An update on the laboratory detection and epidemiology of astrovirus, adenovirus, sapovirus, and enterovirus in gastrointestinal disease

Christopher McIver, Principal Hospital Scientist, Microbiology Department (New South Wales Health Pathology), St George Hospital

Outline

Literature review since 2000

Emergence of novel strains

Astrovirus

Adenovirus

Enterovirus

Sapovirus

Outline

Literature review since 2000

Emergence of novel strains

Astrovirus

Adenovirus

Enterovirus

Sapovirus

Established molecular methods need to be updated to ensure coverage

Pathology (2001) 33, pp. 353–358

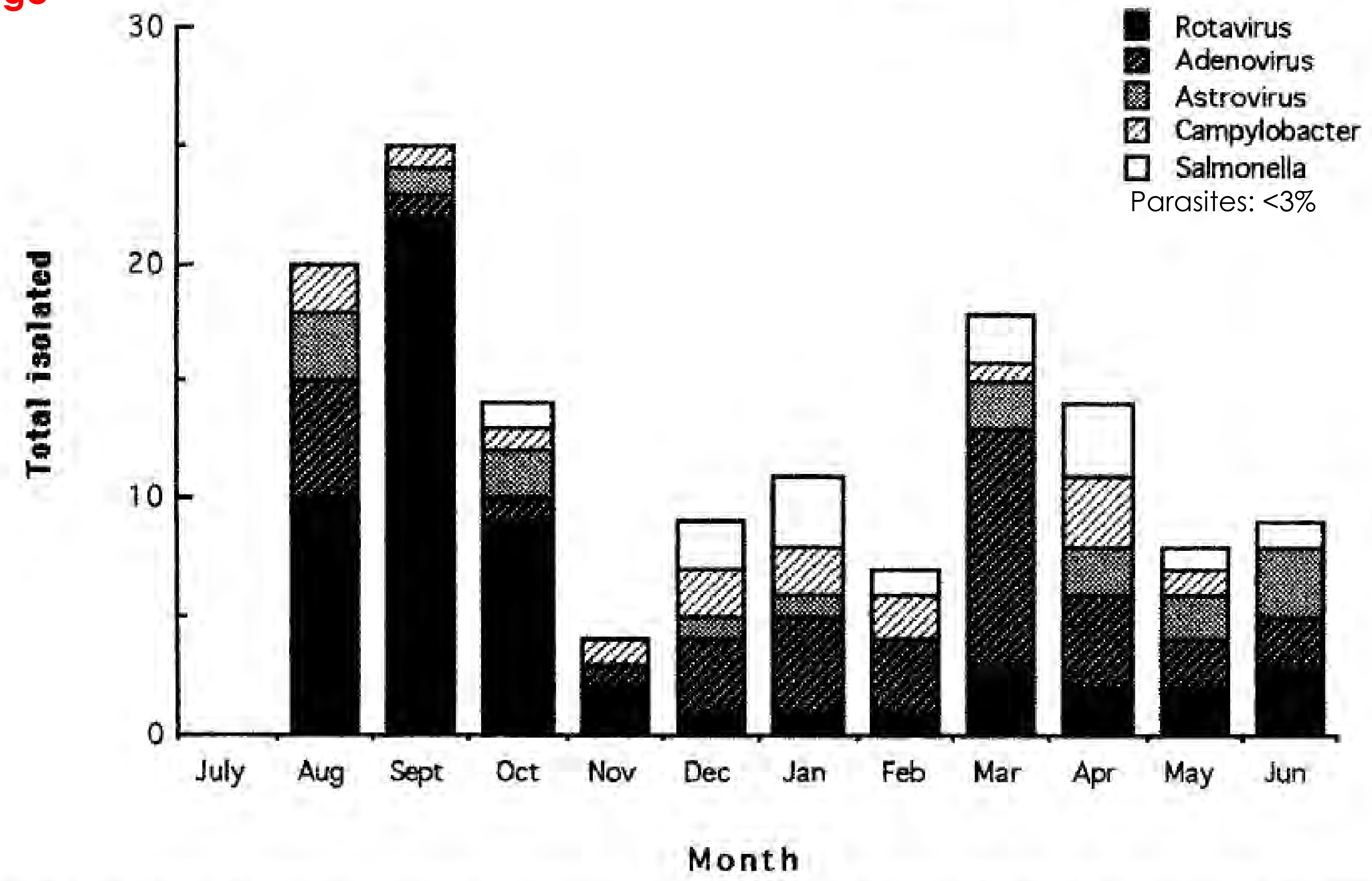
DIAGNOSIS OF ENTERIC PATHOGENS IN CHILDREN WITH GASTROENTERITIS

Christopher J. McIver*, Grant Hansman*, Peter White*, Jennifer C. Doultree†, Michael Catton‡ and William D. Rawlinson*

*Virology Division, Department of Microbiology (SEALS), The Prince of Wales Hospital, Randwick, and School of Pathology, University of NSW; †Immunobiology Department, TGA, Narrabundah, ACT; ‡VIDRL North Western Health, North Melbourne, Victoria, Australia

McIver *et al.* 2001. Pathology, 33: 353

N = 412
< 6 years of age



Parasites: <3%

McIver *et al.* 2001. Pathology, 33: 353

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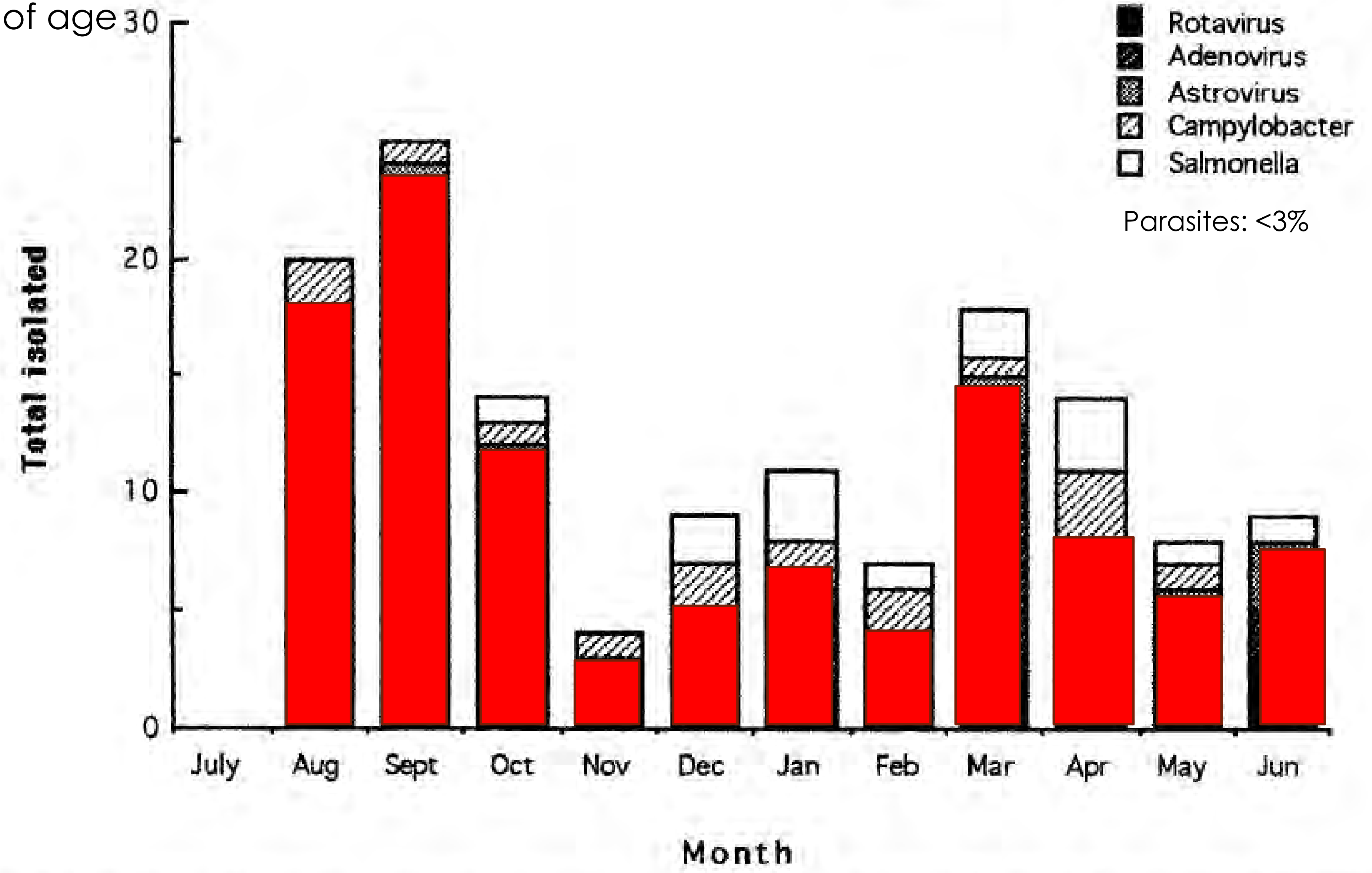

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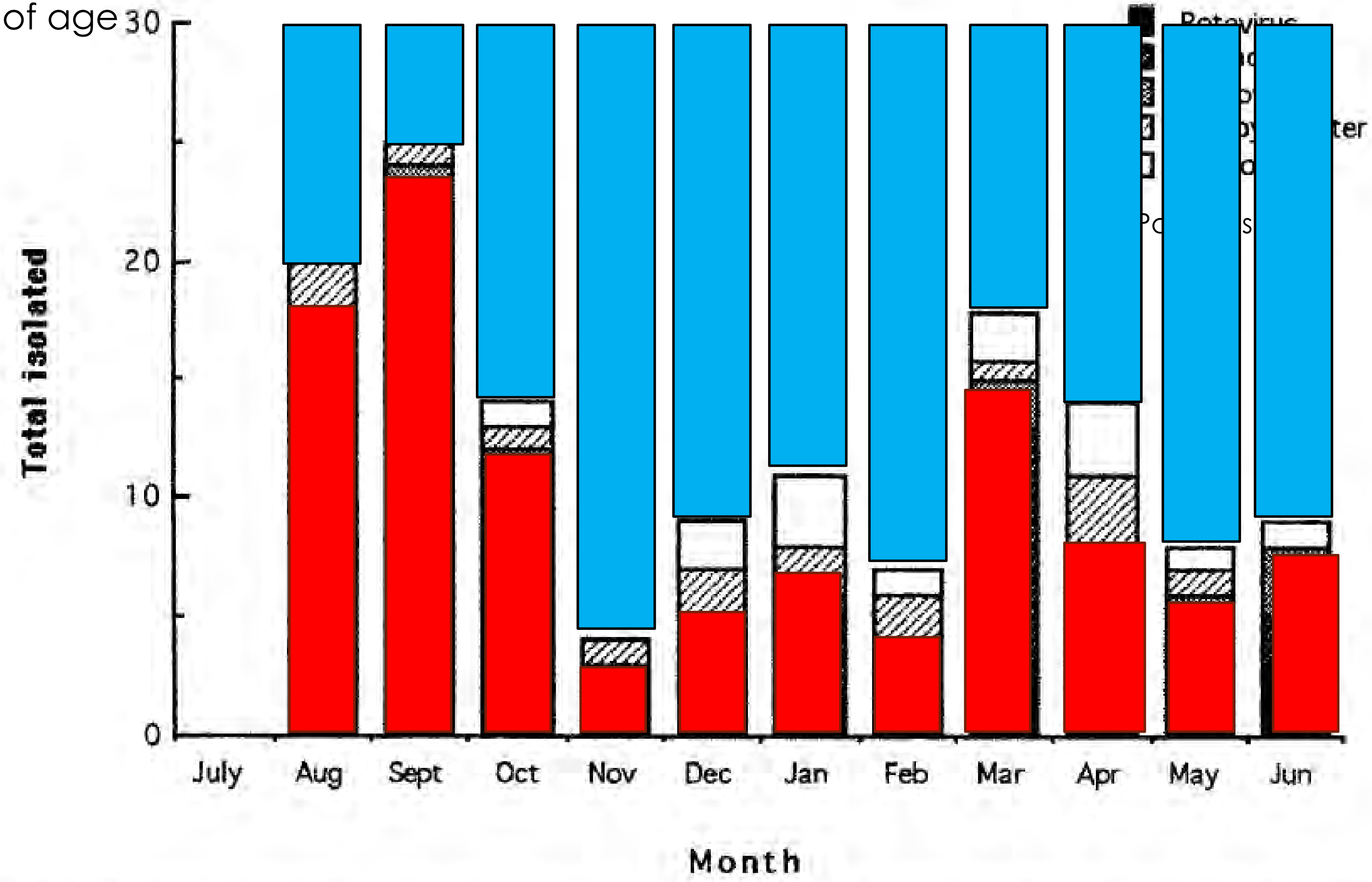

Table 2 Results of electron microscopy of samples from outpatient and hospitalised children between July 1997 and March 1998

Viruses detected	Age group						Origin		Total detected
	< 1	1	2	3	4	5	Isolation	OPD*	
Adenovirus, Parvovirus (SRV)	0	1	0	0	0	0	0	1	1
Adenovirus, Rotavirus	1	0	0	0	0	0	0	1	1
Adenovirus, Small viral-like particles	1	0	0	0	0	0	1	0	1
Adenovirus alone	6	6	1	0	0	0	1	12	13
Adenovirus, Small viral-like particles	1	0	0	0	0	0	0	1	1
Astrovirus alone	2	3	1	0	0	0	0	6	6
Calicivirus (SRV)	1	0	0	0	0	0	0	1	1
Norwalk-like viral particles (SRV)	0	1	0	0	0	0	0	1	1
Parvovirus (SRV)	0	0	0	0	0	1	0	1	1
Rotavirus, Norwalk-like viral particles (SRV)	0	1	0	0	0	0	0	1	1
Rotavirus alone	10	19	2	1	1	1	20	14	34
Rotavirus, unidentified SRV	0	0	1	0	0	0	0	1	1
Unidentified SRV	6	4	2	0	1	0	5	8	13
Small viral-like particles	2	2	3	0	0	0	2	5	7
Number of samples from which viruses were detected	30	37	10	1	2	2	29	53	82
Number of samples negative	81	70	33	17	4	4	91	118	209
Total samples examined	111	107	43	18	6	6	120	171	291

*OPD, outpatients department.

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28.2%

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RT-PCR Norwalk-like virus
10/172 (5.8%)

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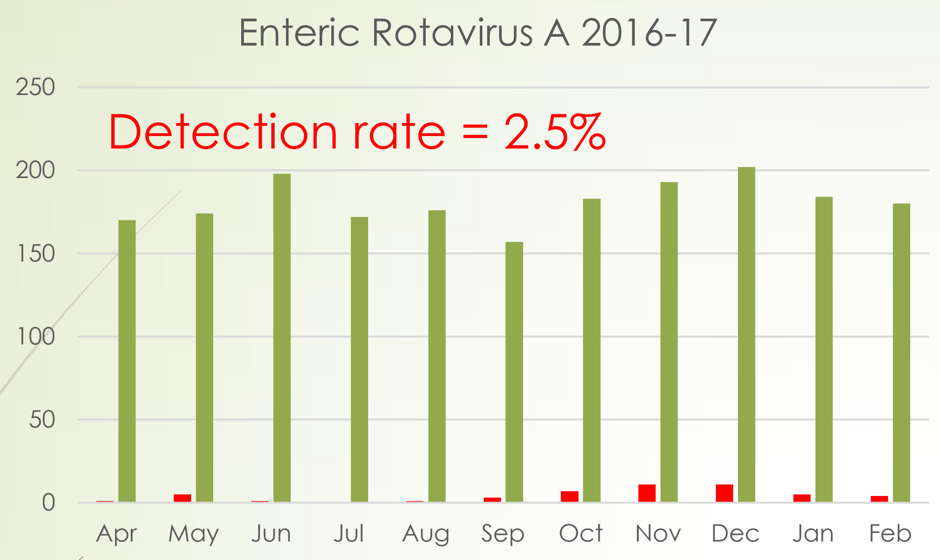
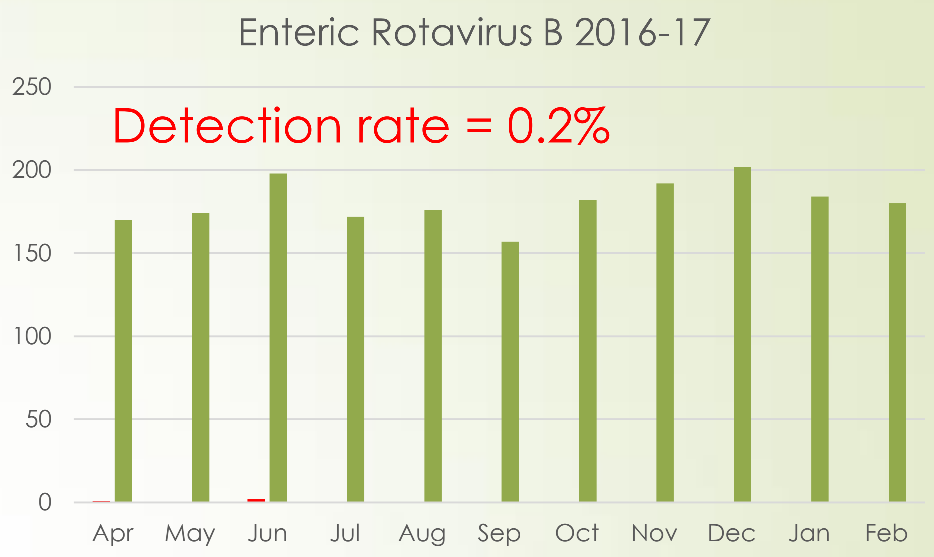
DNA by PCR:

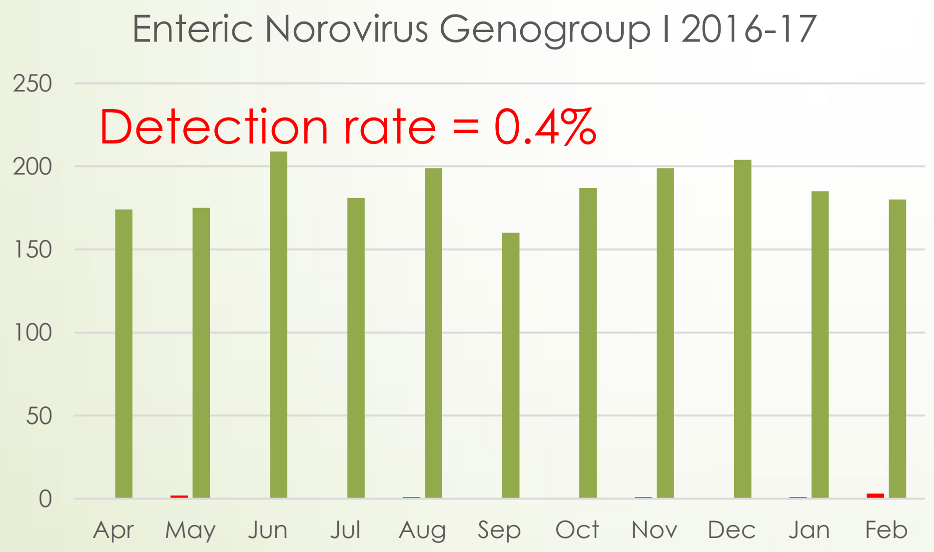
Bacteria :

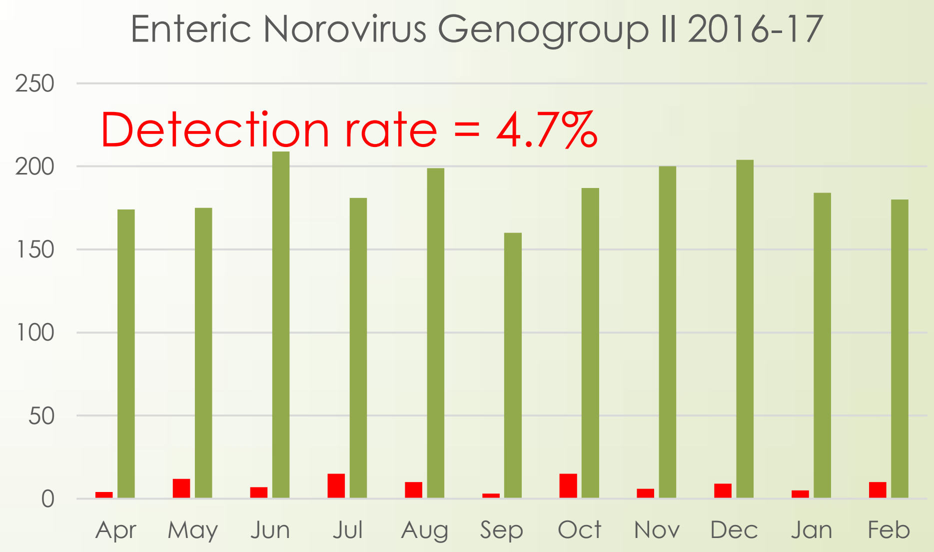
Campylobacter	Not Detected
Salmonella	Not Detected
Shigella/EIEC	Not Detected
Toxigenic C. difficile	Not Detected
Yersinia enterocolitica	Not Detected
Protozoa :	
Dientamoeba fragilis	Not Detected
Cryptosporidium	**DETECTED**
Blastocystis species	Not Detected
Entamoeba histolytica	Not Detected
Giardia intestinalis	Not Detected
Virus:	
Norovirus G1	Not Detected
Norovirus G11	Not Detected
Rotavirus A	Not Detected
Rotavirus B	Not Detected
Astrovirus	Not Detected
Adenovirus 40/41	Not Detected
Enterovirus	Not Detected

Faeces PCR Comment :

