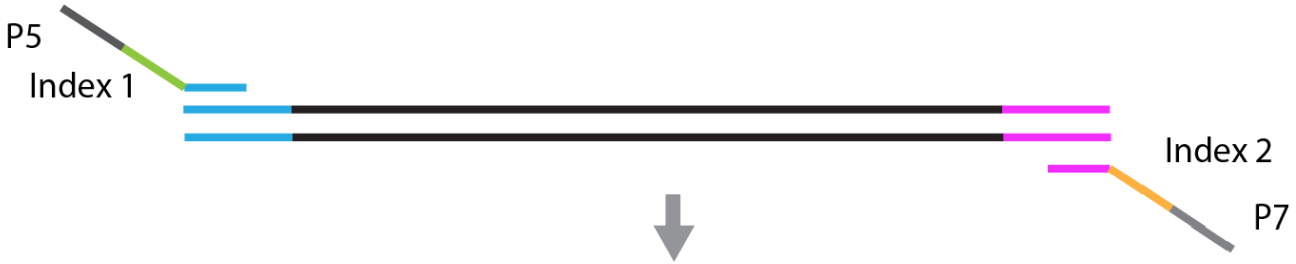


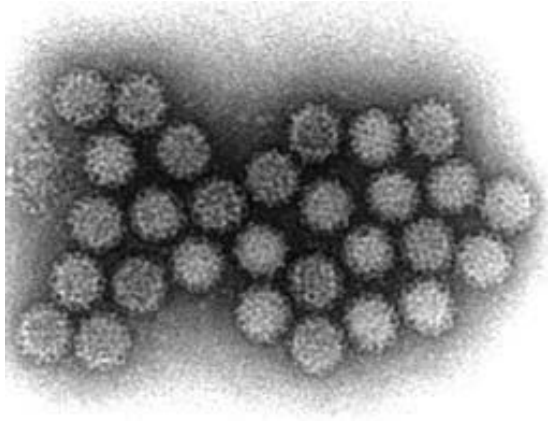
Attach indices and Illumina sequencing adapters using Nextera® XT Index Kit