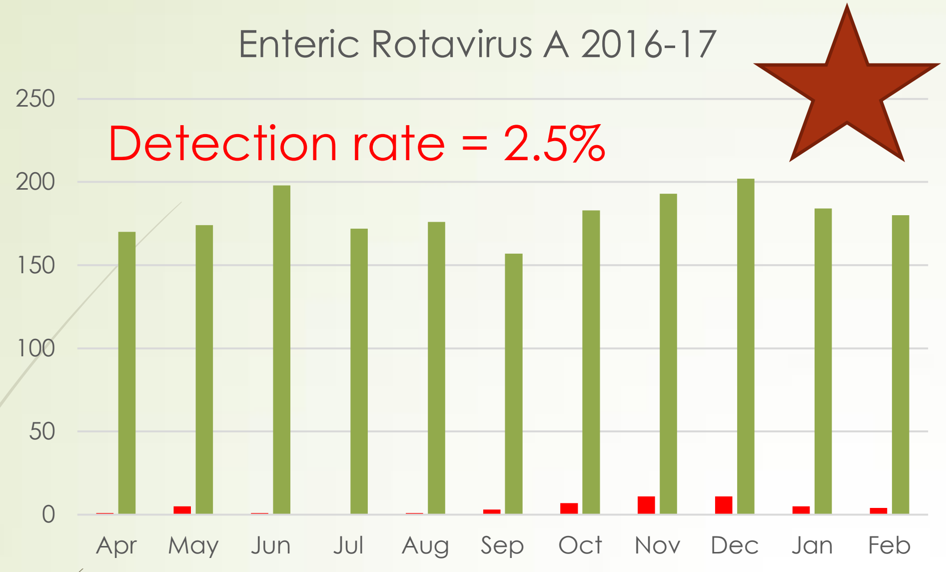


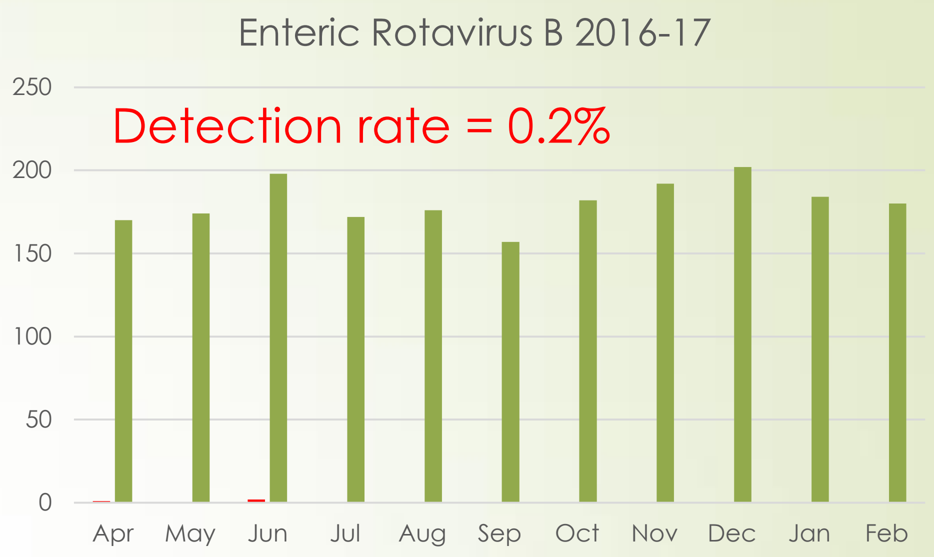


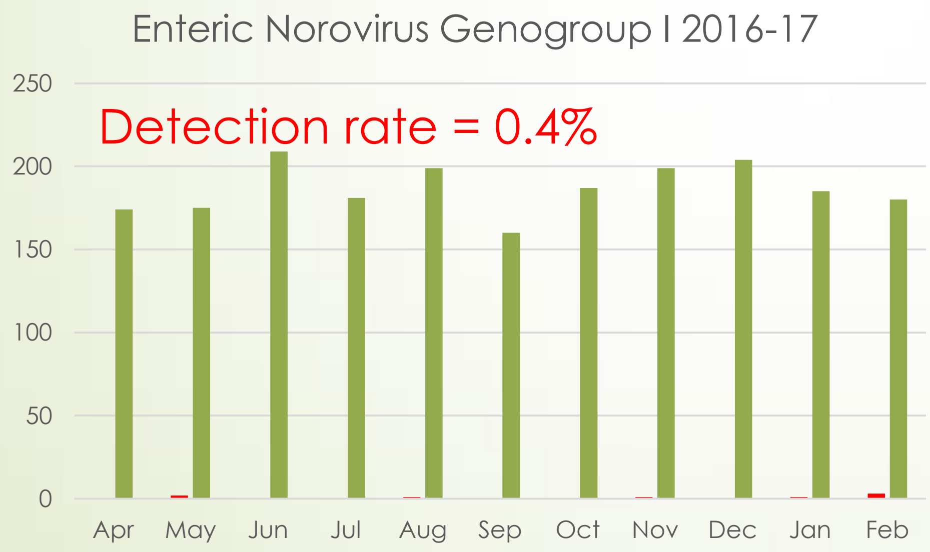


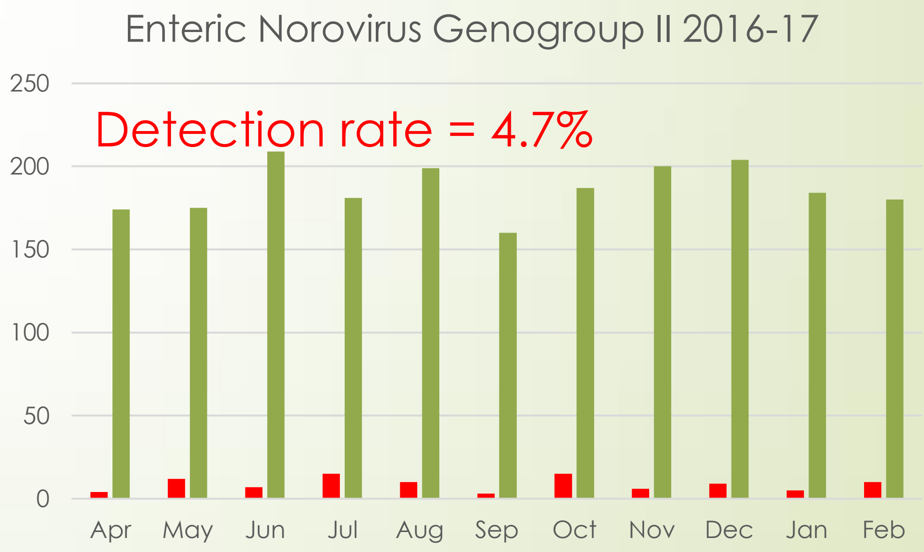


Source: Microbiology Department (SEALS), Prince of Wales Hospital, Randwick









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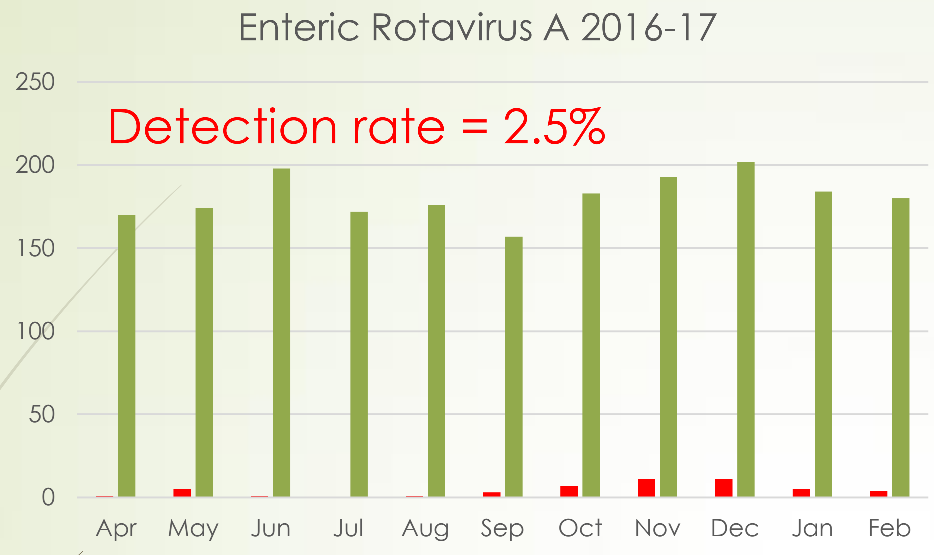

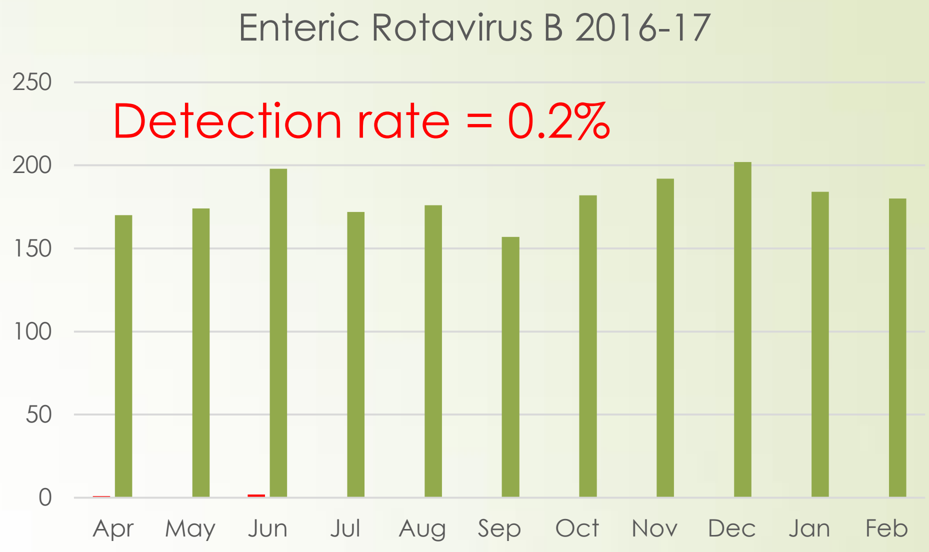

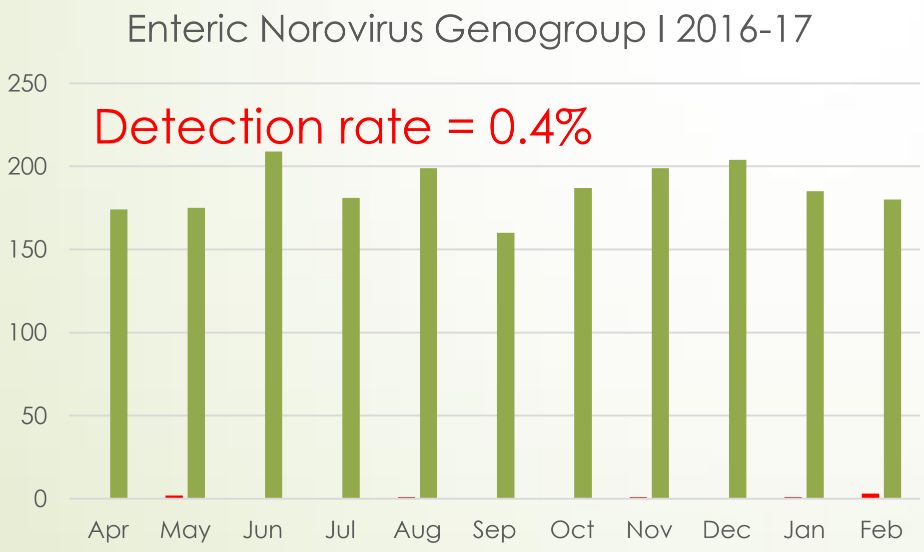

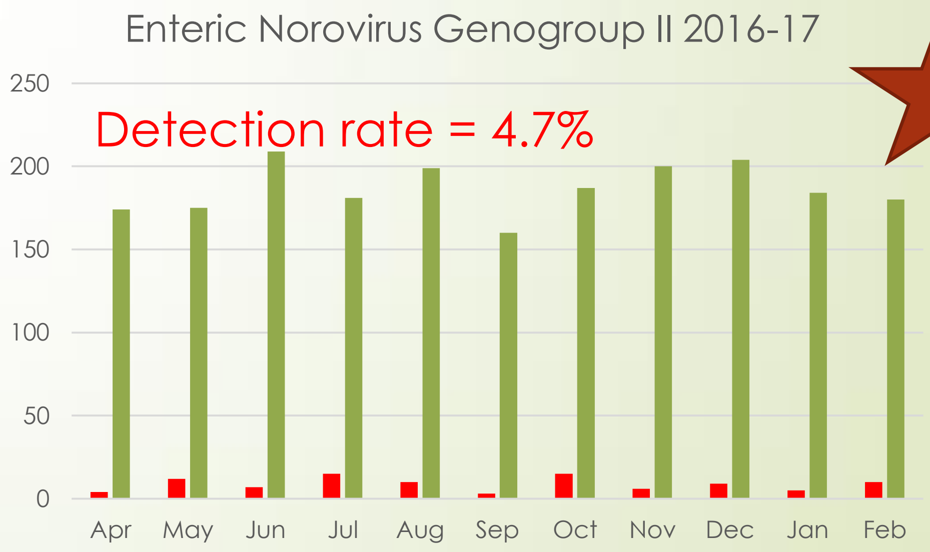

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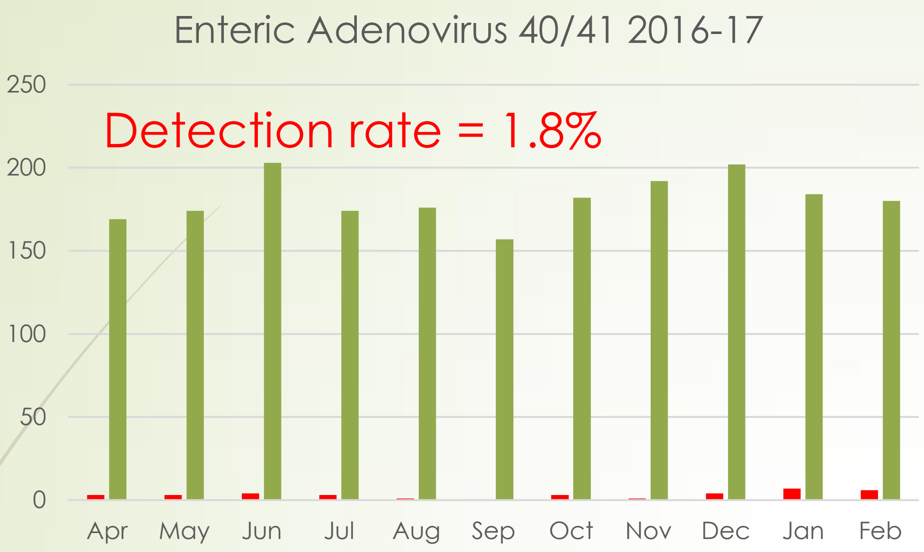

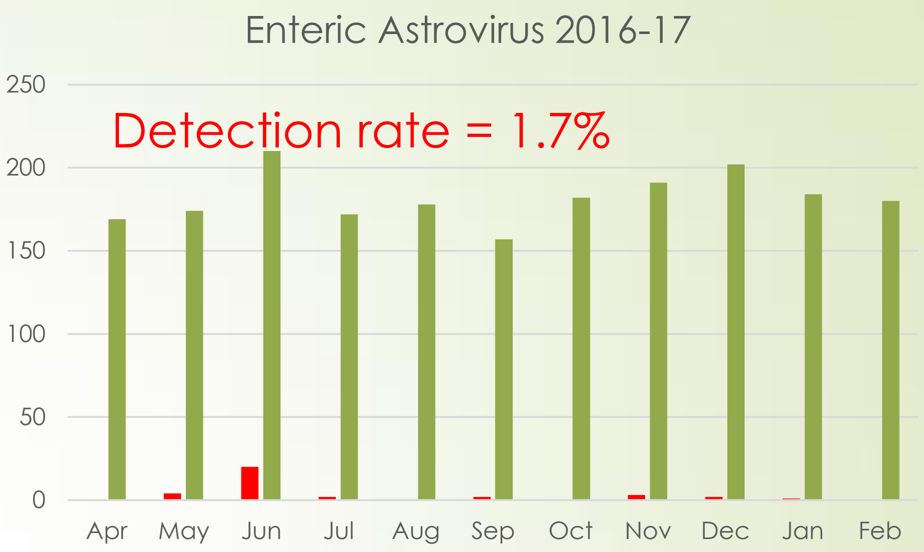

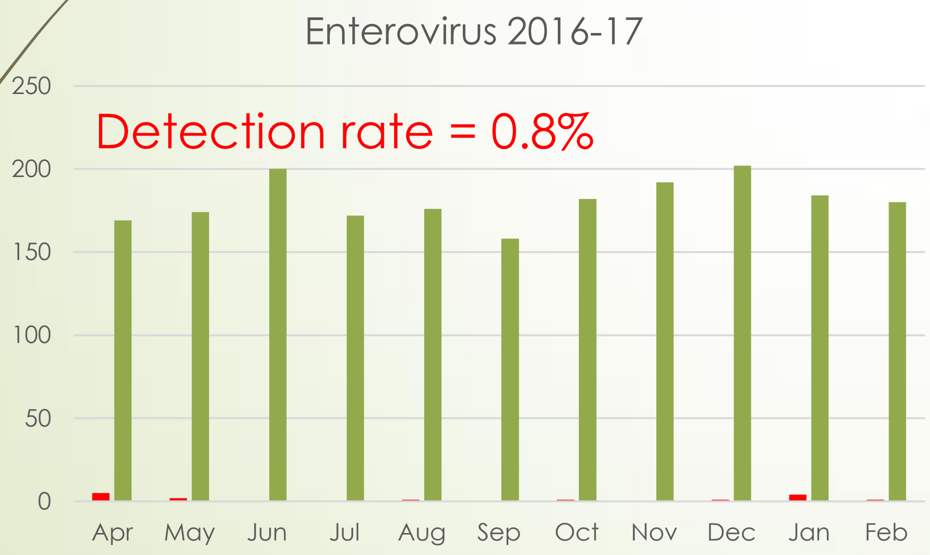

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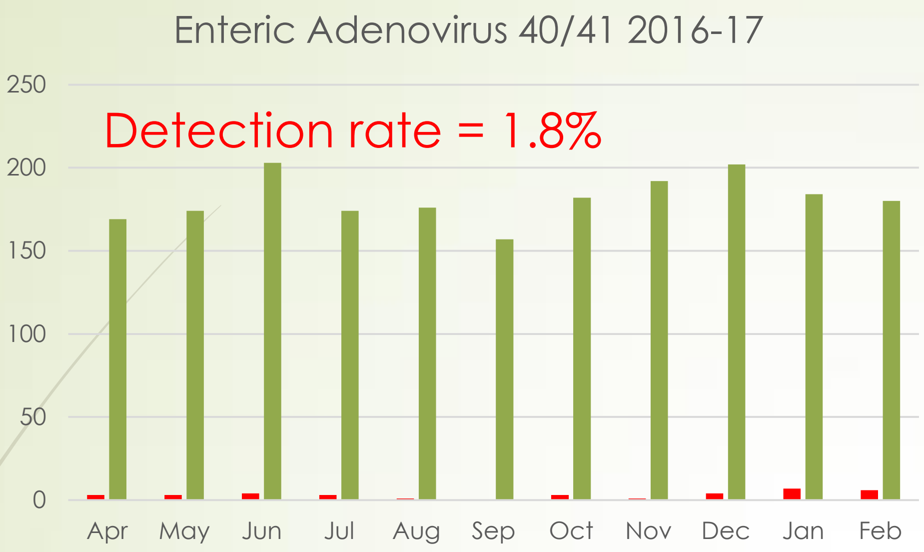
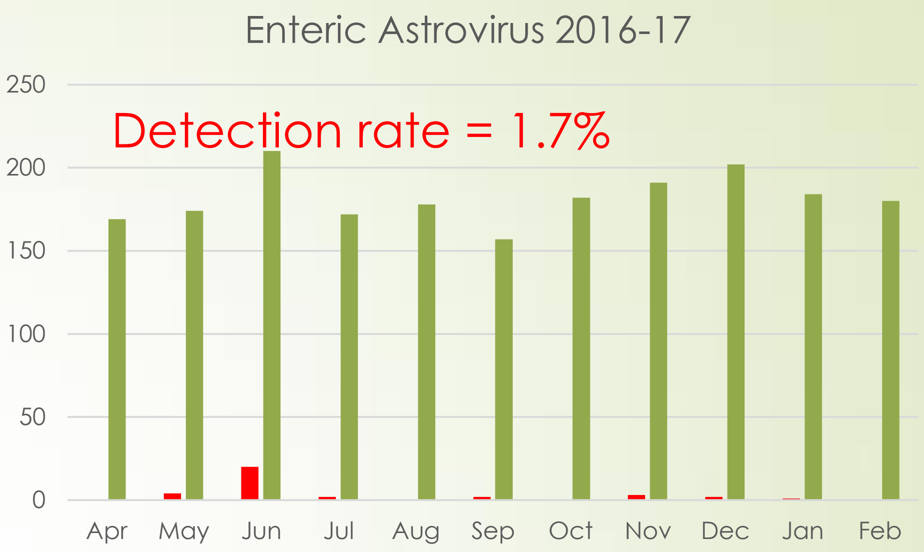
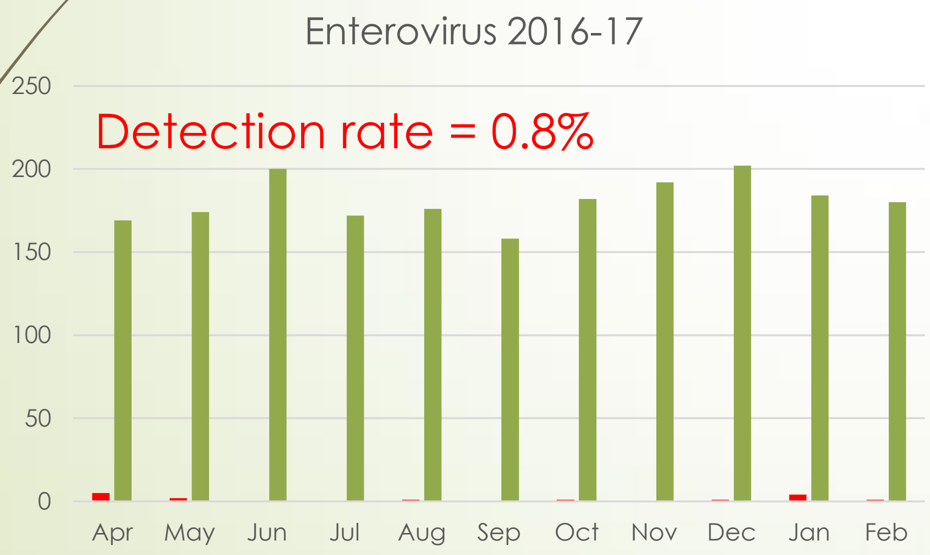




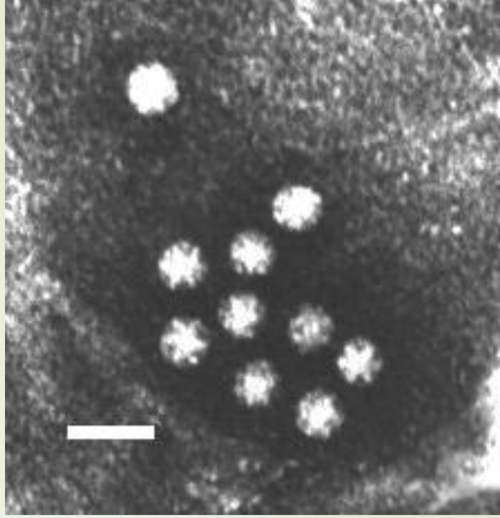


Detection rate = unsure

Calicivirus: Sapovirus

Source: Microbiology Department (SEALS), Prince of Wales Hospital, Randwick

Common characteristics of select enteric viruses



Mild diarrhea
(short duration and without dehydration)

Persist in the convalescence

Epidemics uncommon

Non-encapsulated structures
- Environmentally resilient
- Resistant to disinfectants

Adenovirus DNA

Astrovirus, Enterovirus and Sapovirus: ssRNA

Common characteristics of select enteric viruses

Journal of Clinical Microbiology, June 2008, p. 2119–2121 Vol. 46, No. 6
0095-1137/08/$08.00+0 doi:10.1128/JCM.02198-07

Norovirus Excretion in an Aged-Care Setting▽

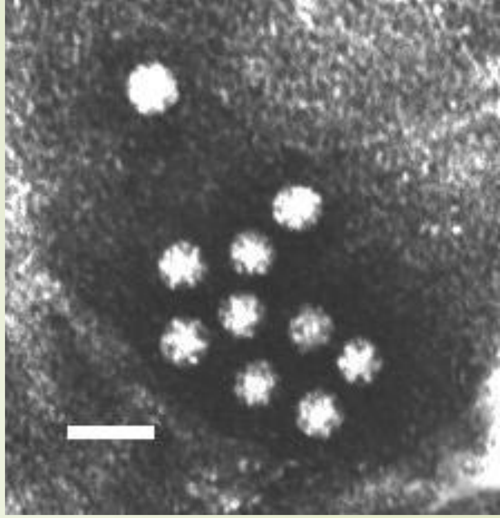
Elise T.-V. Tu,[1,3] Rowena A. Bull,[1,3] Mi-Jurng Kim,[1] Christopher J. McIver,[1,2,3] Leon Heron,[4] William D. Rawlinson,[1,2,3] and Peter A. White[1]*

School of Biotechnology and Biomolecular Sciences, Faculty of Science,[1] and School of Medical Sciences, Faculty of Medicine,[2] The University of New South Wales, UNSW, Sydney 2052, Australia; Virology Division, SESIAHS, Department of Microbiology, Prince of Wales Hospital, Sydney 2031, Australia[3]; and National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases, Research Building, The Children's Hospital at Westmead, Westmead 2145, Australia[4]

Received 13 November 2007/Returned for modification 11 January 2008/Accepted 9 April 2008

Norovirus genogroup II excretion during an outbreak of gastroenteritis was investigated in an aged-care facility. Viral shedding peaked in the acute stage of illness and continued for an average of 28.7 days. The viral decay rate was 0.76 per day, which corresponds to a viral half-life of 2.5 days.

Common characteristics of select enteric viruses



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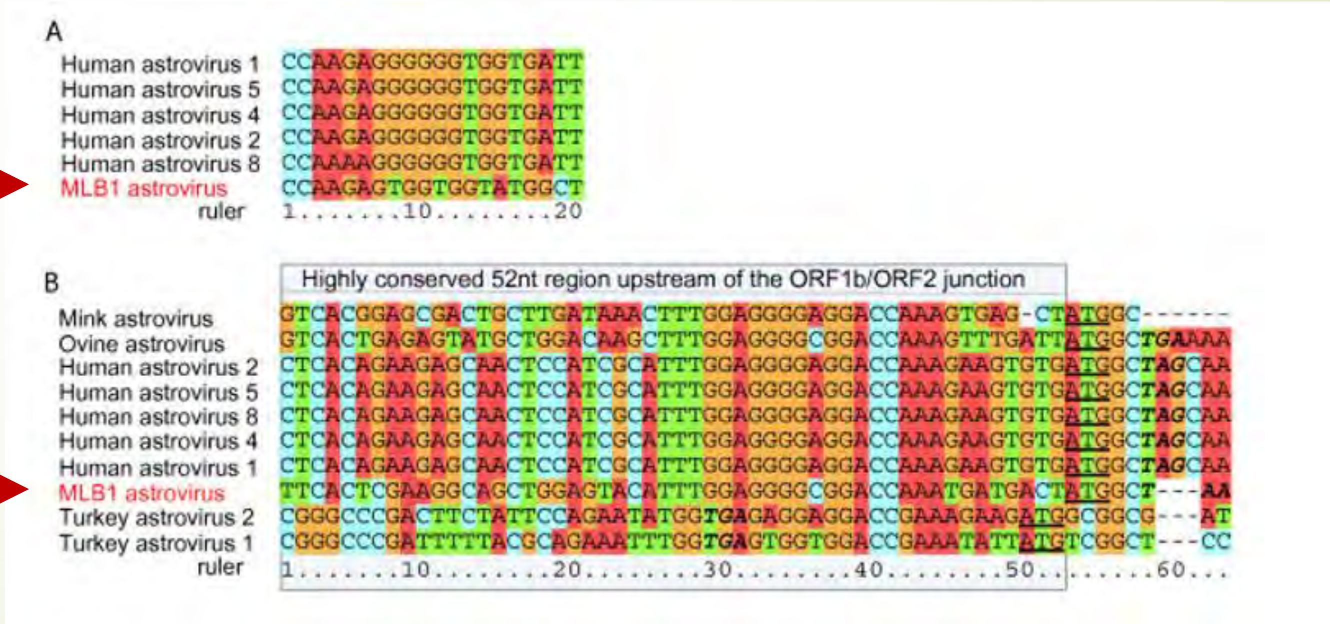
Enteric viruses have a high predisposition to mutations and recombination

New strains are always emerging



Astrovirus

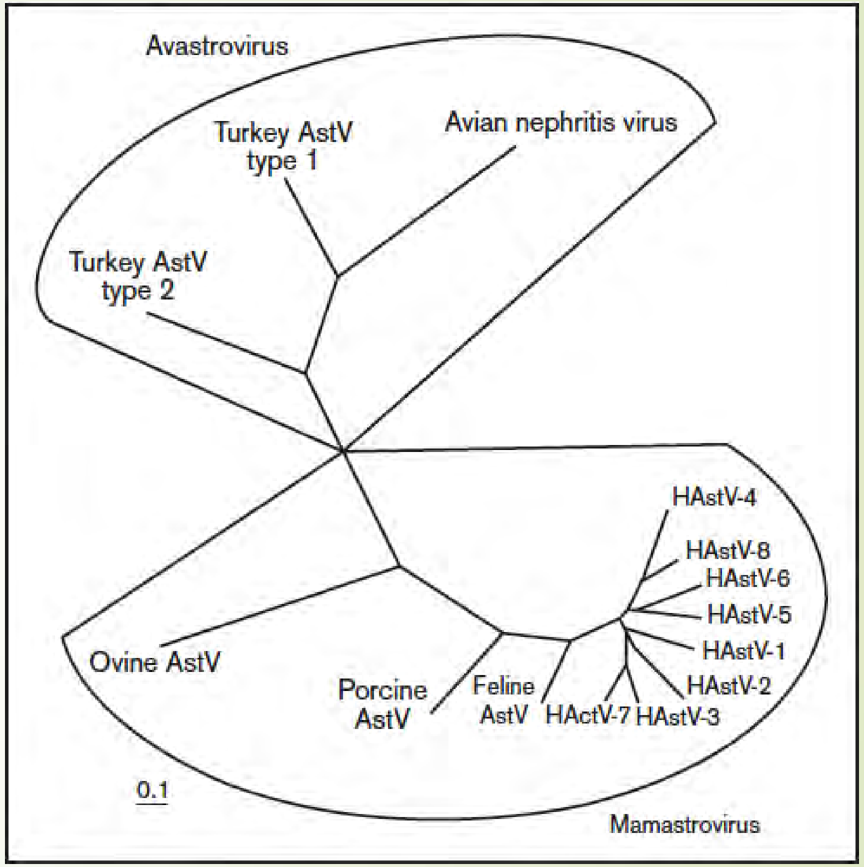
Appleton and Higgins (1975) cause of diarrhea outbreak in a maternity unit, UK

Clinical and serological studies (Kurtz et al. 1979)

8 serotypes associated with human infections

Subsequently been isolated in the faeces of animals

Testing (EIA and RT-PCR) have established association with diarrhea and vomiting in infants and elderly



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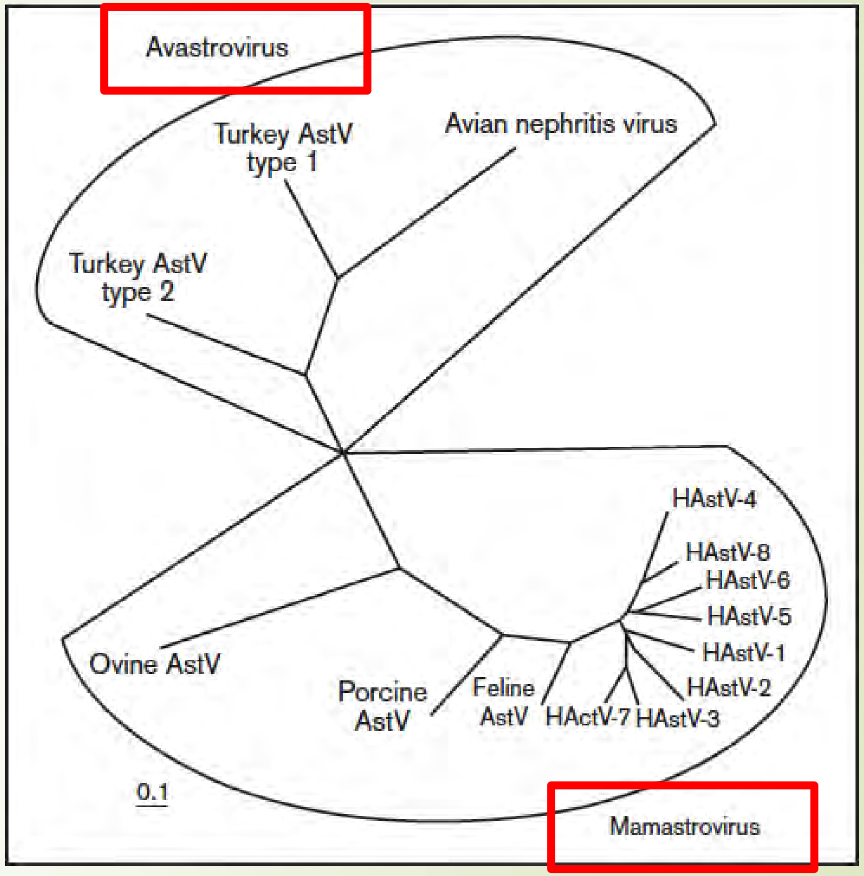

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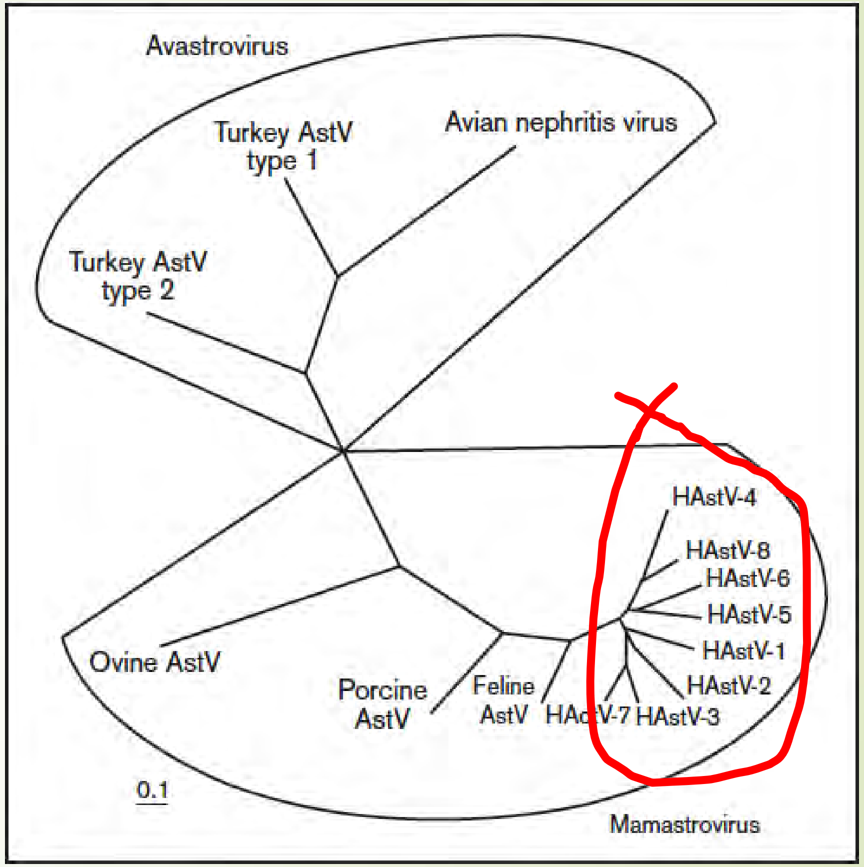

Children presenting in outpatients (*n* = 238) and hospital (*n* = 176) **Detection rate: 4.6%**

Results of confirmatory tests and serotyping for all stool samples tested positive for astrovirus by EIA[a]

Sample code	Season	Origin	Age	EM	NB	Culture /RT-PCR	Serotype	Percentage identities with prototype astroviruses[b]						
								1	2	3	4	5	6	7
SCH 210	Winter 1997	ED	2	Astro	+	+	1	**91.7**	78.4	78.2	78.7	79.8	84.8	79.6
SCH 217	Winter 1997	ED	<1	Astro	+	+	1	**91.7**	78.4	78.2	78.7	79.8	84.8	79.6
SCH 355	Winter 1997	DC	1	Astro	+	+	1	**91.7**	78.4	78.2	78.7	79.8	84.8	79.6
SCH 359	Winter 1997	ED	1	Astro	+	+	1	**91.7**	78.4	78.2	78.7	79.8	84.8	79.6
SCH 475	Spring 1997	ED	<1	Astro	+	+	1	**91.4**	78.3	77.9	78.4	79.8	84.5	79.3
SCH 562	Spring 1997	DC	1	Astro	+	+	1	**91.7**	78.4	78.2	78.7	79.8	84.8	79.6
SCH 605	Spring 1997	DC	2	SRV	+	+	1	**91.7**	78.4	78.2	78.7	79.8	84.8	79.6
SCH 609	Spring 1997	DC	2	Astro	+	+	1	**91.7**	78.4	78.2	78.7	79.8	84.8	79.6
SCH 838	Summer 1997	ISO	1	Neg	+	+	1	**91.4**	77.9	78.2	78.7	79.5	84.2	79.9
SCH 1097	Summer 1997	ED	1	Neg	−	+	4	77.3	79.9	80.5	**92.3**	78.9	81.0	79.8
SCH 1333	Autumn 1998	ED	2	SRV	+	+	4	77.3	79.9	80.5	**92.3**	78.9	81.0	79.8
SCH 1366	Autumn 1998	ED	2	SRV	+	+	4	77.3	79.9	80.5	**92.3**	78.9	81.0	79.8
SCH 1433	Autumn 1998	ED	1	Astro	+	+	4	77.3	79.9	80.5	**92.3**	78.9	81.0	79.8
SCH 1468	Autumn 1998	ED	<1	NE	+	+	1	**89.4**	80.7	78.4	76.9	79.8	83.3	81.0
SCH 1604	Autumn 1998	ISO	1	NE	+	+	4	77.6	80.7	77.9	**95.4**	77.5	81.3	81.3
SCH 1660	Autumn 1998	ED	1	NE	+	+	4	77.3	79.9	80.5	**92.3**	78.9	81.0	79.8
SCH 1759	Winter 1998	ISO	4	NE	+	+	4	78.2	81.0	78.7	**96.3**	77.5	81.3	80.8
SCH 1749	Winter 1998	ED	3	NE	+	+	3	79.3	79.0	**96.5**	80.0	80.0	81.0	78.7
SCH 1766	Winter 1998	ISO	2	NE	+	+	1	**90.8**	77.9	77.9	78.4	79.3	84.5	80.2

McIver *et al.* 2000. *J Virol. Meth.* 84: 99-105

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SCH 210	Winter 1997	ED	2	Astro	+	+	1	**91.7**	78.4	78.2	78.7	79.8	84.8	79.6
SCH 217	Winter 1997	ED	<1	Astro	+	+	1	**91.7**	78.4	78.2	78.7	79.8	84.8	79.6
SCH 355	Winter 1997	DC	1	Astro	+	+	1	**91.7**	78.4	78.2	78.7	79.8	84.8	79.6
SCH 359	Winter 1997	ED	1	Astro	+	+	1	**91.7**	78.4	78.2	78.7	79.8	84.8	79.6
SCH 475	Spring 1997	ED	<1	Astro	+	+	1	**91.4**	78.3	77.9	78.4	79.8	84.5	79.3
SCH 562	Spring 1997	DC	1	Astro	+	+	1	**91.7**	78.4	78.2	78.7	79.8	84.8	79.6
SCH 605	Spring 1997	DC	2	SRV	+	+	1	**91.7**	78.4	78.2	78.7	79.8	84.8	79.6
SCH 609	Spring 1997	DC	2	Astro	+	+	1	**91.7**	78.4	78.2	78.7	79.8	84.8	79.6
SCH 838	Summer 1997	ISO	1	Neg	+	+	1	**91.4**	77.9	78.2	78.7	79.5	84.2	79.9
SCH 1097	Summer 1997	ED	1	Neg	−	+	4	77.3	79.9	80.5	**92.3**	78.9	81.0	79.8
SCH 1333	Autumn 1998	ED	2	SRV	+	+	4	77.3	79.9	80.5	**92.3**	78.9	81.0	79.8
SCH 1366	Autumn 1998	ED	2	SRV	+	+	4	77.3	79.9	80.5	**92.3**	78.9	81.0	79.8
SCH 1433	Autumn 1998	ED	1	Astro	+	+	4	77.3	79.9	80.5	**92.3**	78.9	81.0	79.8
SCH 1468	Autumn 1998	ED	<1	NE	+	+	1	**89.4**	80.7	78.4	76.9	79.8	83.3	81.0
SCH 1604	Autumn 1998	ISO	1	NE	+	+	4	77.6	80.7	77.9	**95.4**	77.5	81.3	81.3
SCH 1660	Autumn 1998	ED	1	NE	+	+	4	77.3	79.9	80.5	**92.3**	78.9	81.0	79.8
SCH 1759	Winter 1998	ISO	4	NE	+	+	4	78.2	81.0	78.7	**96.3**	77.5	81.3	80.8
SCH 1749	Winter 1998	ED	3	NE	+	+	3	79.3	79.0	**96.5**	80.0	80.0	81.0	78.7
SCH 1766	Winter 1998	ISO	2	NE	+	+	1	**90.8**	77.9	77.9	78.4	79.3	84.5	80.2

McIver *et al.* 2000. *J Virol. Meth.* 84: 99-105

Children presenting in outpatients (*n* = 238) and hospital (*n* = 176) **Detection rate: 4.6%**

Results of confirmatory tests and serotyping for all stool samples tested positive for astrovirus by EIA[a]

Sample code	Season	Origin	Age	EM	NB	Culture /RT-PCR	Serotype	Percentage identities with prototype astroviruses[b]						
								1	2	3	4	5	6	7
SCH 210	Winter 1997	ED	2	Astro	+	+	1	**91.7**	78.4	78.2	78.7	79.8	84.8	79.6
SCH 217	Winter 1997	ED	<1	Astro	+	+	1	**91.7**	78.4	78.2	78.7	79.8	84.8	79.6
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SCH 475	Spring 1997	ED	<1	Astro	+	+	1	**91.4**	78.3	77.9	78.4	79.8	84.5	79.3
SCH 562	Spring 1997	DC	1	Astro	+	+	1	**91.7**	78.4	78.2	78.7	79.8	84.8	79.6
SCH 605	Spring 1997	DC	2	SRV	+	+	1	**91.7**	78.4	78.2	78.7	79.8	84.8	79.6
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McIver *et al.* 2000. *J Virol. Meth.* 84: 99-105

2014

TABLE 3 Prevalence of serotypes (genotypes) of classic human astrovirus

Region and country (setting, period of study)	Prevalence (%) of serotype:								Reference
	1	2	3	4	5	6	7	8	
North Africa									
Egypt (rural, 1995–1998)	43.3	3.6	12.0	4.8	15.7	7.2	0	12.0	237
Asia									
Vietnam (urban, 2005–2006)	100.0	0	0	0	0	0	0	0	241
India (urban, 2004–2008)	6707	9.7	0	0	6.5[a]	0	0	16.0	308
Japan (urban 2008–2009)	91.0	0	9.0	0	0	0	0	0	250
North America									
USA (urban, 1993–1994)	55.0	17.0	—[b]	—	—	—	—	—	266
South America									
Brazil (urban, 1990–1992)	45.5	27.3	12.1	12.1	0	3.0	0	0	265
Argentina (urban, 1995–1998)	41.0	13.0	13.0	25.0	8.0	0	0	0	267
Europe									
Spain (urban, 1997–2000)	38.0	6.0	19.0	26.0	0	0	0	11.0	164
Oceania									
Australia (urban, 1995)	85.0	0	0	15.0	0	0	0	0	35

[a] ORF1a/ORF2 recombinant strains.
[b] —, there were no data on this genotype in the study.

Source: Bosch *et al*. 2014. Clin. Microbiol Rev 2014, 27 (4):1048.

2014

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North America									
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South America									
Brazil (urban, 1990–1992)	45.5	27.3	12.1	12.1	0	3.0	0	0	265
Argentina (urban, 1995–1998)	41.0	13.0	13.0	25.0	8.0	0	0	0	267
Europe									
Spain (urban, 1997–2000)	38.0	6.0	19.0	26.0	0	0	0	11.0	164
Oceania									
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[a] ORF1a/ORF2 recombinant strains.
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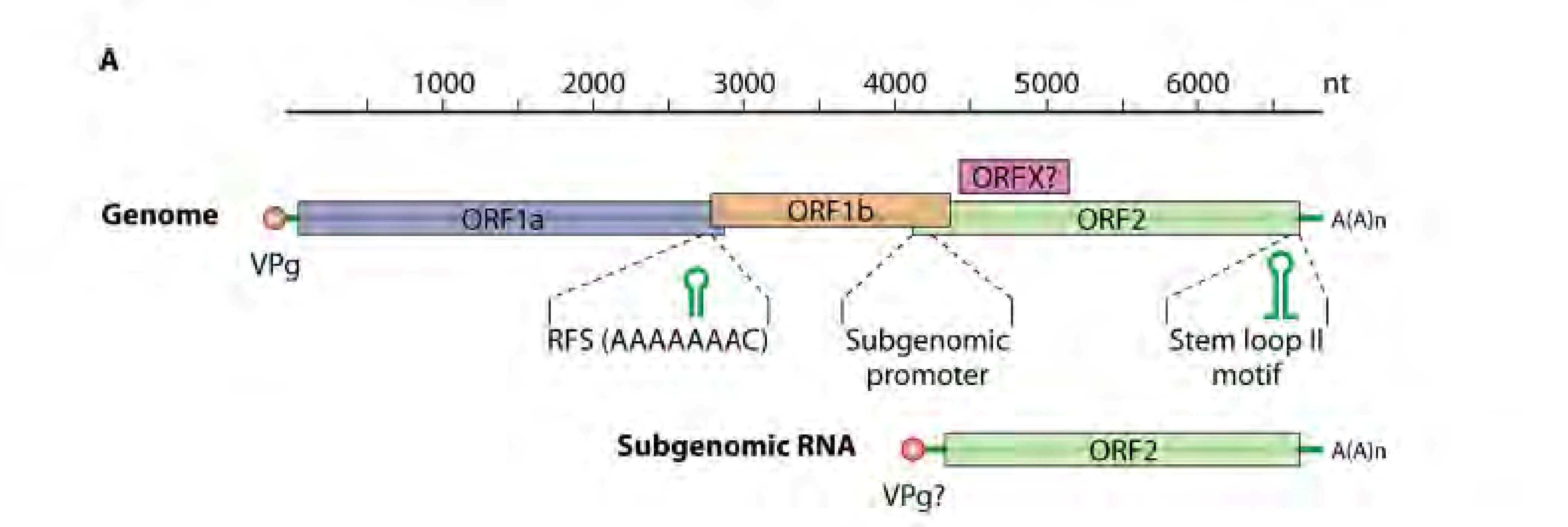
Genomic structure of Astrovirus



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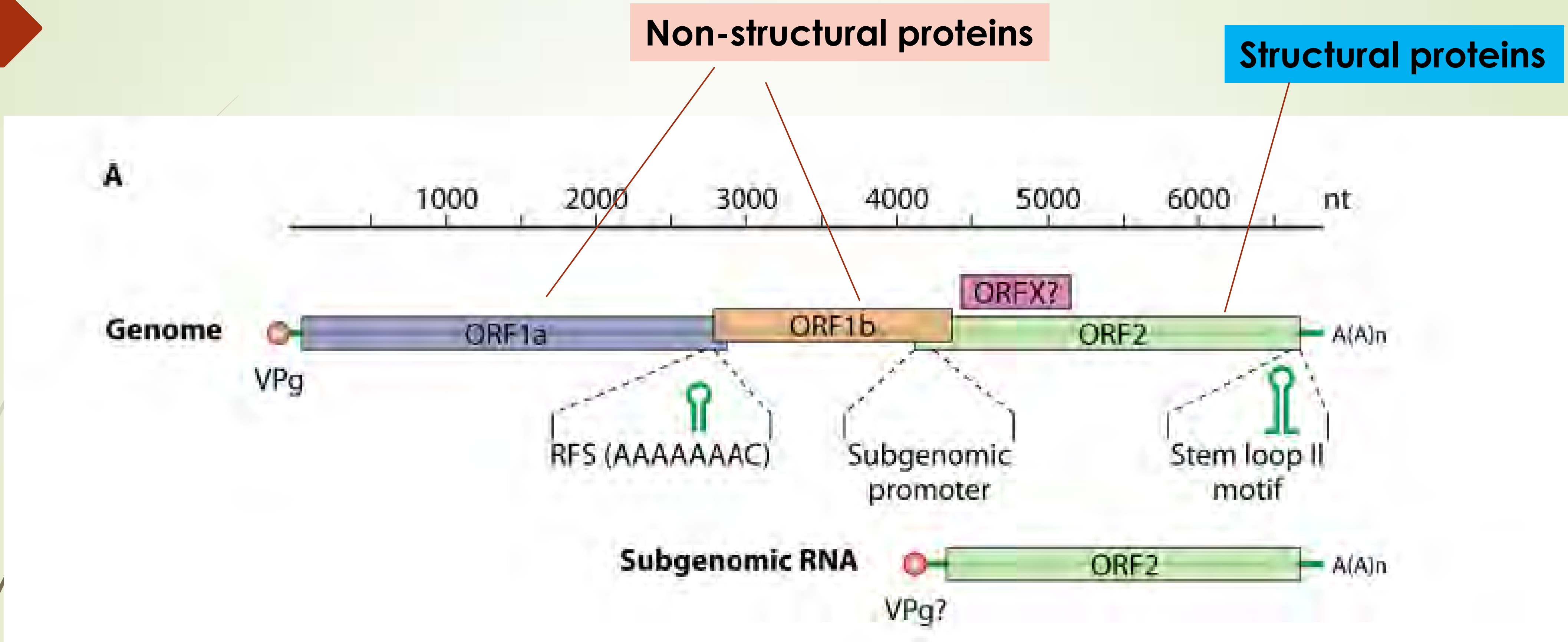
Genomic structure of Astrovirus



Source: Bosch *et al*. 2014. Clin. Microbiol Rev 2014, 27 (4):1048.