Normalise and pool libraries

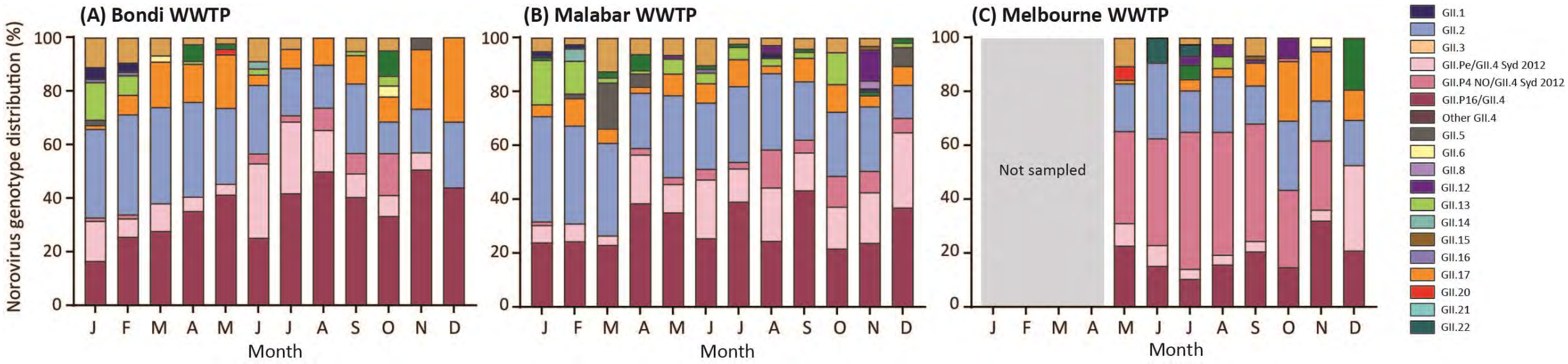
Sequence

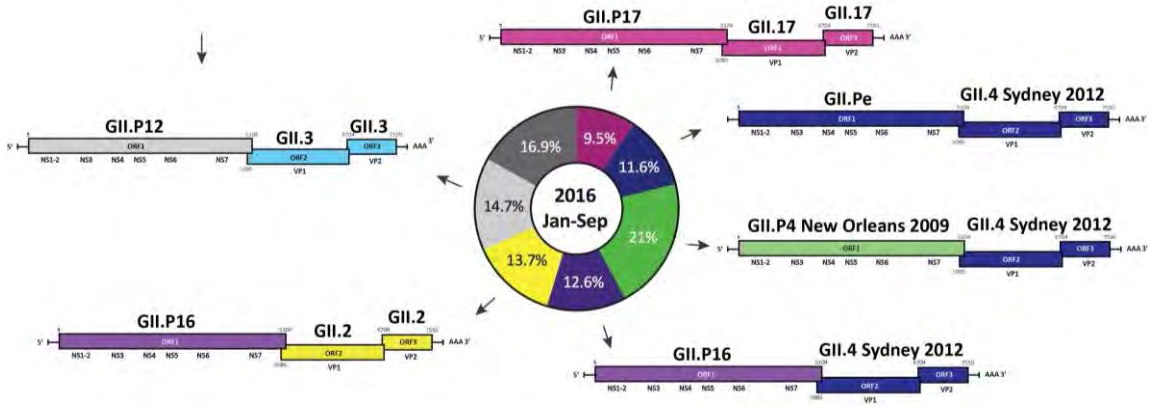
NGS data analysis



- Average of 580,000 reads per sample
- Geneious program for data analysis
- Paired end reads merged, primers trimmed
- Reads mapped to NoV reference sequences

Norovirus genotype distribution in wastewater samples 2017





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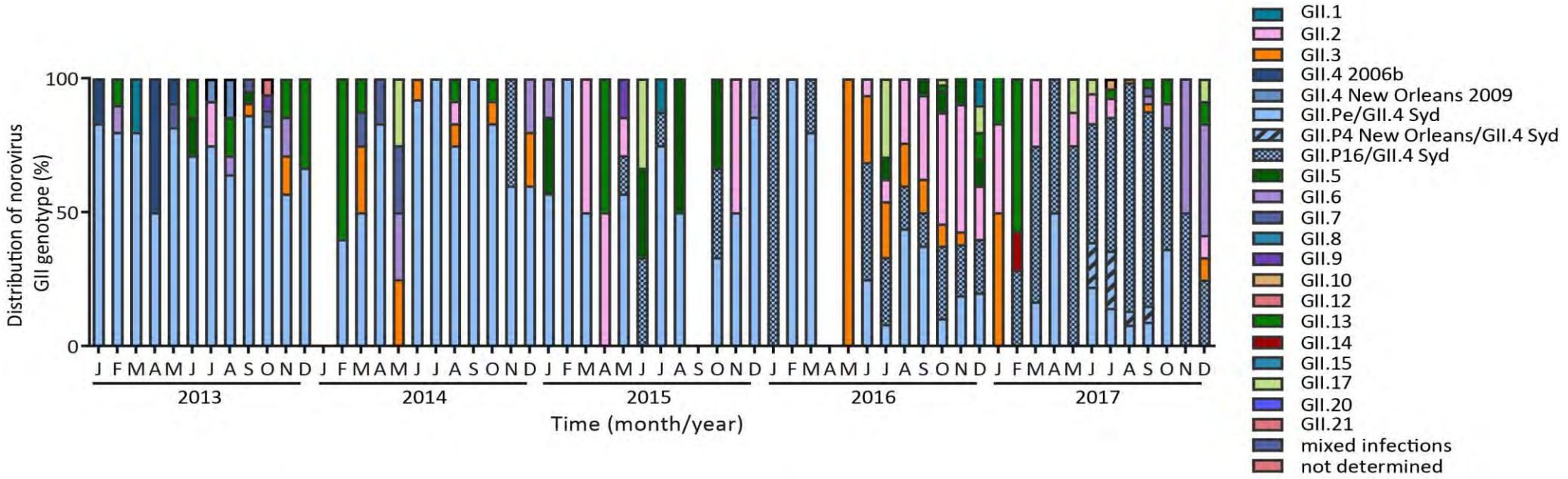
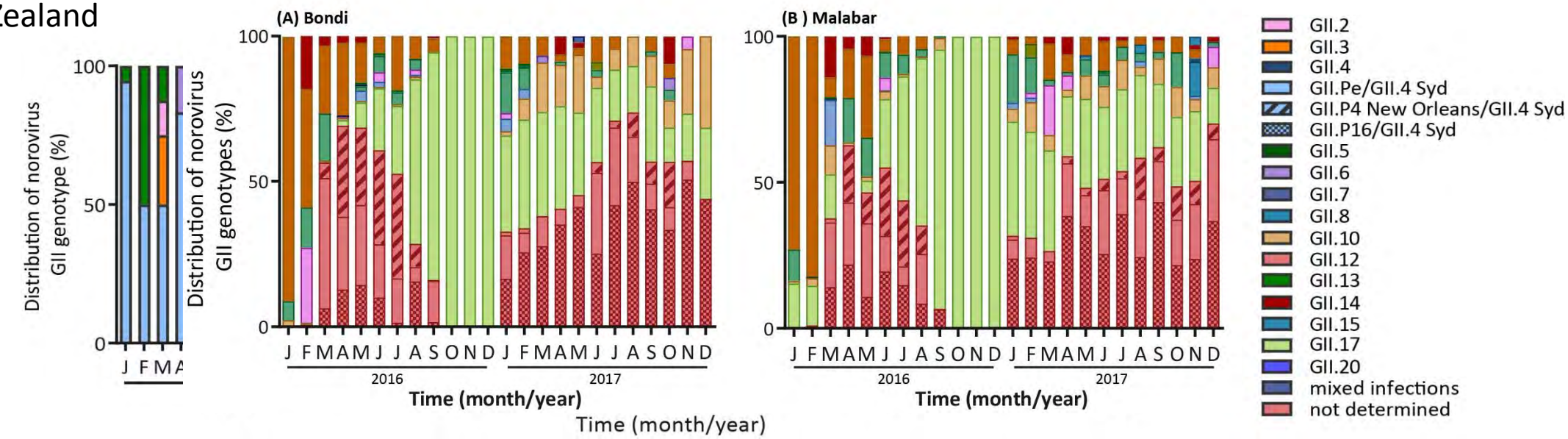