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Metagenomic Analysis of Human Diarrhea: Viral Detection and Discovery

Stacy R. Finkbeiner[1,2¶], Adam F. Allred[1,2¶], Phillip I. Tarr[3], Eileen J. Klein[4], Carl D. Kirkwood[5], David Wang[1,2]*

1 Departments of Molecular Microbiology and Pathology & Immunology, Washington University School of Medicine, St. Louis, Missouri, United States of America, 2 Department of Pathology & Immunology, Washington University School of Medicine, St. Louis, Missouri, United States of America, 3 Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri, United States of America, 4 Department of Emergency Medicine, Children's Hospital and Regional Medical Center, Seattle, Washington, United States of America, 5 Enteric Virus Research Group, Murdoch Childrens Research Institute, Royal Children's Hospital, Victoria, Australia

"Micro-mass sequencing" to systematically identify viruses present in stools

Finkbeiner *et al. 2008. PLoS Pathogens.* 4:e1000011

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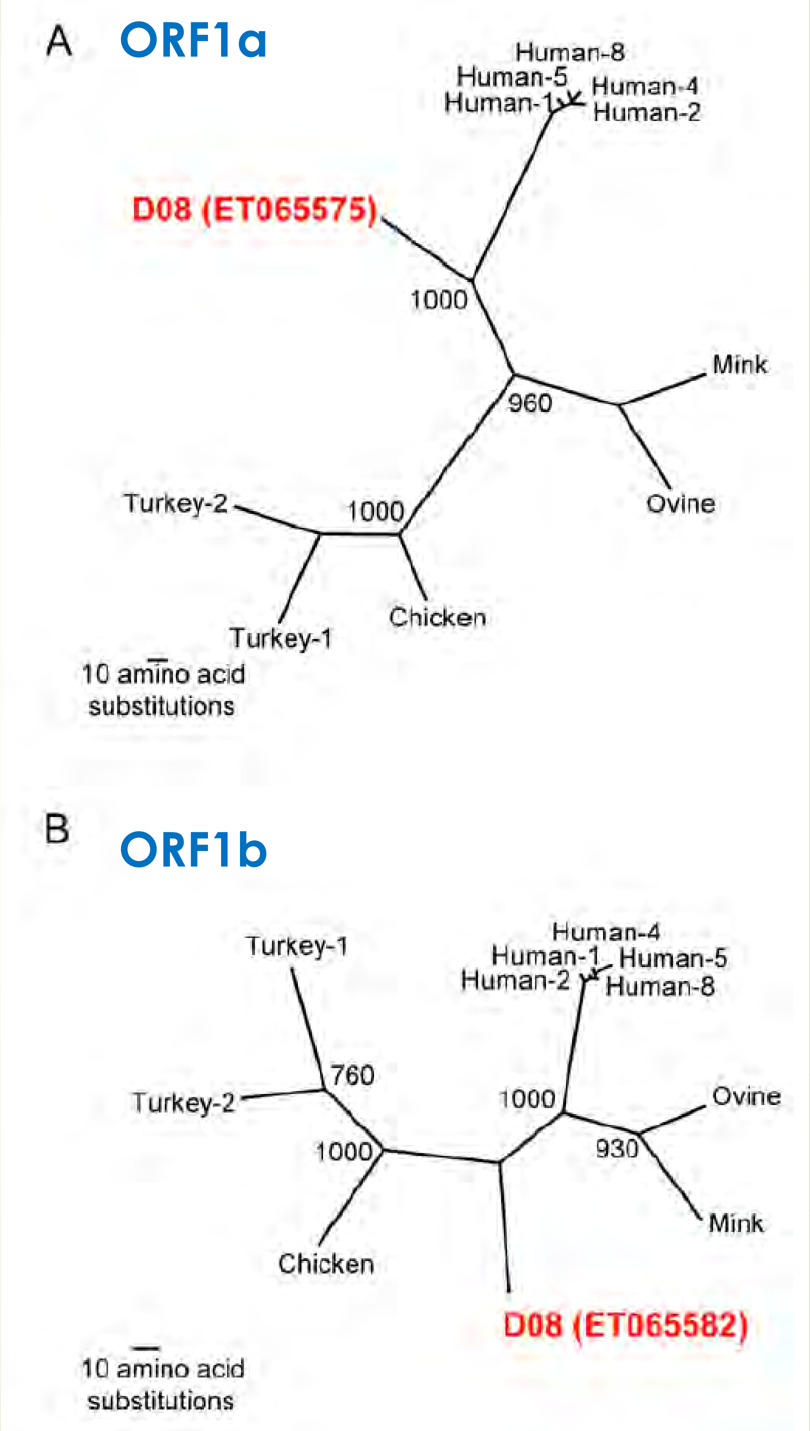

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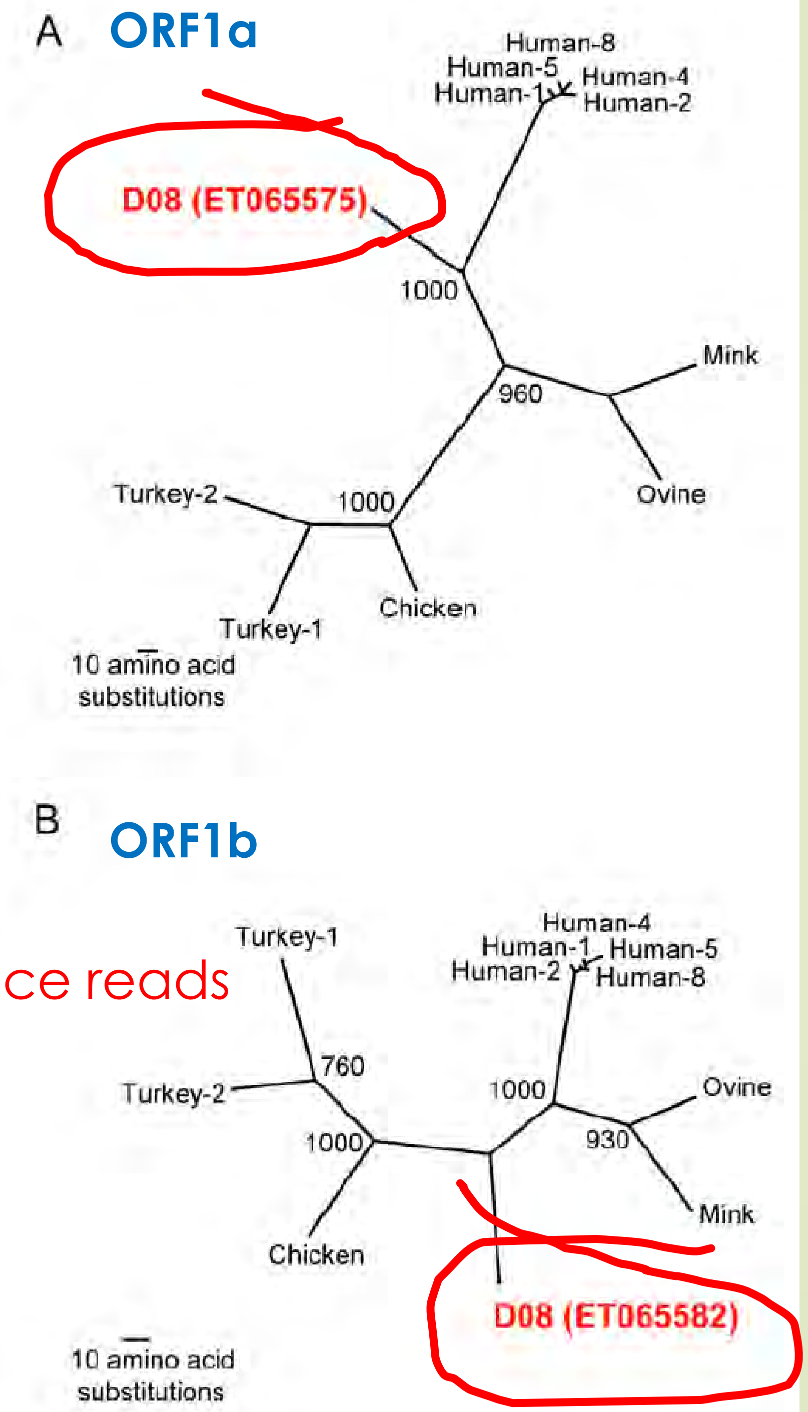
Phylogenetic analysis of highly divergent astrovirus-like sequence reads



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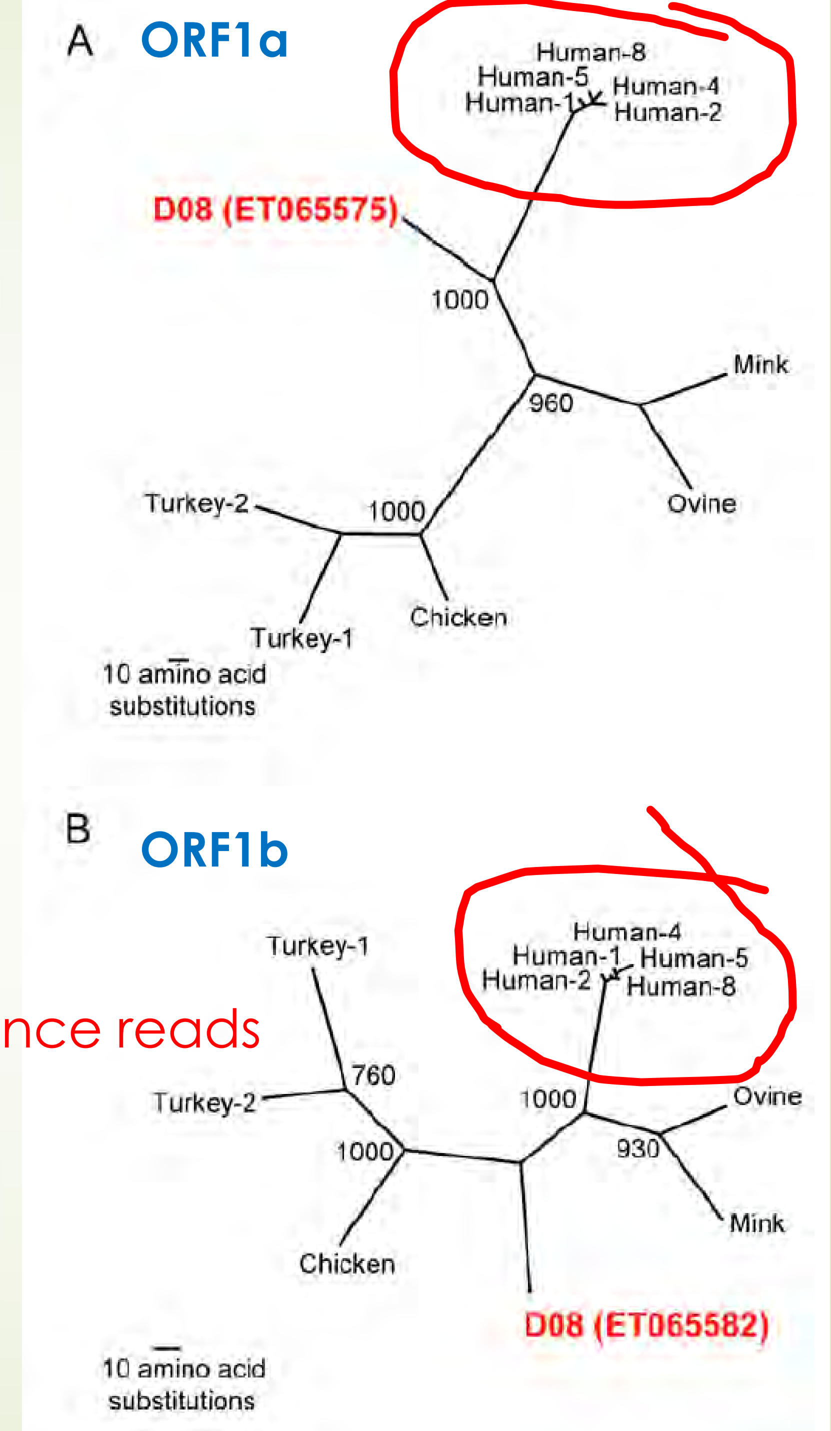


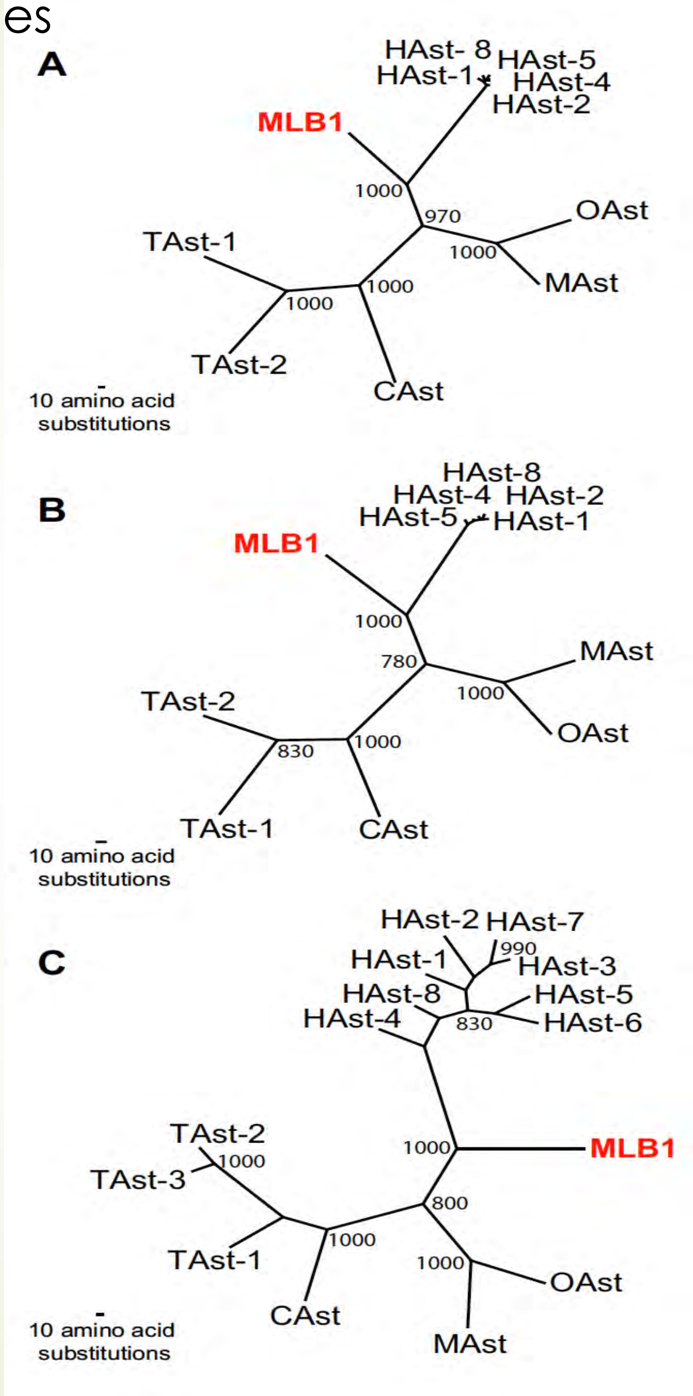
Phylogenetic analysis of highly divergent astrovirus-like sequence reads

Phylogenetic analysis of open reading frames

ORF1a
Serine protease

ORF1b
polymerase

ORF2
capsid



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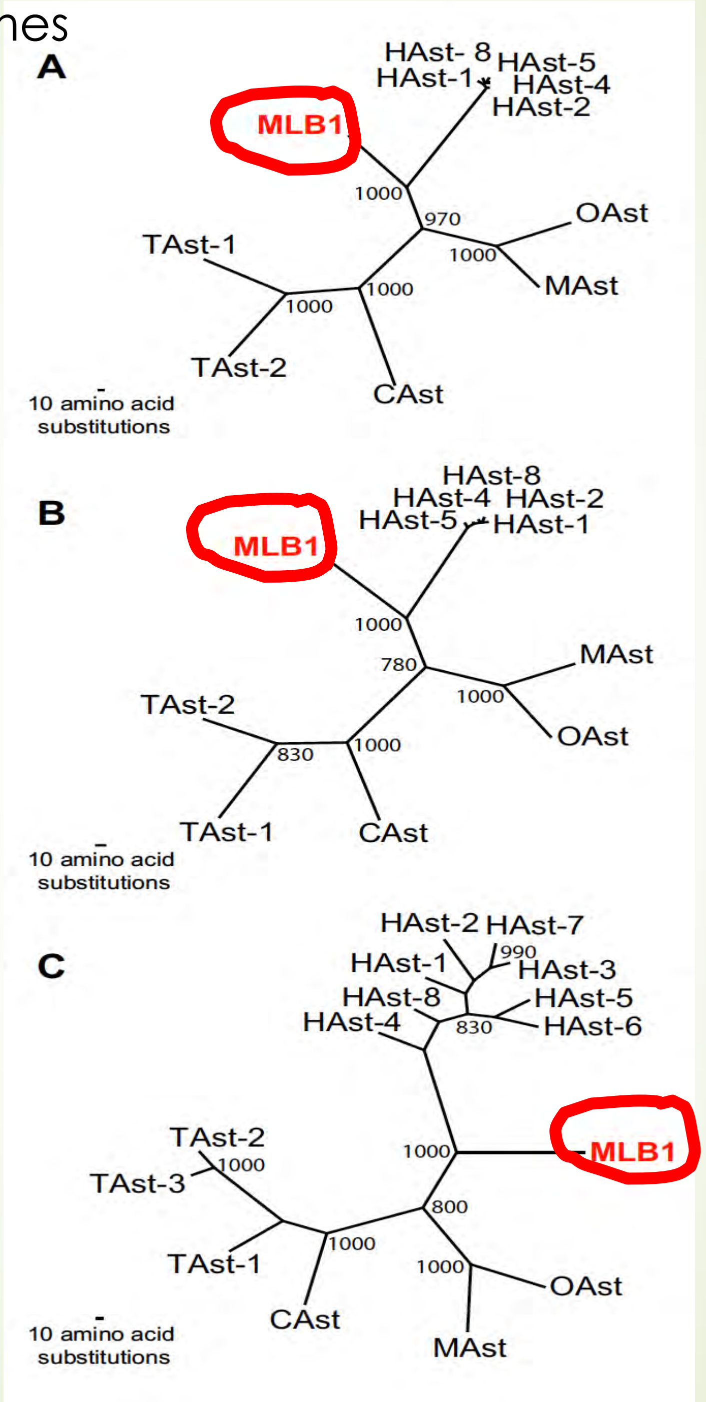

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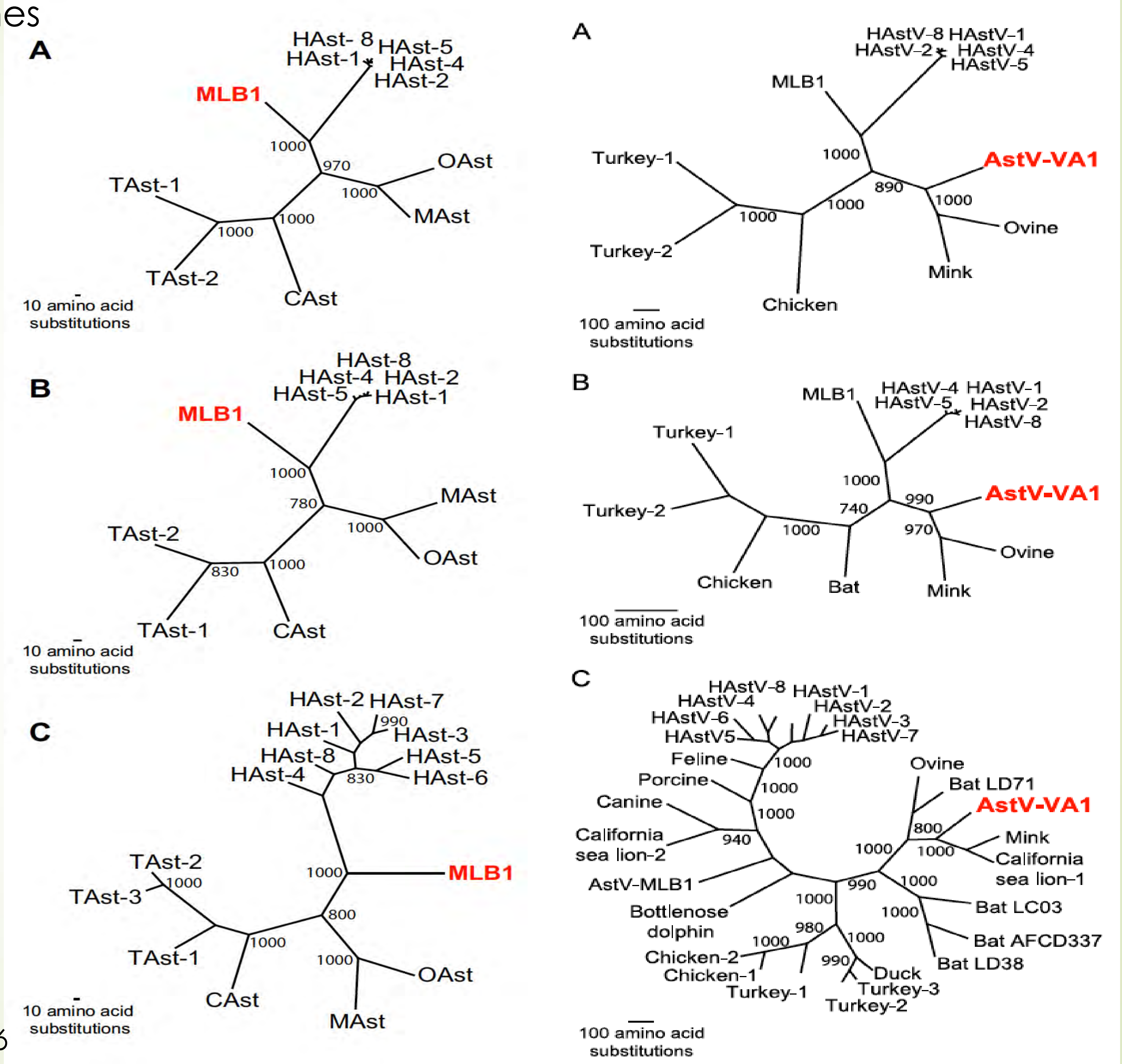

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Finkbeiner *et al. 2009.* J Virol. *83: 10836*

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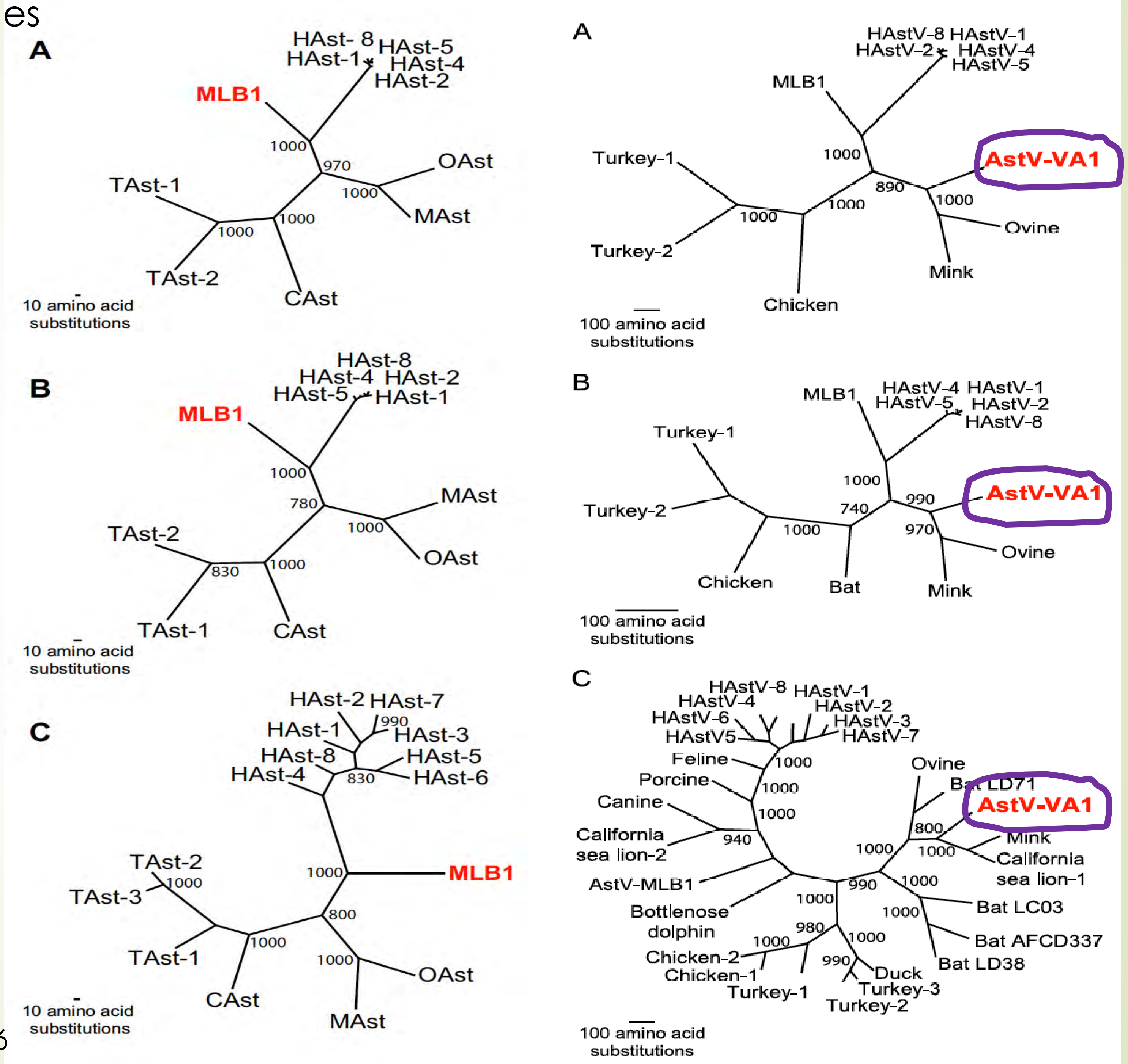

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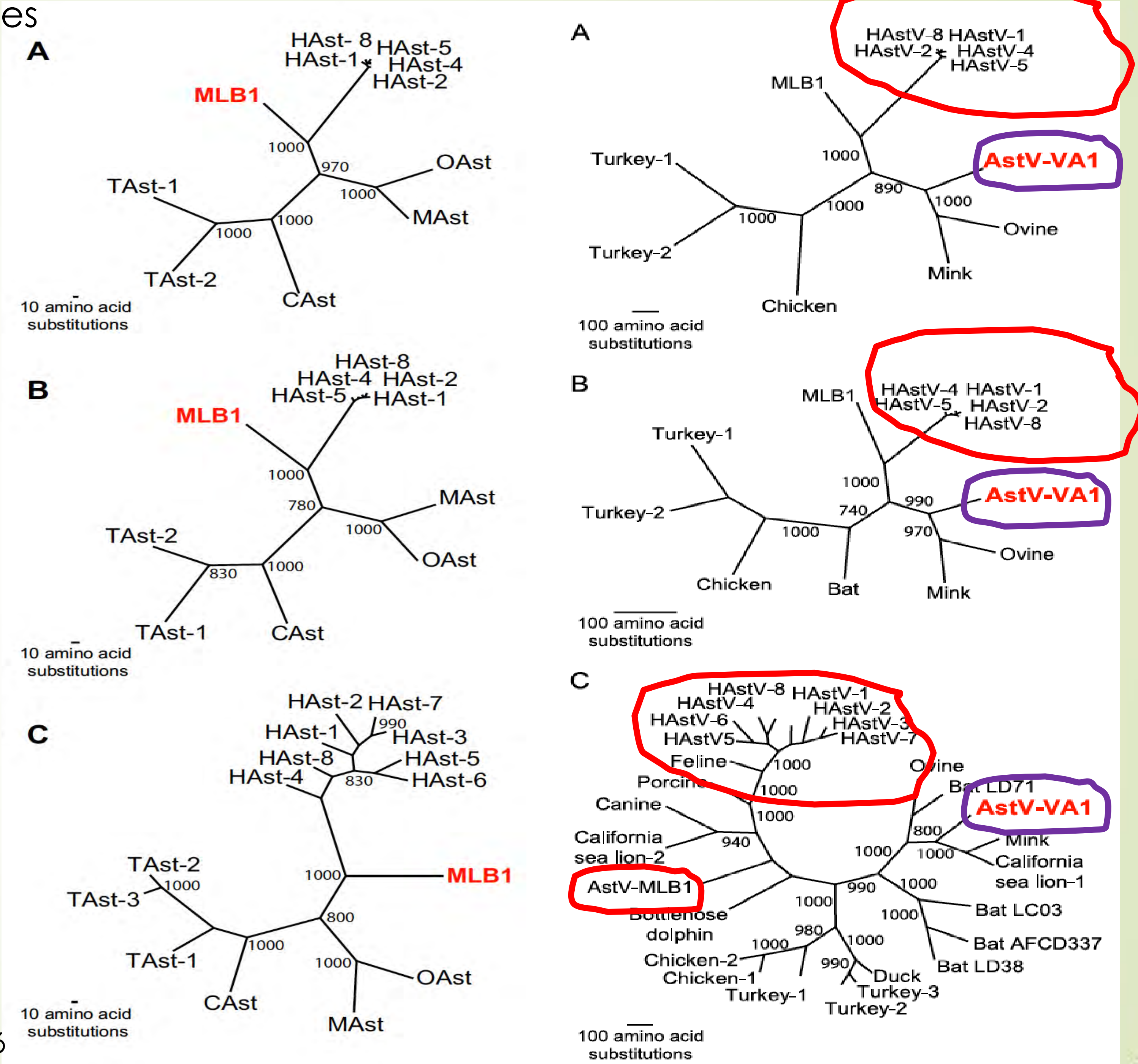

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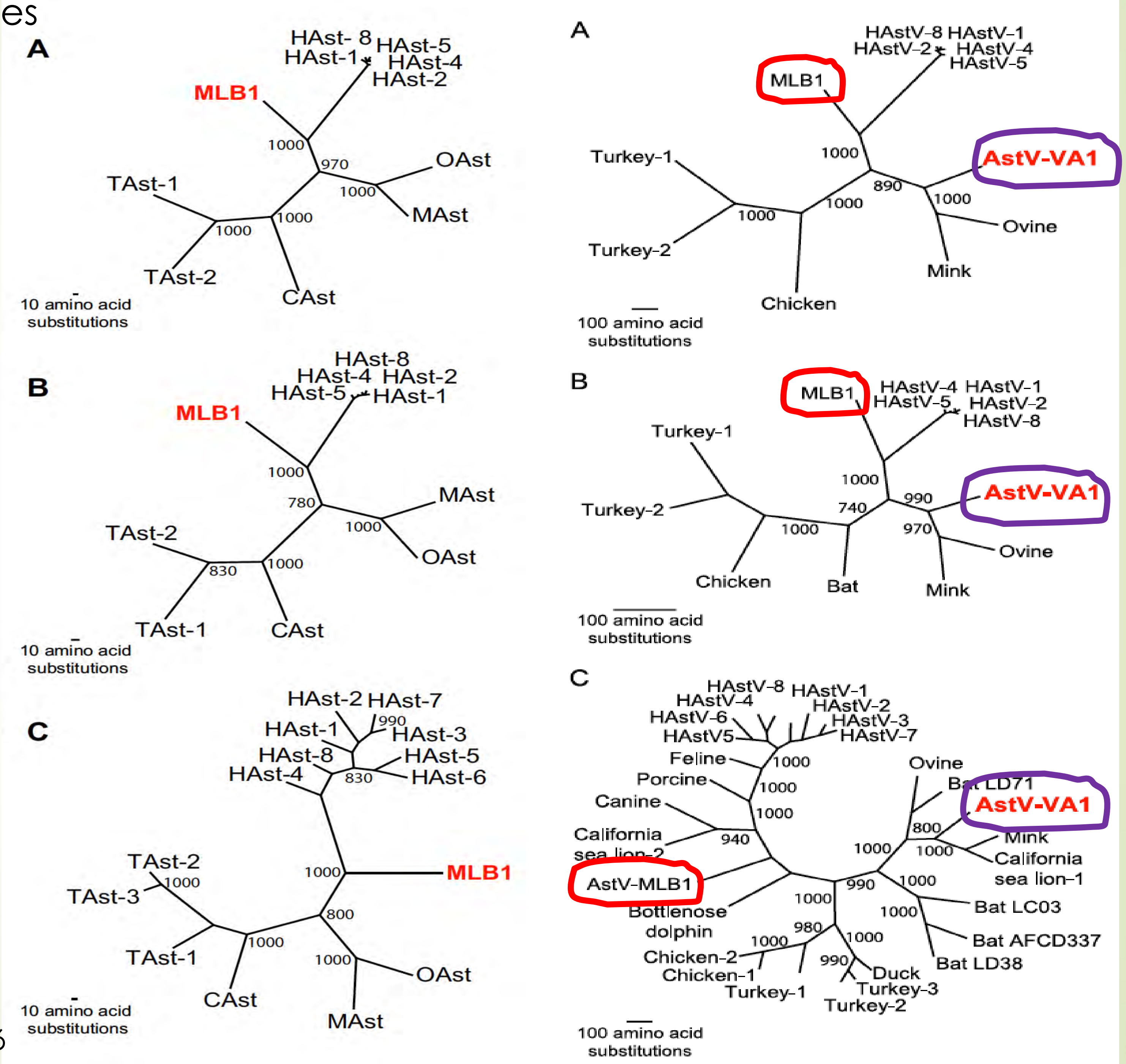

Finkbeiner *et al. 2008. Virol J.* 5:117
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Multiple Astrovirus MLB1, MLB2, VA2 Clades, and Classic Human Astrovirus in Children With Acute Gastroenteritis in Japan

Pattara Khamrin,[1,2,3*] Aksara Thongprachum,[2] Shoko Okitsu,[2,3] Satoshi Hayakawa,[3] Niwat Maneekarn,[1] and Hiroshi Ushijima[2,3]

[1]*Department of Microbiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand*
[2]*Department of Developmental Medical Sciences, School of International Health, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan*
[3]*Division of Microbiology, Department of Pathology and Microbiology, Nihon University School of Medicine, Tokyo, Japan*

Khamrin *et al. 2016. J Med Virol. 88:356*

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[3]*Division of Microbiology, Department of Pathology and Microbiology, Nihon University School of Medicine, Tokyo, Japan*

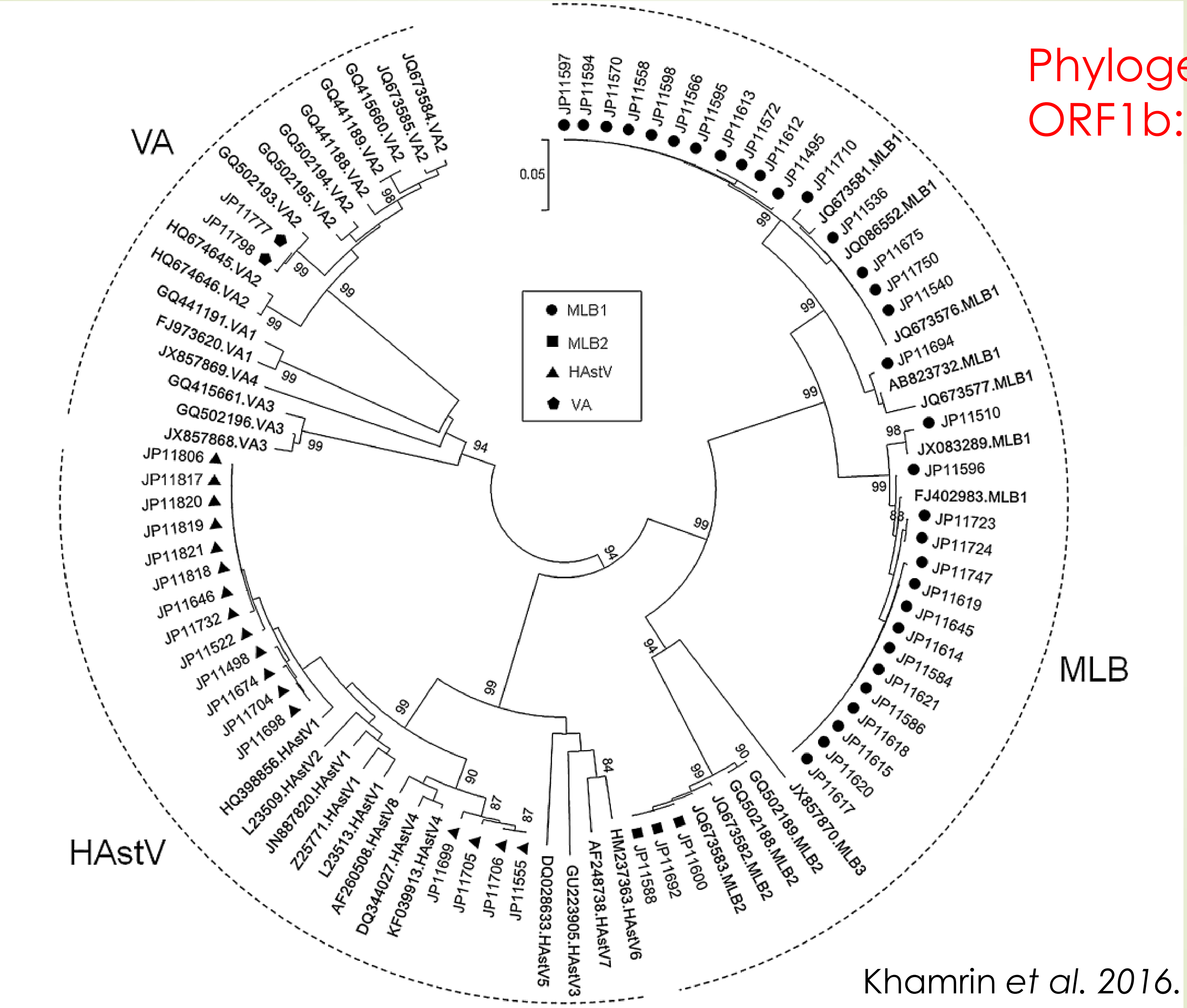
JAPAN: 2012 – 2013

Finkbeiner (2009) target:
Pan-astrovirus consensus primers targeting RdRp

54/330 (16.4%) stools from children with acute gastroenteritis

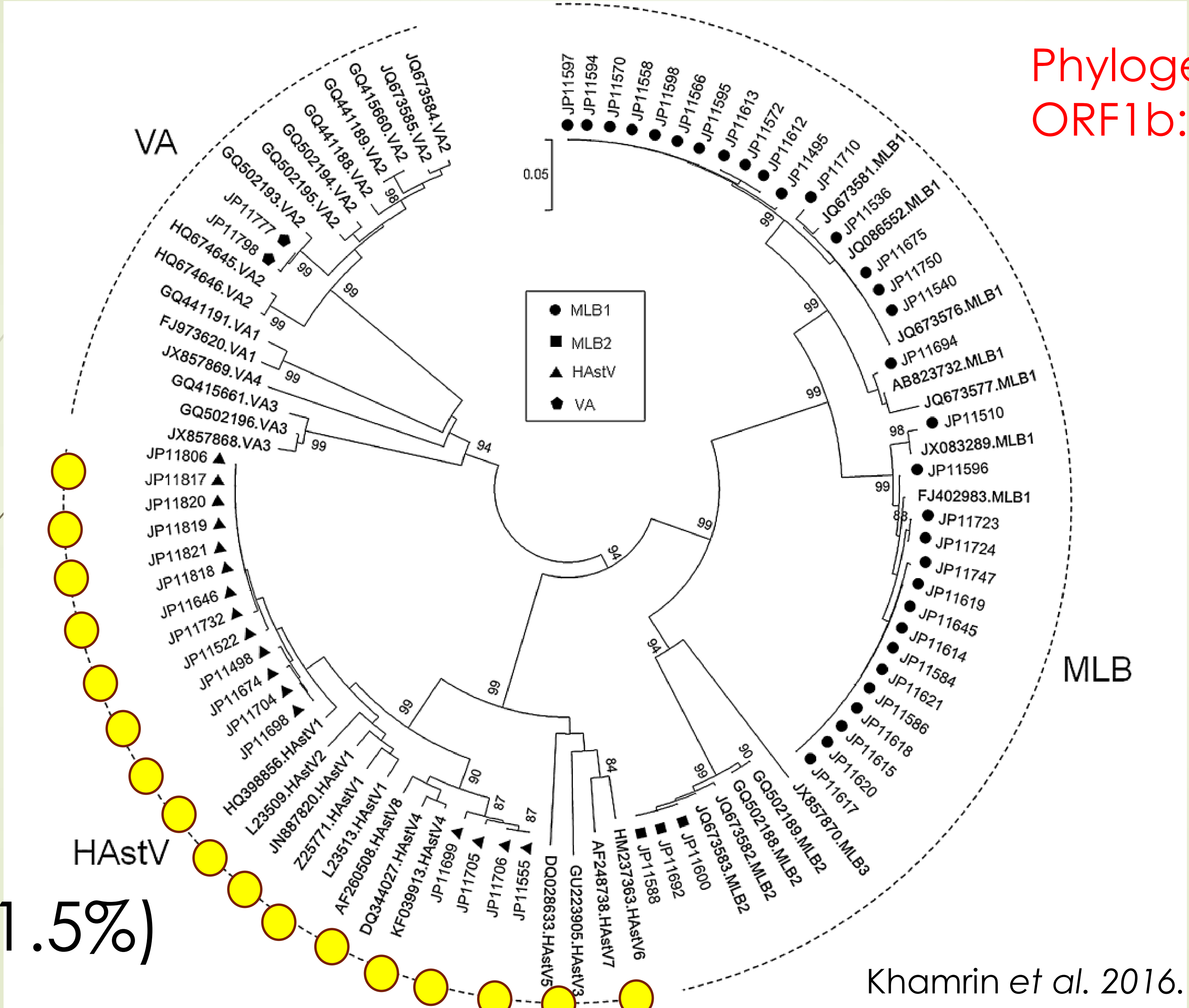
Khamrin *et al.* 2016. *J Med Virol.* 88:356

Phylogenetic analysis ORF1b: RdRp



Khamrin *et al.* 2016. *J Med Virol. 88:356*

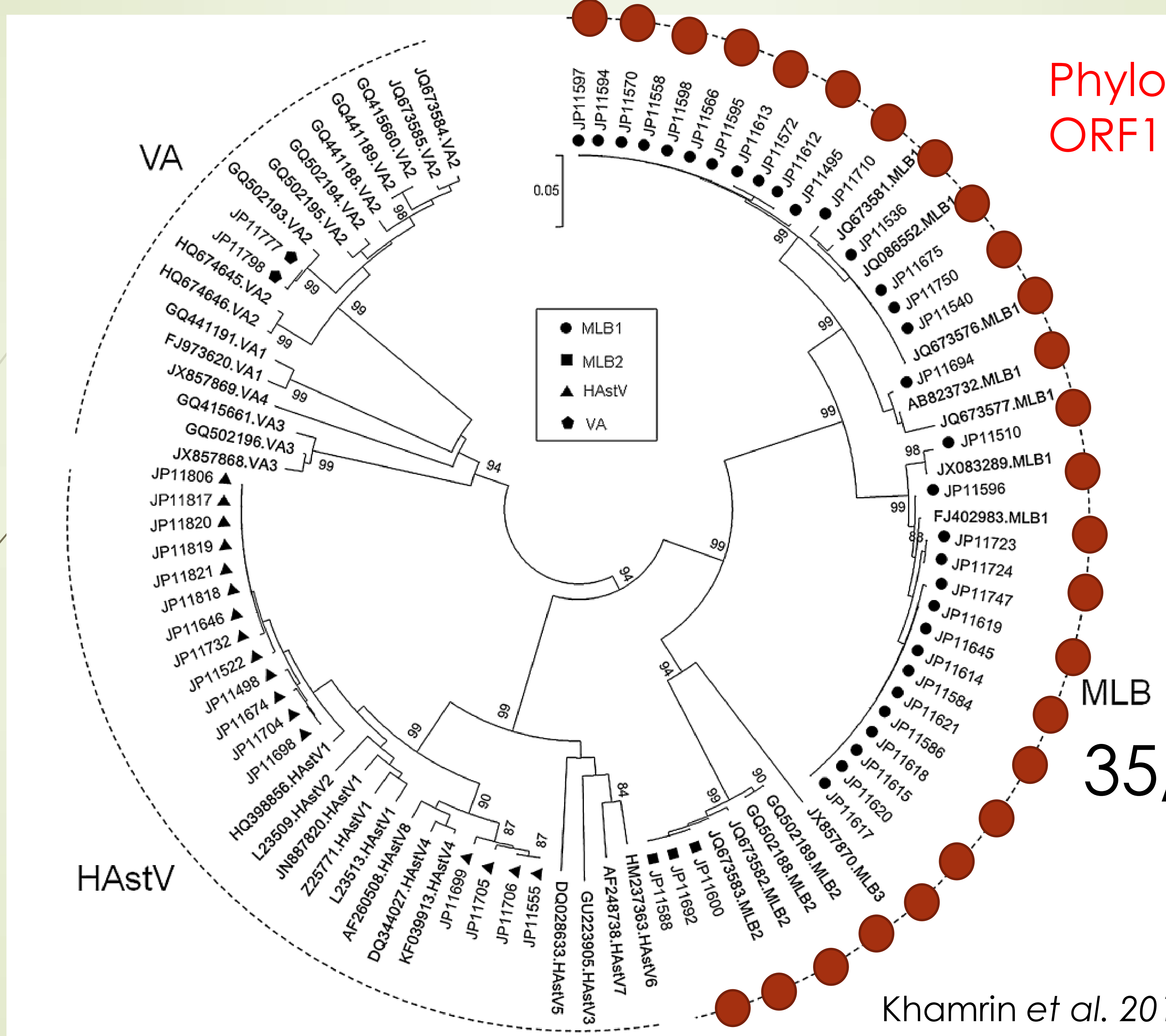
Phylogenetic analysis
ORF1b: RdRp



17/54 (31.5%)