New Zealand



Benefits

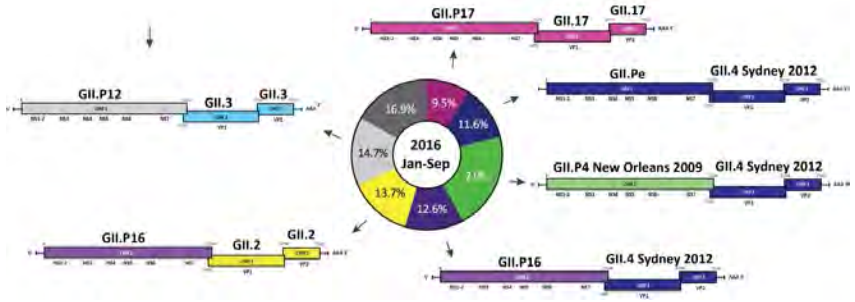
- No ethics
- Less sample to be processed
  = **cheaper**
- Represents a whole population
- Non-biased
- Greater diversity
- Possible predictive tool

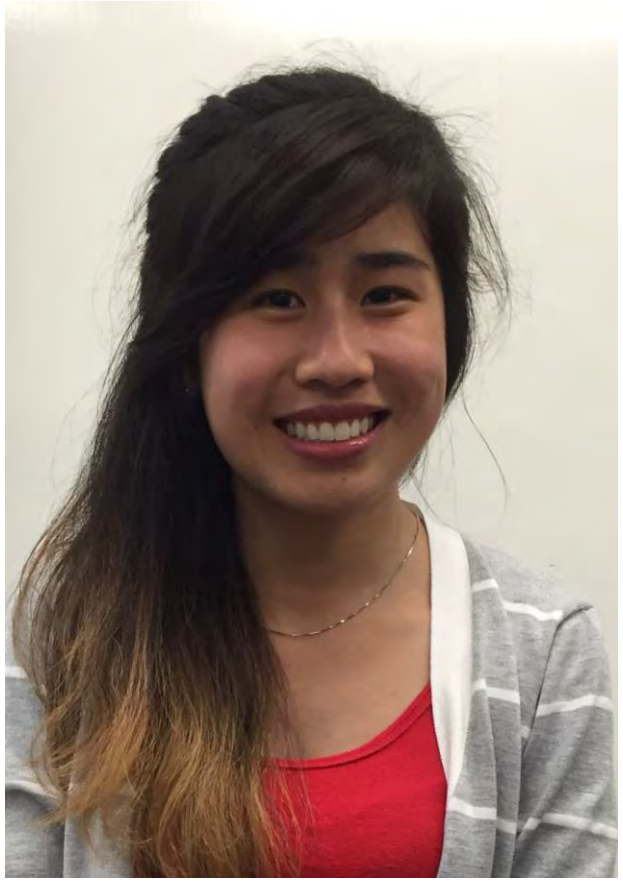
Improvements?

- Recombinant noroviruses
- GI noroviruses
- Other enteric viruses

Summary

- The molecular surveillance of norovirus is important to predict and identify new noroviruses and potential pandemic variants
- Recombinant GII.P16/GII.4 Sydney 2012 is responsible for majority of acute viral gastroenteritis in 2017 – pandemic?
- Clinical samples are biased towards symptomatic cases and over-represent childhood infections
- Wastewater samples provide a better understanding of noroviruses within a population
- Genotype dominance correlates, although not exactly.





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Emerging recombinant noroviruses identified by clinical and waste water screening

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