Khamrin *et al.* 2016. *J Med Virol. 88:356*

Phylogenetic analysis ORF1b: RdRp

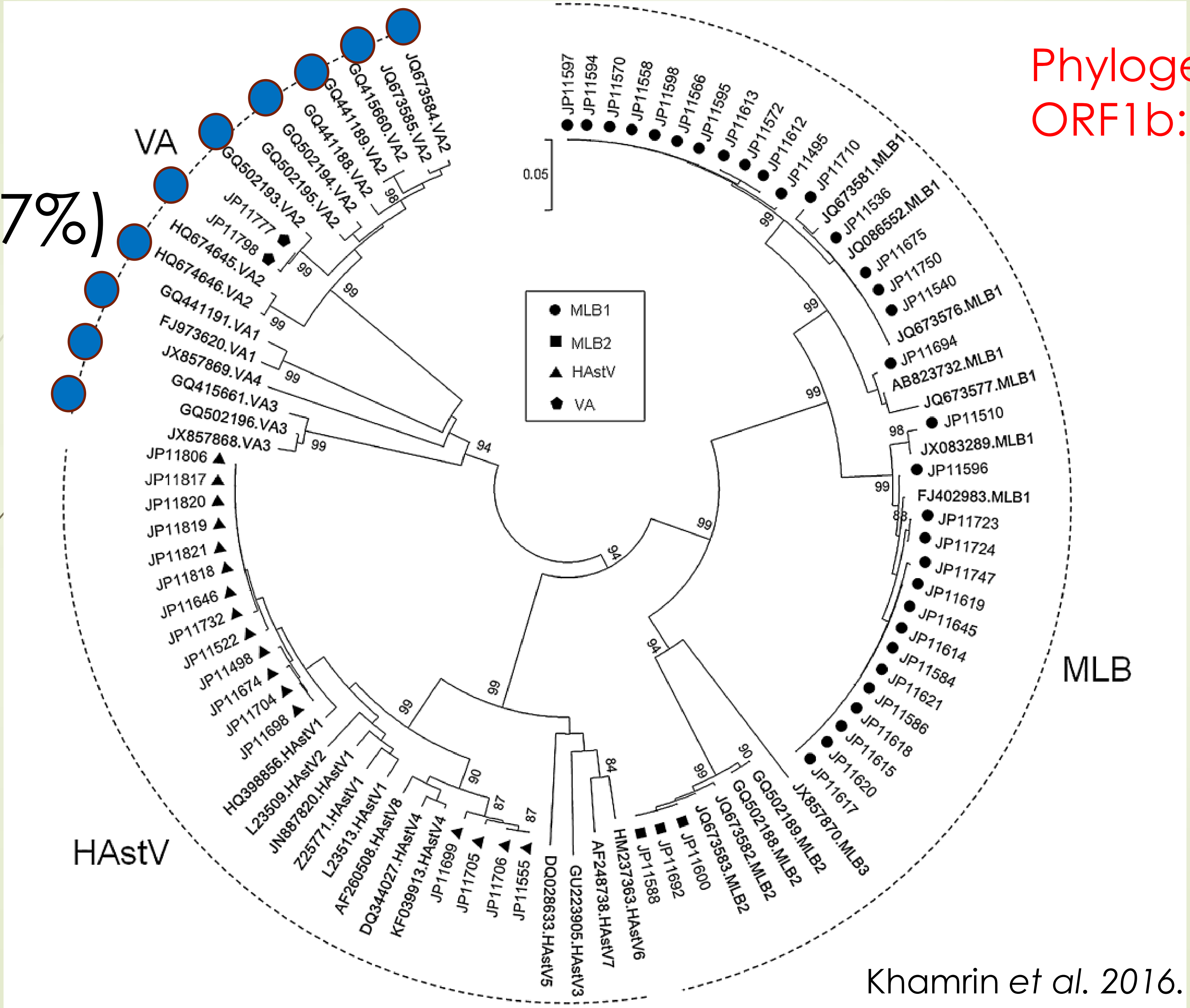


35/54 (64.8%)

Khamrin *et al.* 2016. *J Med Virol.* 88:356

2/54 (3.7%)

Phylogenetic analysis ORF1b: RdRp



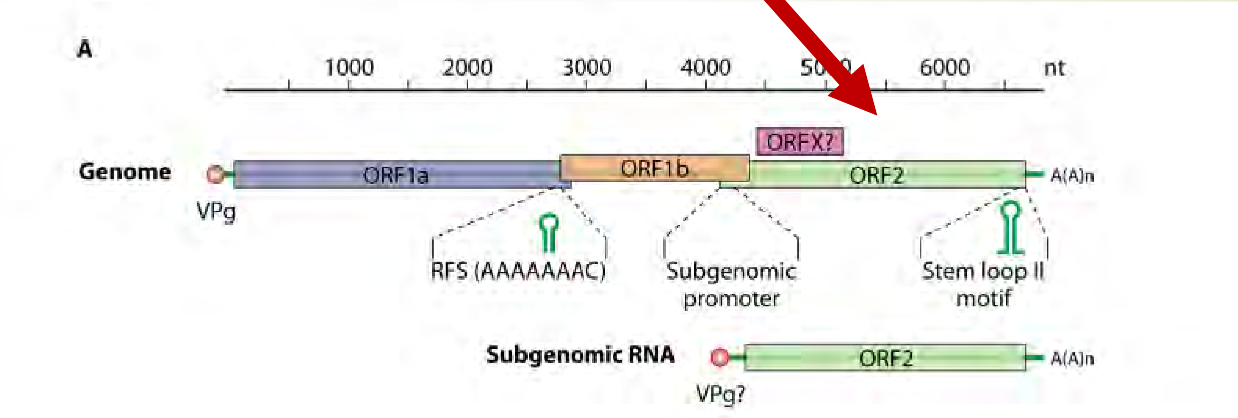
Khamrin *et al.* 2016. *J Med Virol.* 88:356

Molecular detection of astroviruses

8 classic serotypes

- 5' conserved end of ORF2 (detection and sequencing)

Noel *et al*. 1995. *J Clin Microbiol*, 33:797



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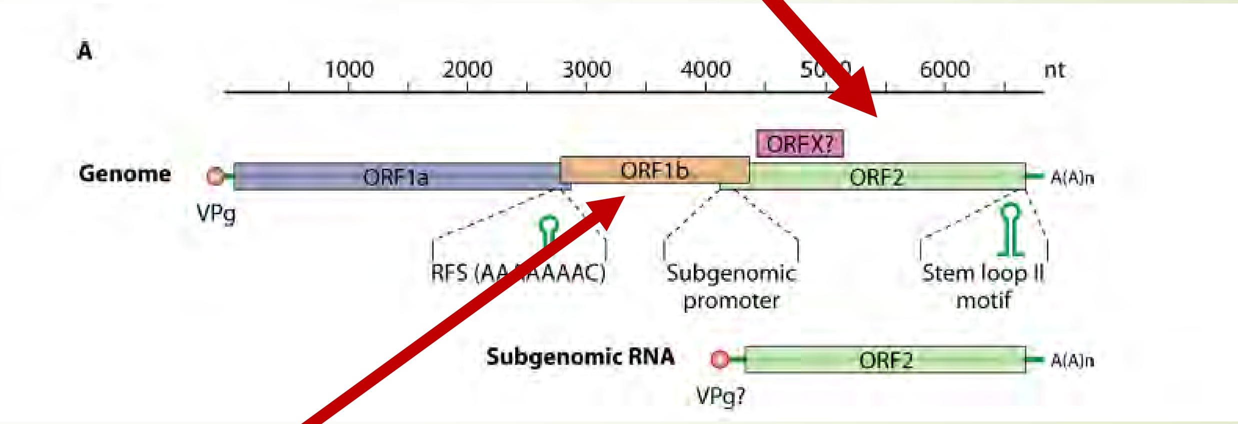
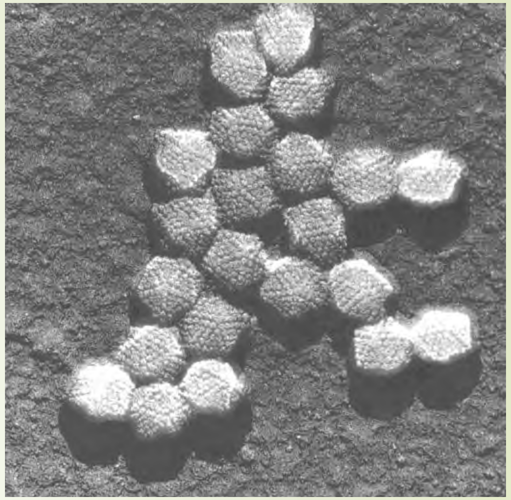


Novel types

- ORF1b

Chu *et al*. 2008. *J Virol*, 82:9107
Kapoor *et al*. 2009. *Gen Virol*, 90:2965
Finkbeiner *et al. 2009. Emerg Infect Dis*, 15:441

Adenovirus



https://www.google.com.au/search?q=adenovirus&client

Non-enveloped DNA viruses, 70-100 nm
Family: *Adenoviridae*
Genus: *Mastadenovirus*

7 known species A-G: encompassing 68 types

Protracted diarrhea (compared to rotavirus), lasting 7 – 8 days

Detection rate is higher in “developing countries” than in “developed”.

Beijing, China 2014
219/2,233 (9.8%) children

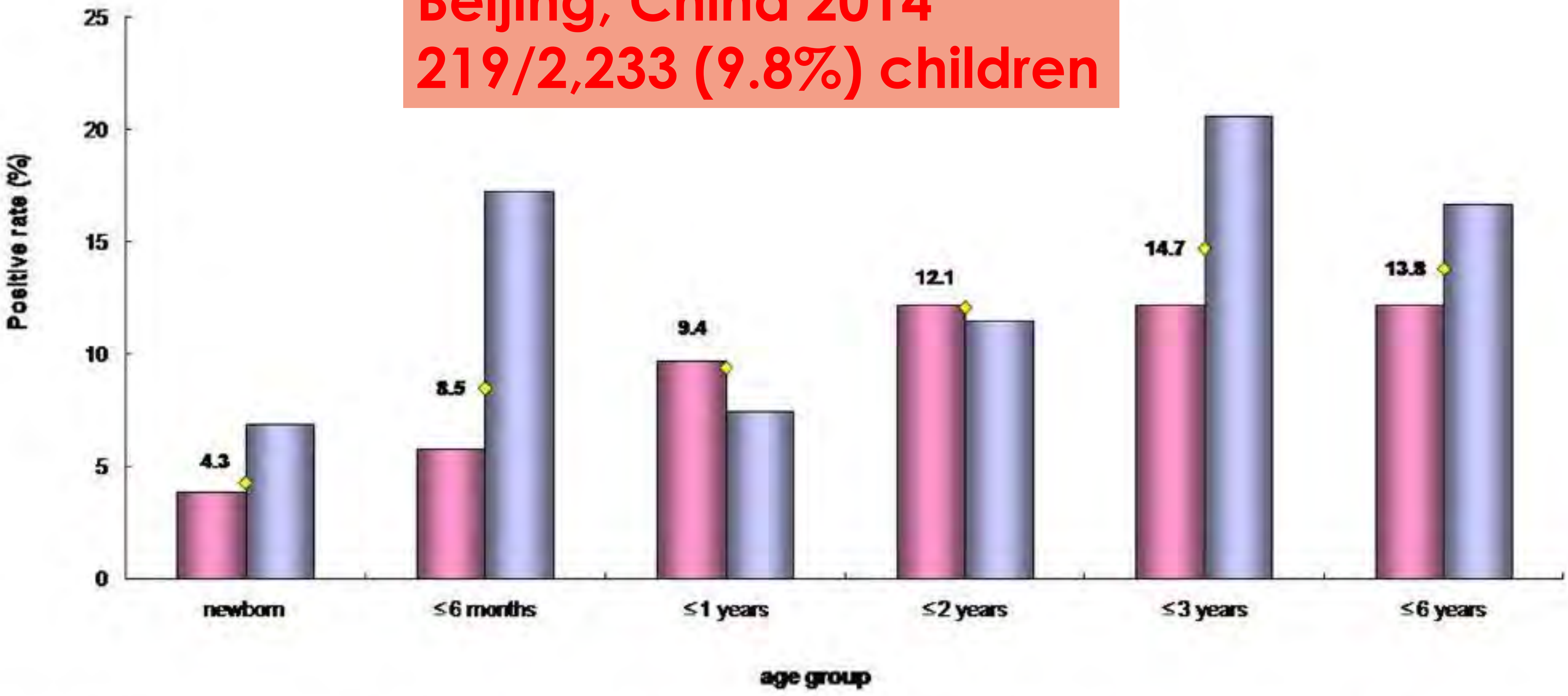
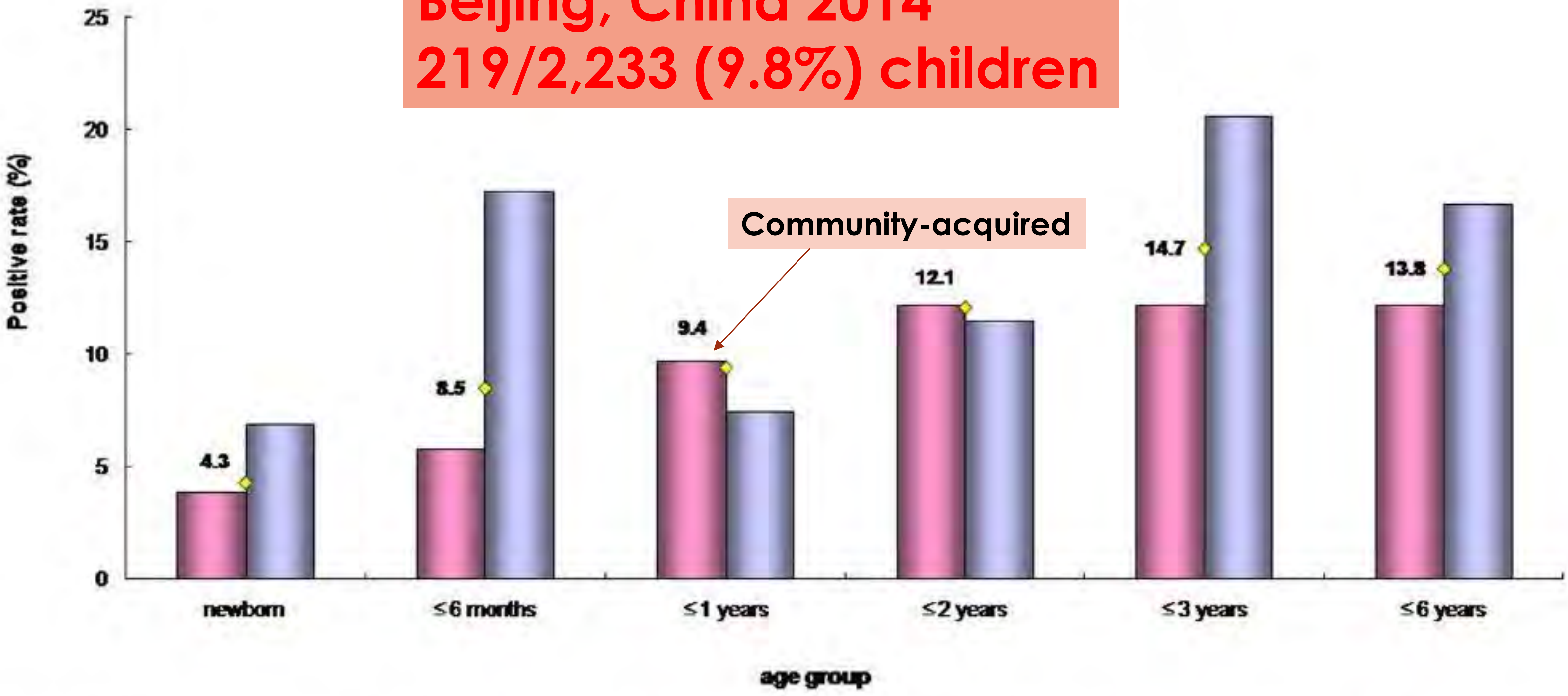


Figure 3. Age distributions of Ad detection rates in children with CAD and HAD. CAD including hospitalized children with CAD (IP-CAD) and outpatient children with CAD (OP-CAD). HAD refer to hospitalized children with HAD (HAD). The range of ages for each age group are indicated in the parentheses as follows: newborn (0–28 days); ≤6 months (28 days–6 months); ≤1 year (6 months–1 year); ≤2 years (1 year–2 years); ≤3years (2 years–3 years); ≤6 years (3 year–6 years).

doi:10.1371/journal.pone.0088791.g003

Liu *et al.* 2014. *PLOS, 9:e88791*

Beijing, China 2014
219/2,233 (9.8%) children



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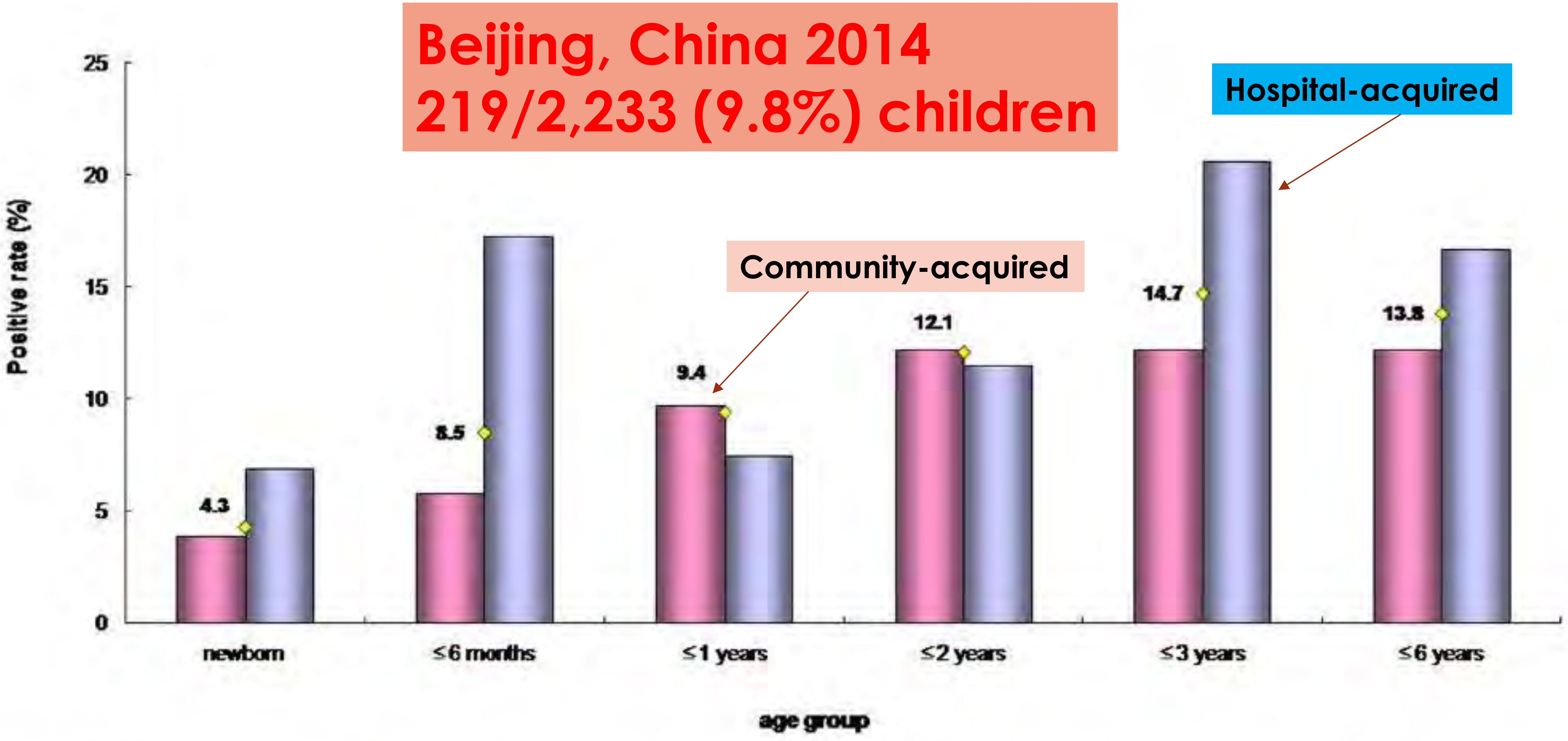
doi:10.1371/journal.pone.0088791.g003

Liu *et al.* 2014. *PLOS, 9:e88791*



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Beijing, China 2014
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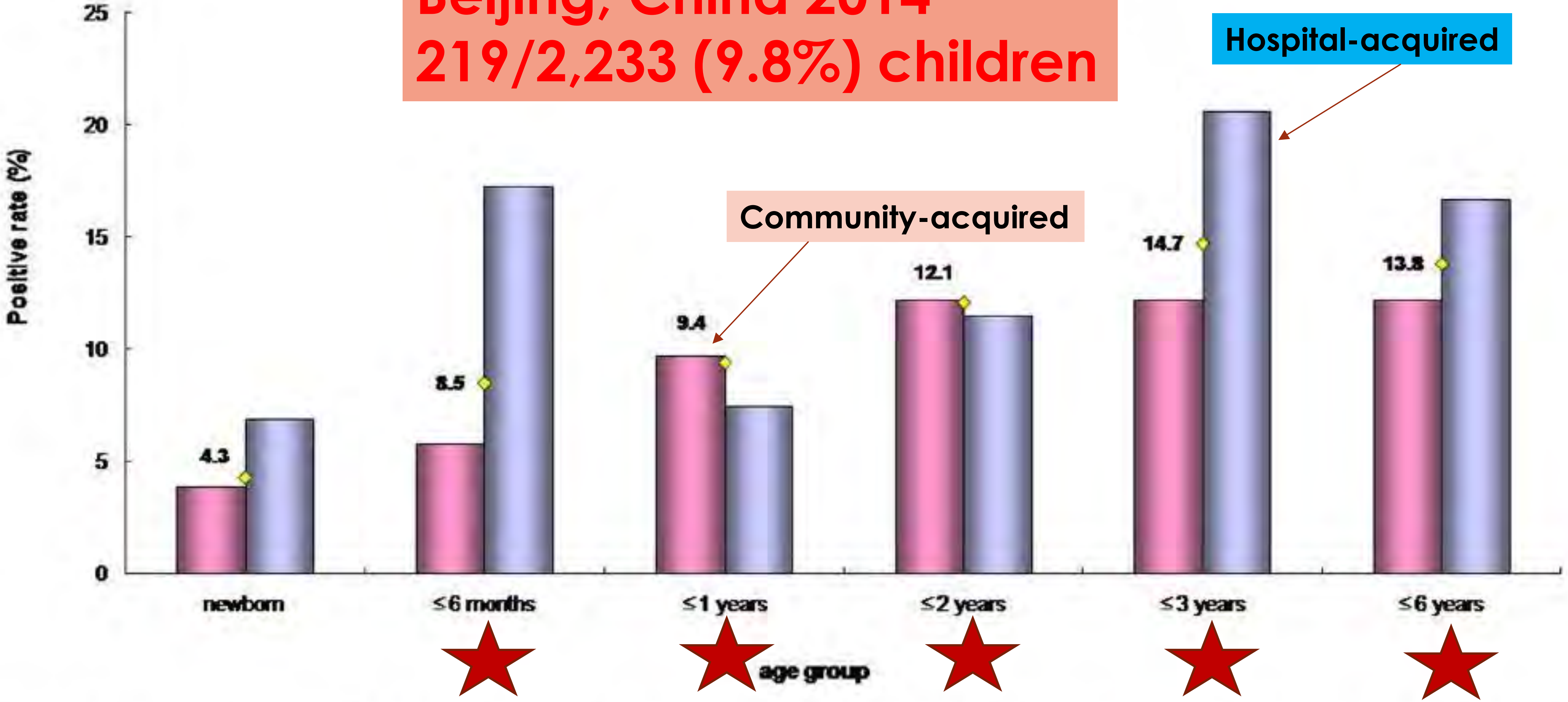

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Liu *et al.* 2014. *PLOS, 9:e88791*

Adenoviruses have broad tropisms

Table 1. Adenovirus serotypes and associated clinical diseases [5–7, 17, 34–37]

HAvD subgroup	Serotype	Type of infection
A	12, 18, 31	gastrointestinal, respiratory, urinary
B, type 1	**3, 7, 16, 21**	keratoconjunctivitis, gastrointestinal, respiratory, urinary
B, type 2	11, 14, 34, 35	gastrointestinal, respiratory, urinary
C	1, 2, 5, 6	respiratory, gastrointestinal including hepatitis, urinary
D	**8–10,13,15,17,19,20,22–30,32,33,36–39,42–49**	keratoconjunctivitis, gastrointestinal
E	**4**	keratoconjunctivitis, respiratory
F	40, 41	gastrointestinal
G	52	gastrointestinal

Ghebremedhin. 2014. Euro J Microbiol and Immunol, 4:26

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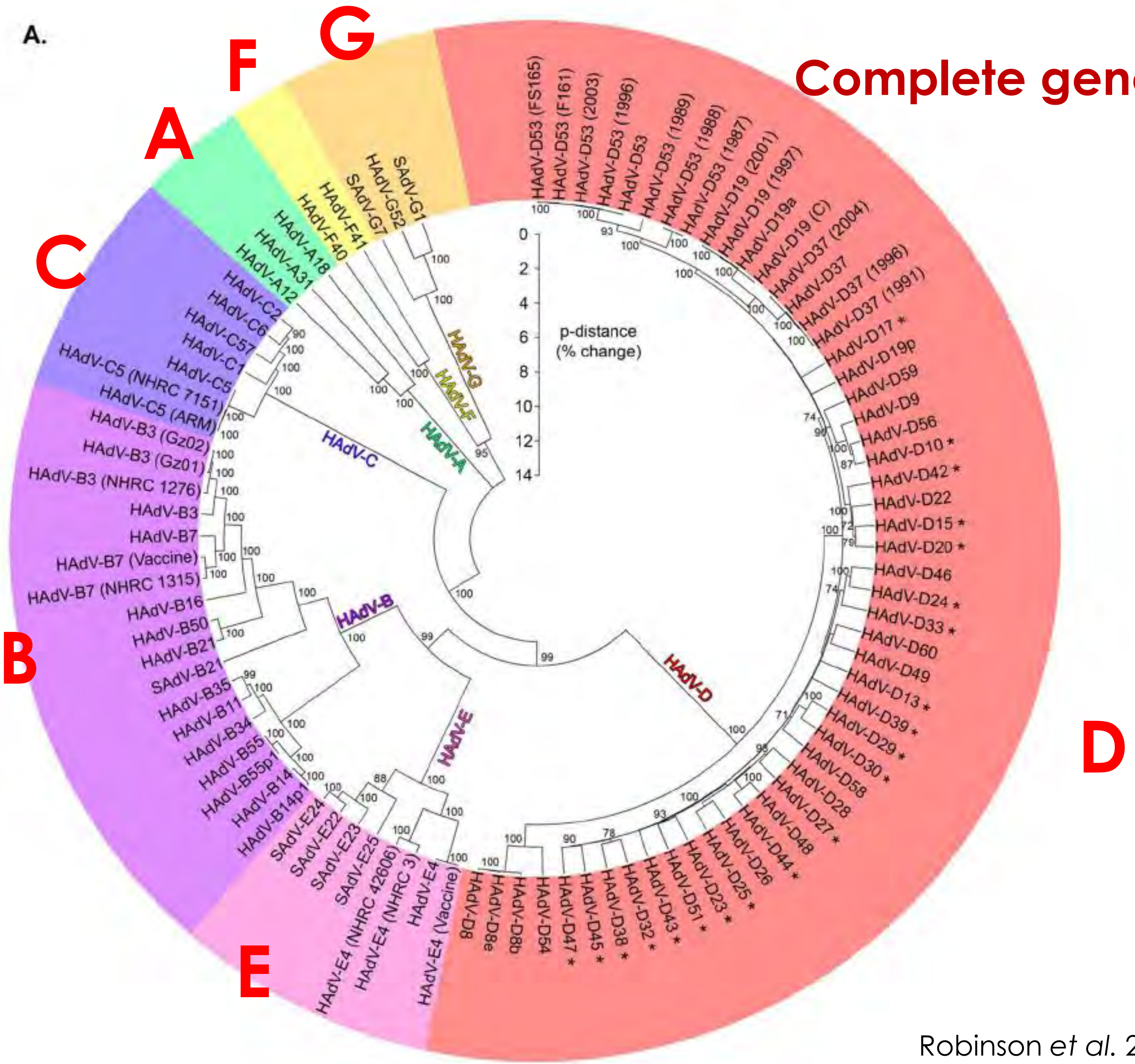
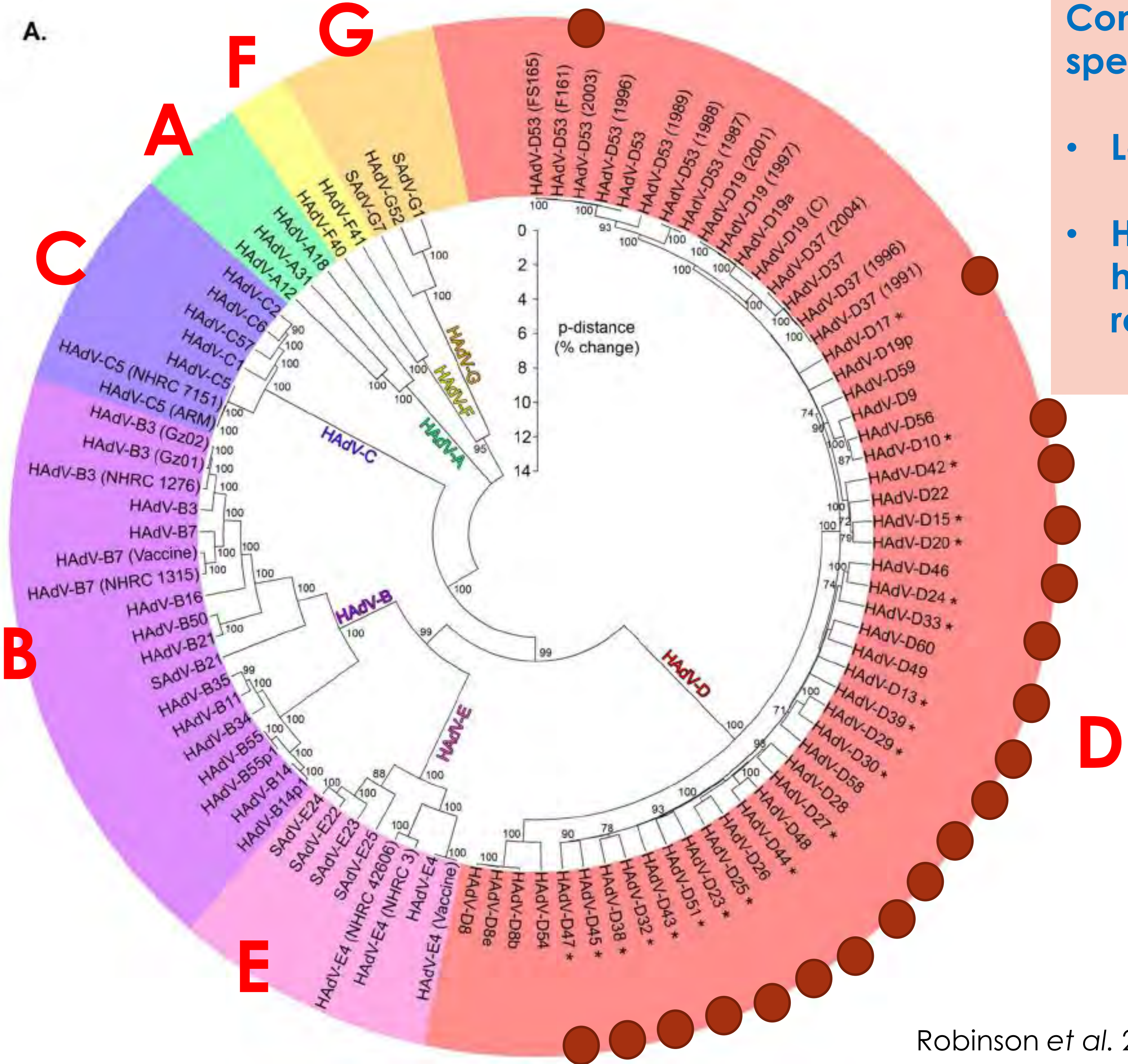
Ghebremedhin. 2014. Euro J Microbiol and Immunol, 4:26

Complete genome sequencing



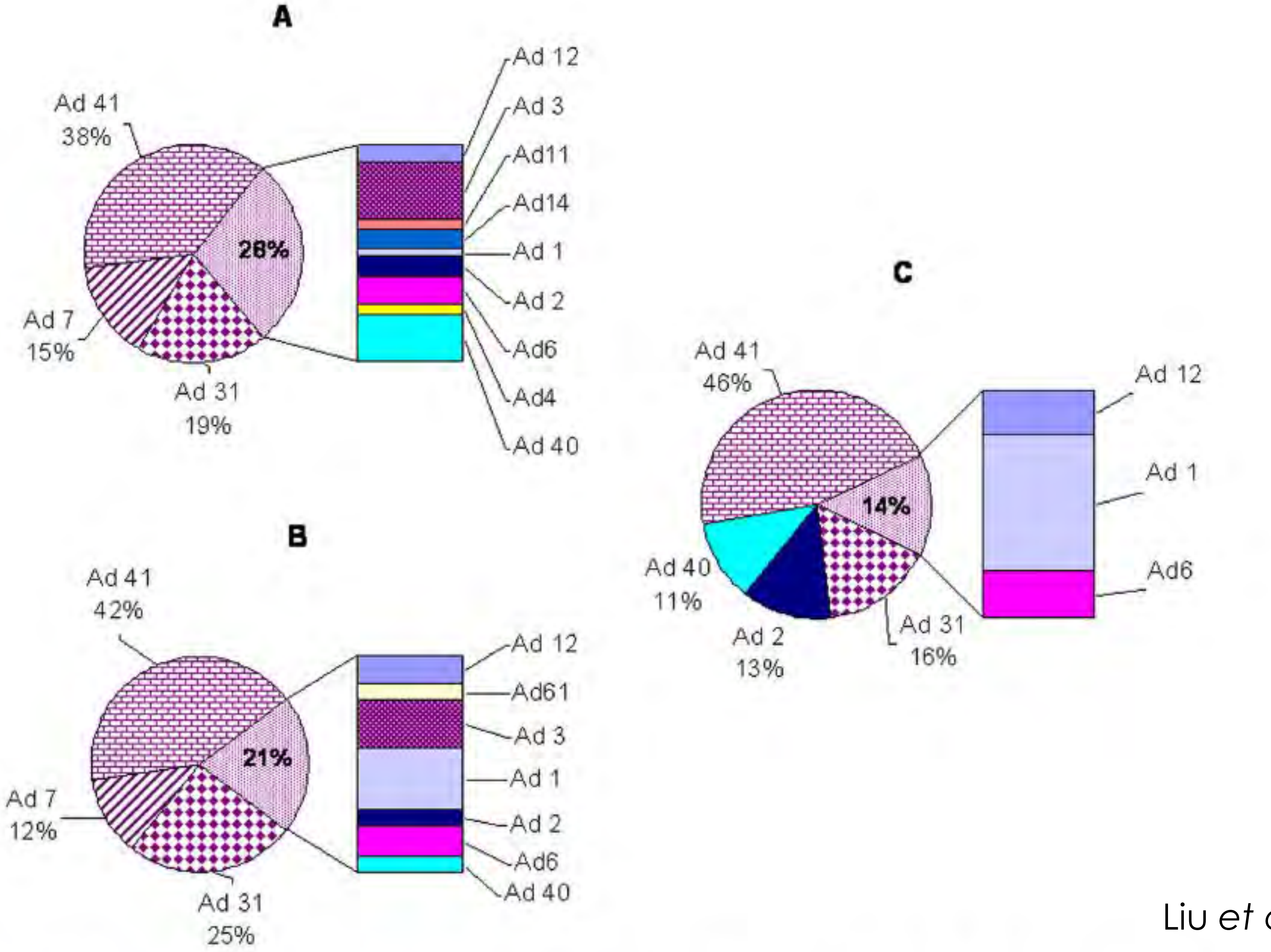
Robinson *et al.* 2013. Scientific Reports, 3:1812



Compared to other species:

- Low mutation rate
- Highly dependent on homologous recombination

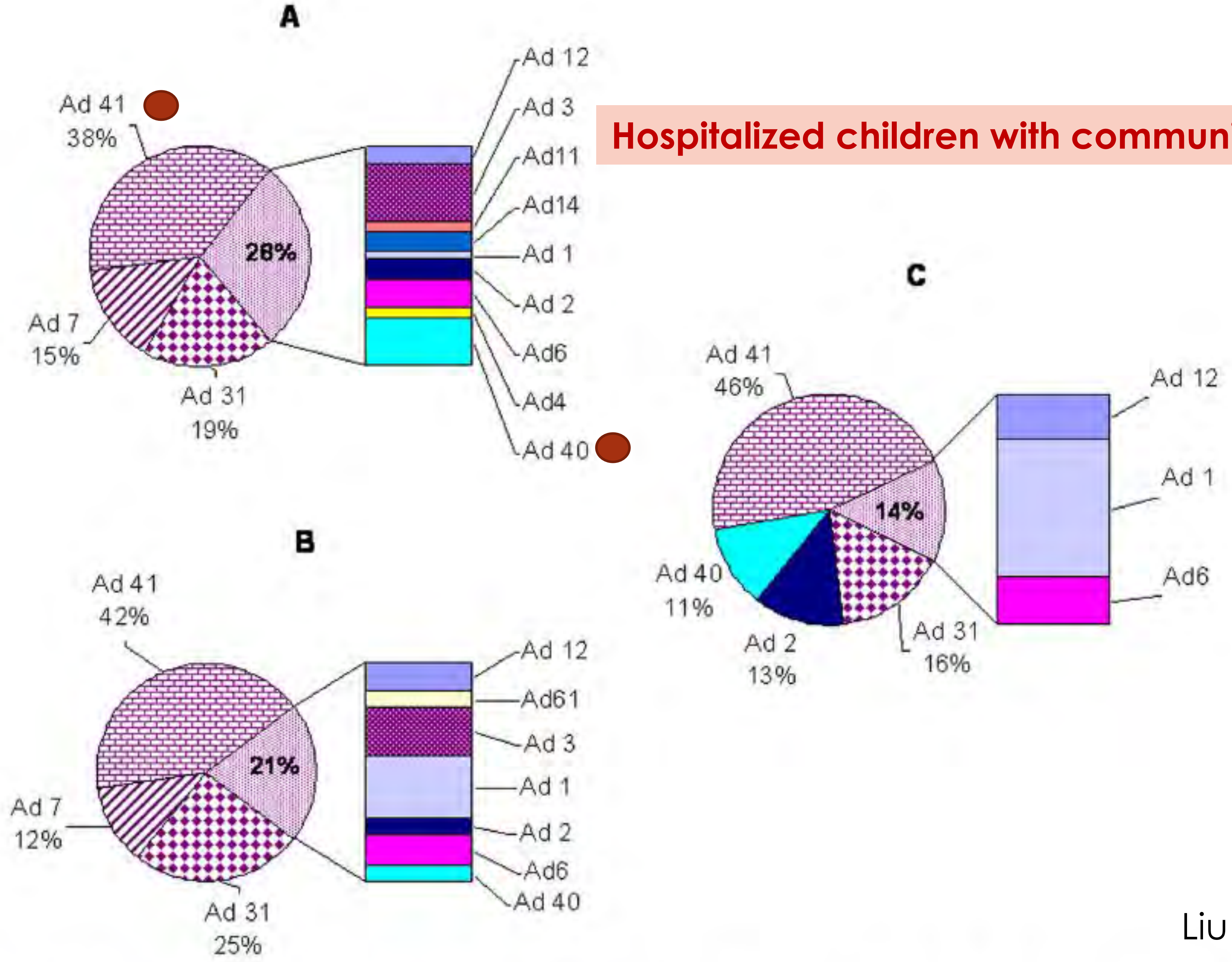
Robinson *et al.* 2013. Scientific Reports, 3:1812



Liu *et al*. 2014. *PLOS, 9:e88791*

Figure 1. The proportions of adenovirus types among children with acute diarrhea during 2011–2012. (A) The hospitalized children with CAD (IP-CAD). (B) The hospitalized children with HAD (HAD). (C) The pediatric outpatients for acute diarrhea (OP-CAD).
doi:10.1371/journal.pone.0088791.g001

Hospitalized children with community-acquired



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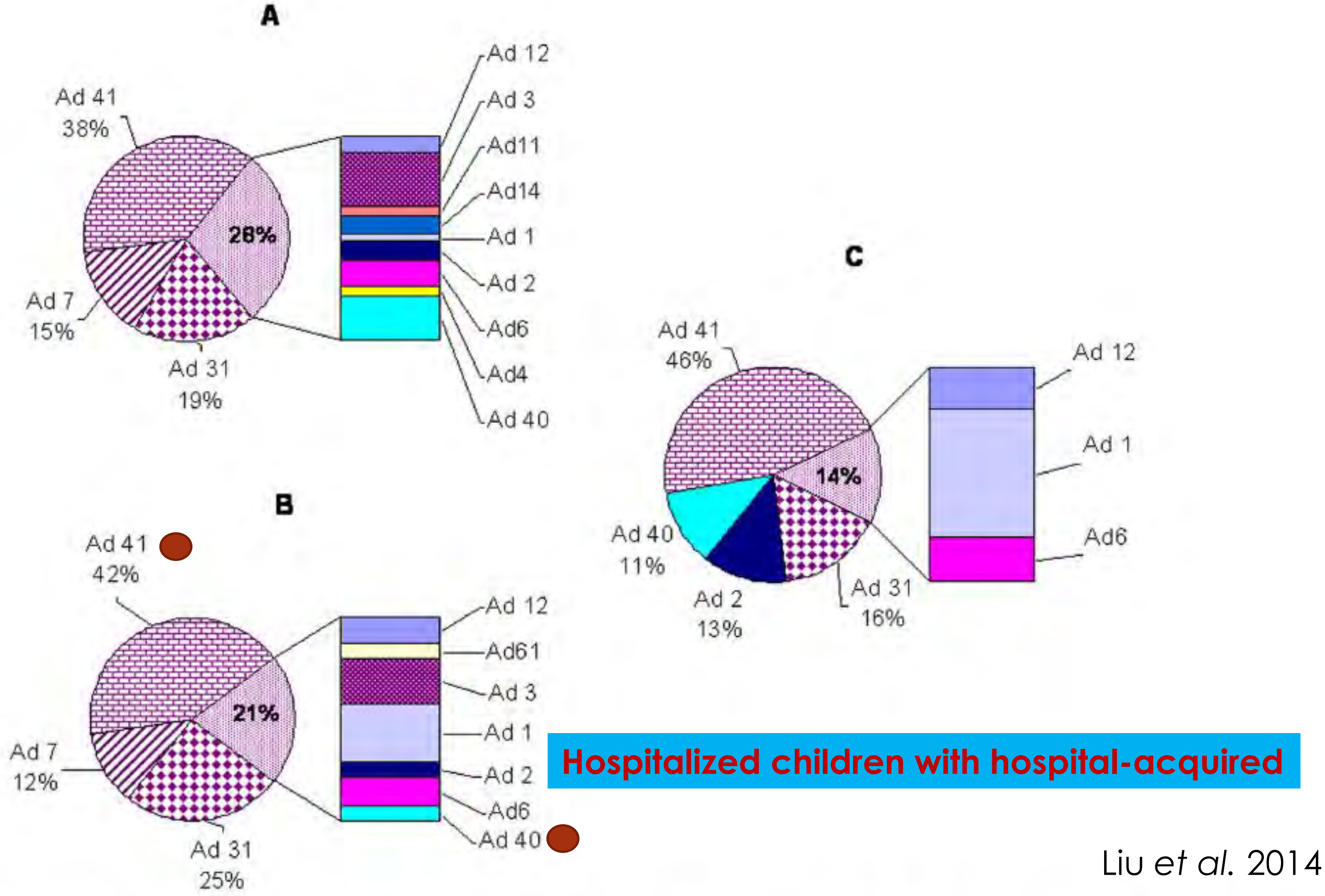

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Liu *et al.* 2014. *PLOS, 9:e88791*

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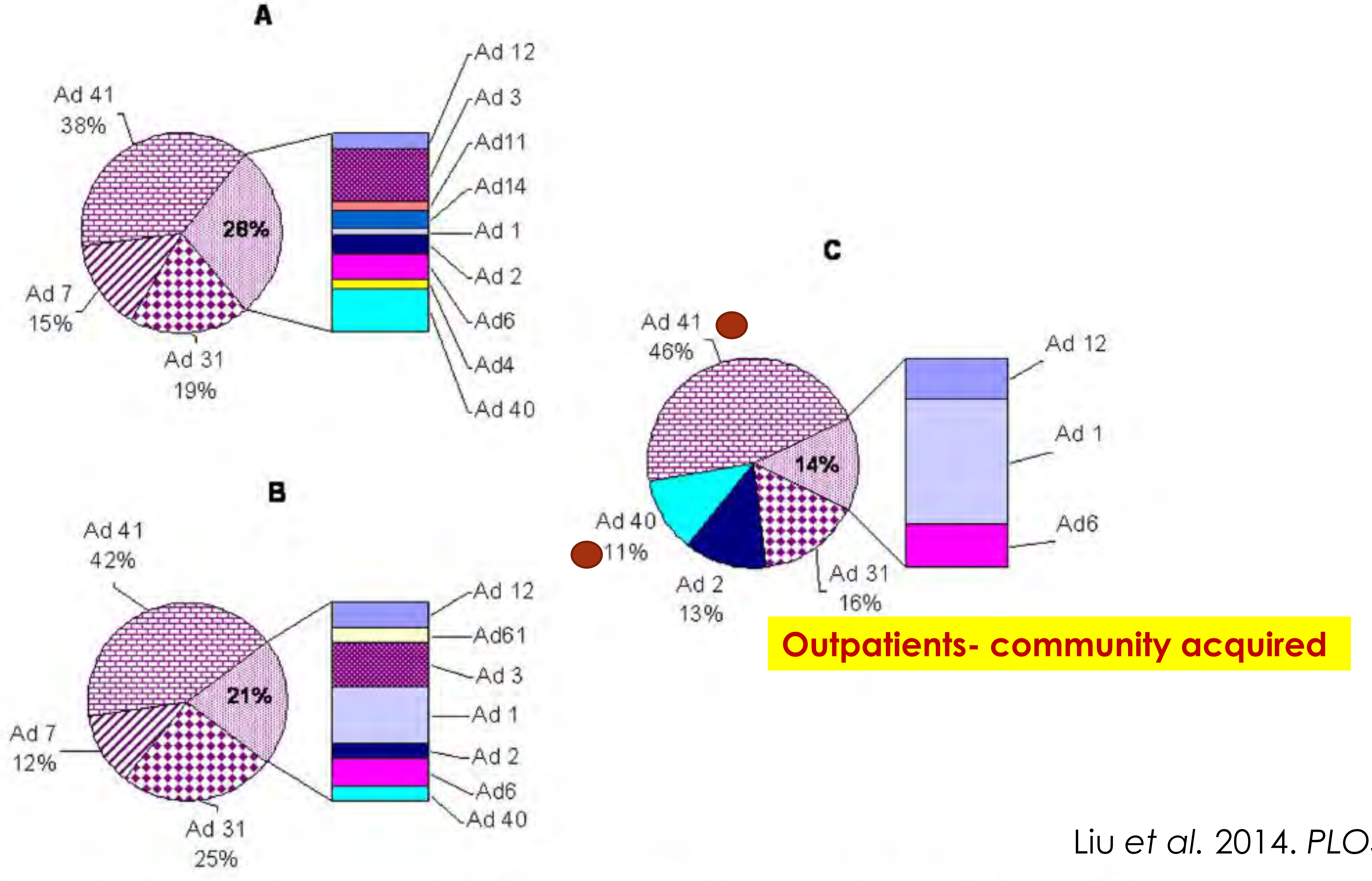

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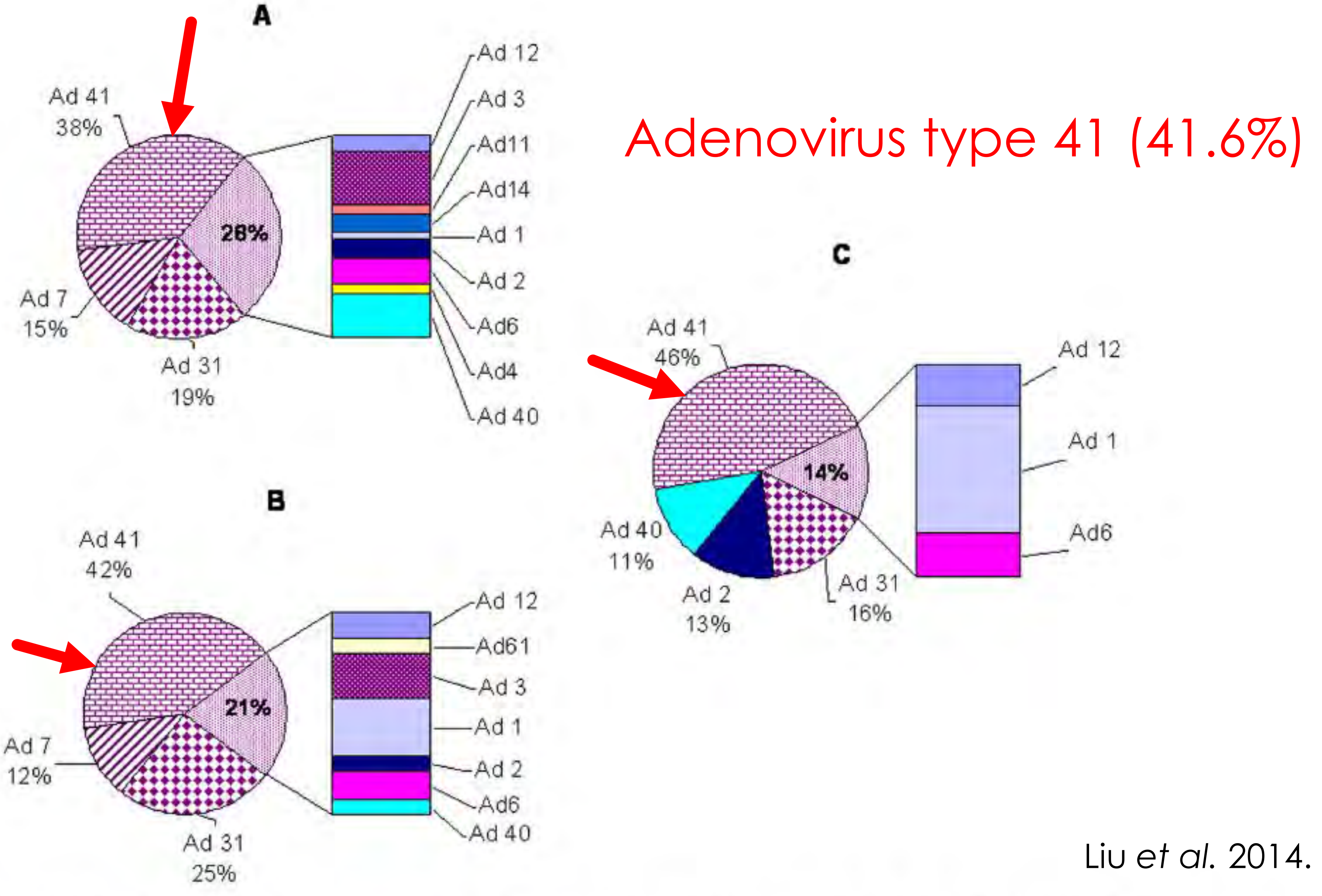

Adenovirus type 41 (41.6%)

Liu *et al.* 2014. *PLOS, 9:e88791*

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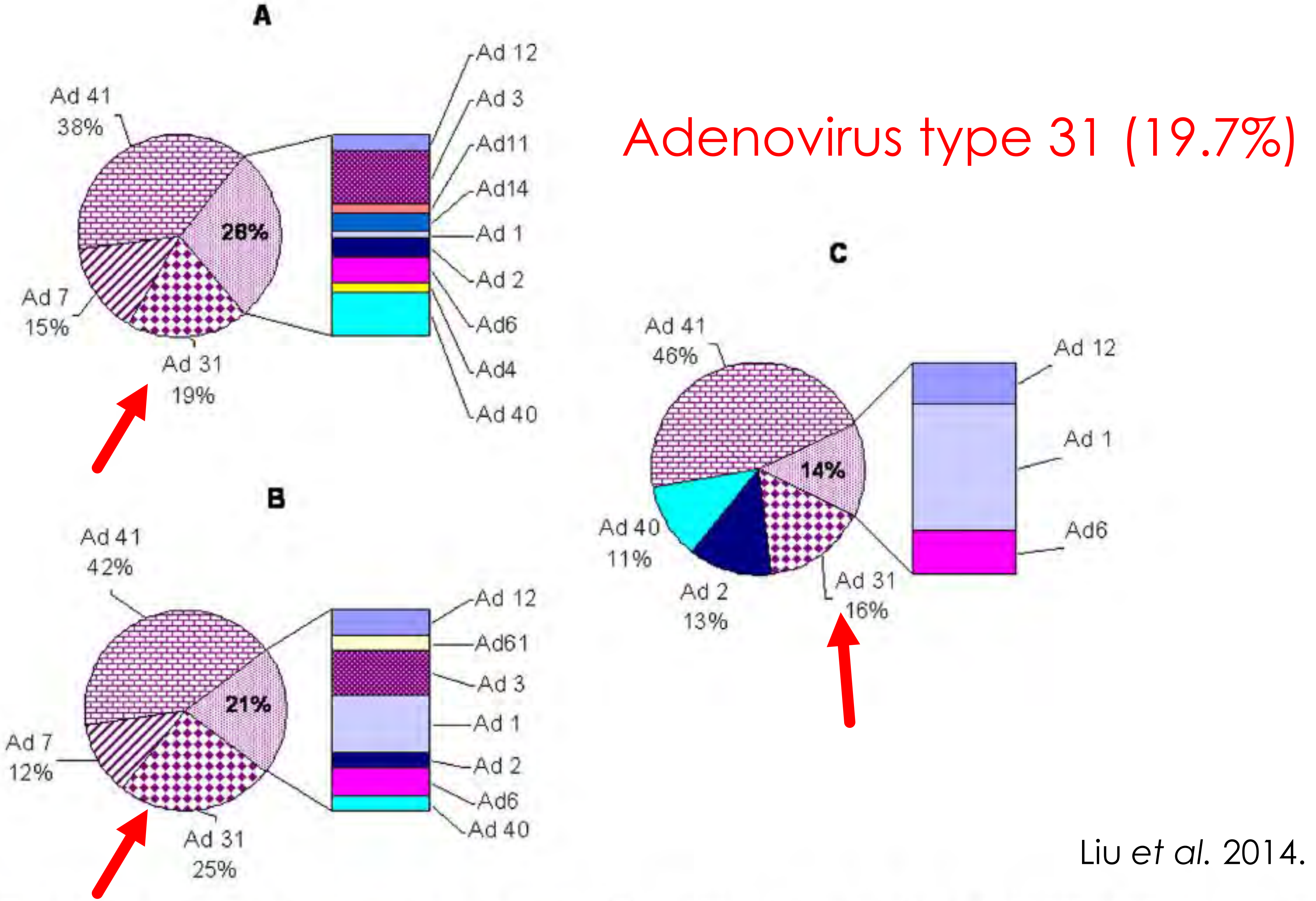
Adenovirus type 31 (19.7%)



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Molecular detection of adenoviruses

Table 1. Primers for Ad identification.

Target genes	Primers	Position	sequences(5′-3′)	length(nt)
Hexon	hexAA1885(+)	21–45	GCCSCARTGGKCWTACATGCACATC	301
	hexAA1913(−)	321–301	CAGCACSCCICGRATGTCAAA	
Fiber	fibF1(+)	396–426	ACTTAATGCTGACACGGGCAC	541(Ad40)
	fibF2(−)	1006–1028	TAATGTTTGTGTTACTCCGCTC	586(Ad41)

note: R = A/G, K = G/T, S = C/G, W = A/T, I = Hypoxanthine.

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Established methods need to be updated to ensure coverage of newly identified types (based on implementation of genomic analyses)

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Enterovirus

Spread by the faecal-oral route

Known to cause hand, foot and mouth disease and herpangina

Complications including:
Meningitis, encephalitis, acute flaccid paralysis, acute cardiopulmonary,
respiratory infection, and myocardial injury

Enterovirus

Diarrhea is less prominent

- low prevalence and self-limiting nature

Enterovirus is not often included in a pathogen screen (before multiplexing PCR)

Epidemiologic studies of gastroenteritis

- Have shown a clinical correlation with detection

- Most cases were mild without severe symptoms

Prevalence studies of enterovirus

Japan, 1985-99

13 321 patients with gastroenteritis

Echoviruses accounted for 625 (4.7%) of infections

Infectious agents surveillance report in Japan, 1985 to 1999, Tokyo, Japan Administration of Health and Welfare

2008-12, India

Children < 9 years (n = 2,330)

Table 1
Year-wise analysis of diarrhea samples positive for enterovirus and rotavirus.

Culture RT-PCR (degenerate primers)

Year	Total patients	Rotavirus- positive patients	No. EV-positive patients (%)		
			EV-RD- Positive (%)	EV-PCR-positive (%)	EV-PCR-negative
2008	584	60 (10.27)	111 (19.00)	91 (81.98)	20 (18.02)
2009	452	51 (11.28)	88 (19.47)	70 (79.55)	18 (20.45)
2010	564	86 (15.25)	106 (18.79)	77 (72.64)	29 (27.36)
2011	508	107 (21.06)	54 (10.63)	40 (74.07)	14 (25.92)
2012	222	18 (8.1)	21 (9.45)	16 (76.19)	5 (23.81)
Total (% average)	2330	322 (13.82)	380 (16.31)	294 (77.37)	86 (22.63)

Of 300 randomly selected diarrhea stool samples, which were negative for EV in cell culture, examined directly by RT-PCR using primers specific for CV-As, 8 were positive suggesting association of NPEVs with additional 2.7% of the diarrheal cases. Note that about 86 (22.63%) of the RD-positive isolates were negative for VP1 RT-PCR using the primer sets.

Rao *et al.* 2013. *Infect., Genetics and Evol. 17:153*

2008-12, India

Children < 9 years (n = 300)

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Rotavirus
13.8%

Enterovirus
16.3%

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Rotavirus
13.8%

Enterovirus
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77.4% confirmed
by
RT-PCR
Broad specificity

Rao et al. 2013. Infect., Genetics and Evol. 17:153

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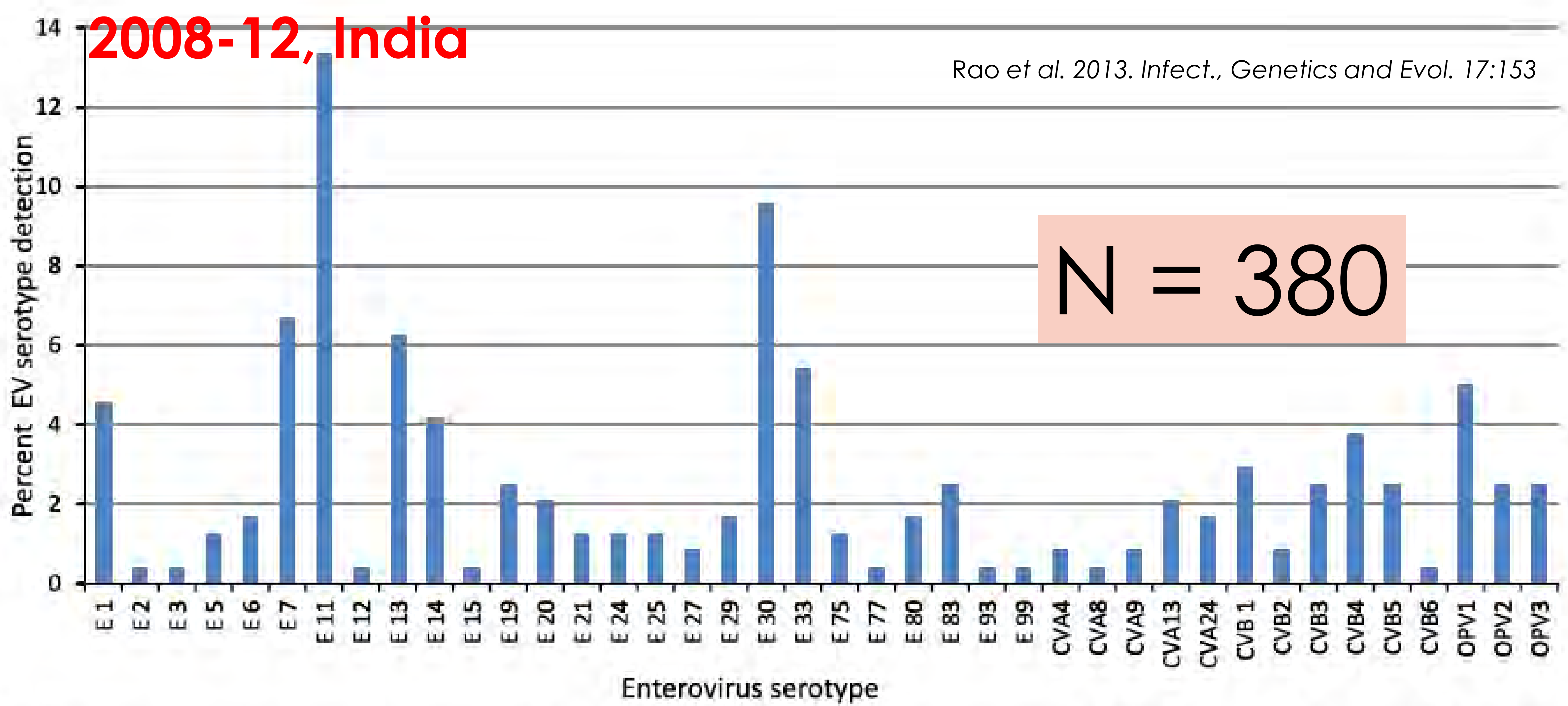
Rotavirus
13.8%

Enterovirus
16.3%

Serotyped (genotyped) according to the VP1 sequence analysis

Rao *et al.* 2013. *Infect., Genetics and Evol. 17:153*



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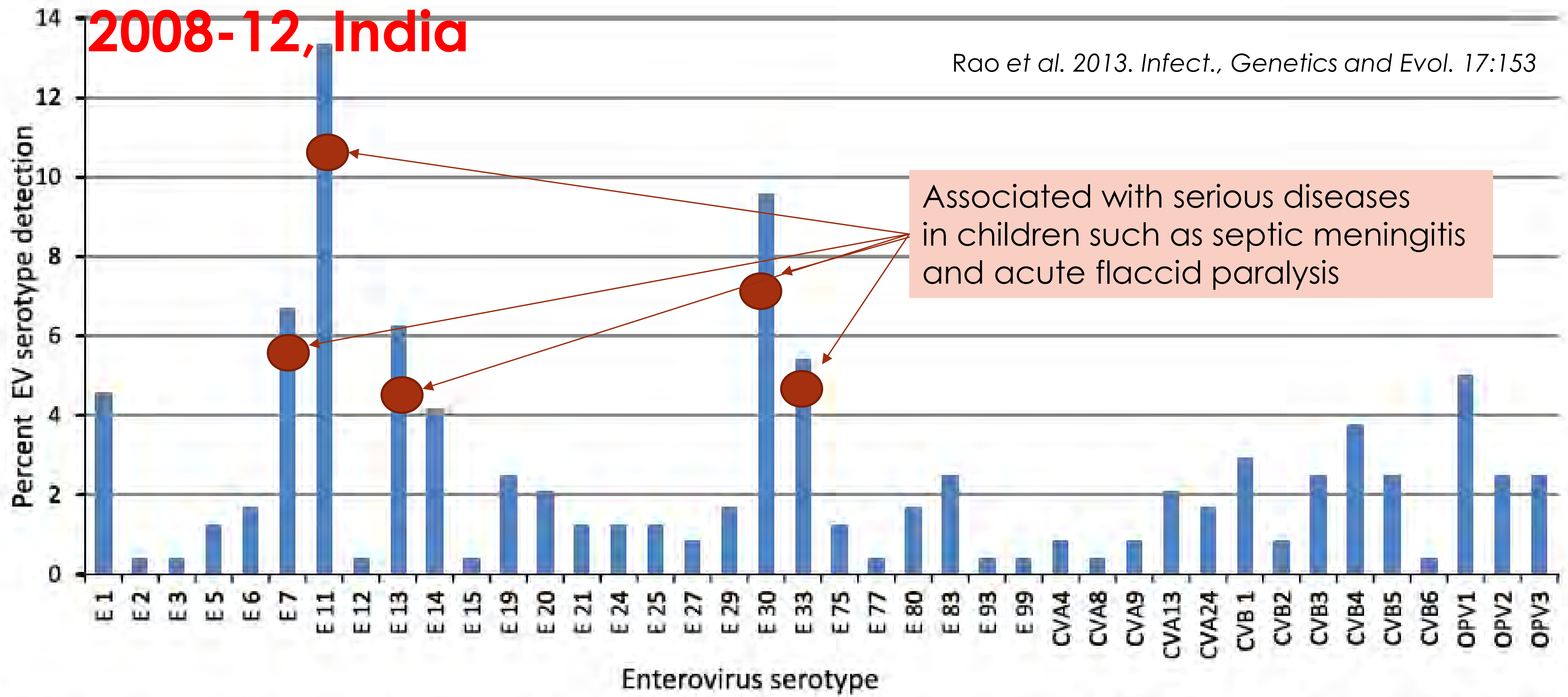
2008-12, India

Rao et al. 2013. *Infect., Genetics and Evol.* 17:153



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Non-polio acute flaccid paralysis: 66 serotypes, EV71, E13 and CBV5 frequently detected
In contrast, only 37 serotypes besides the 3 OPV types are detected in diarrheal children

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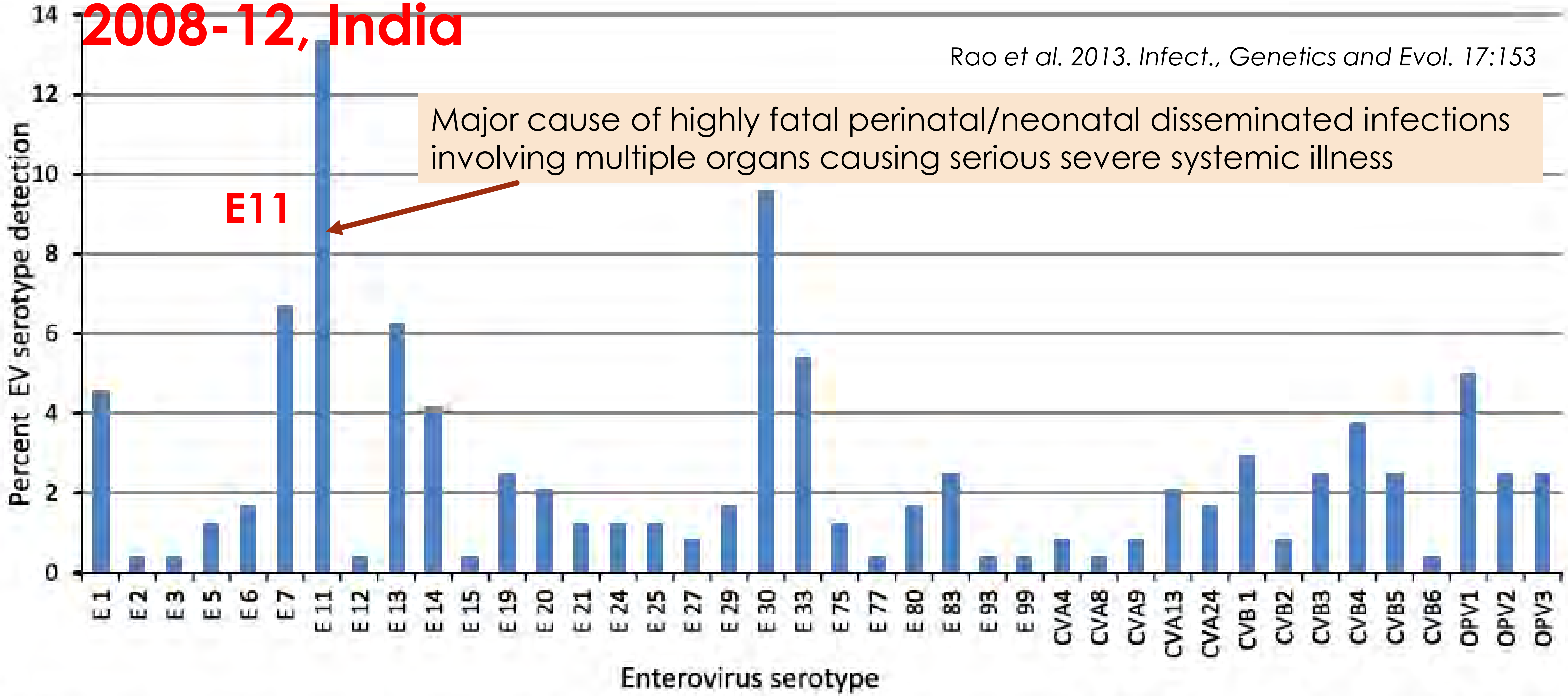
Rao et al. 2013. *Infect., Genetics and Evol. 17:153*



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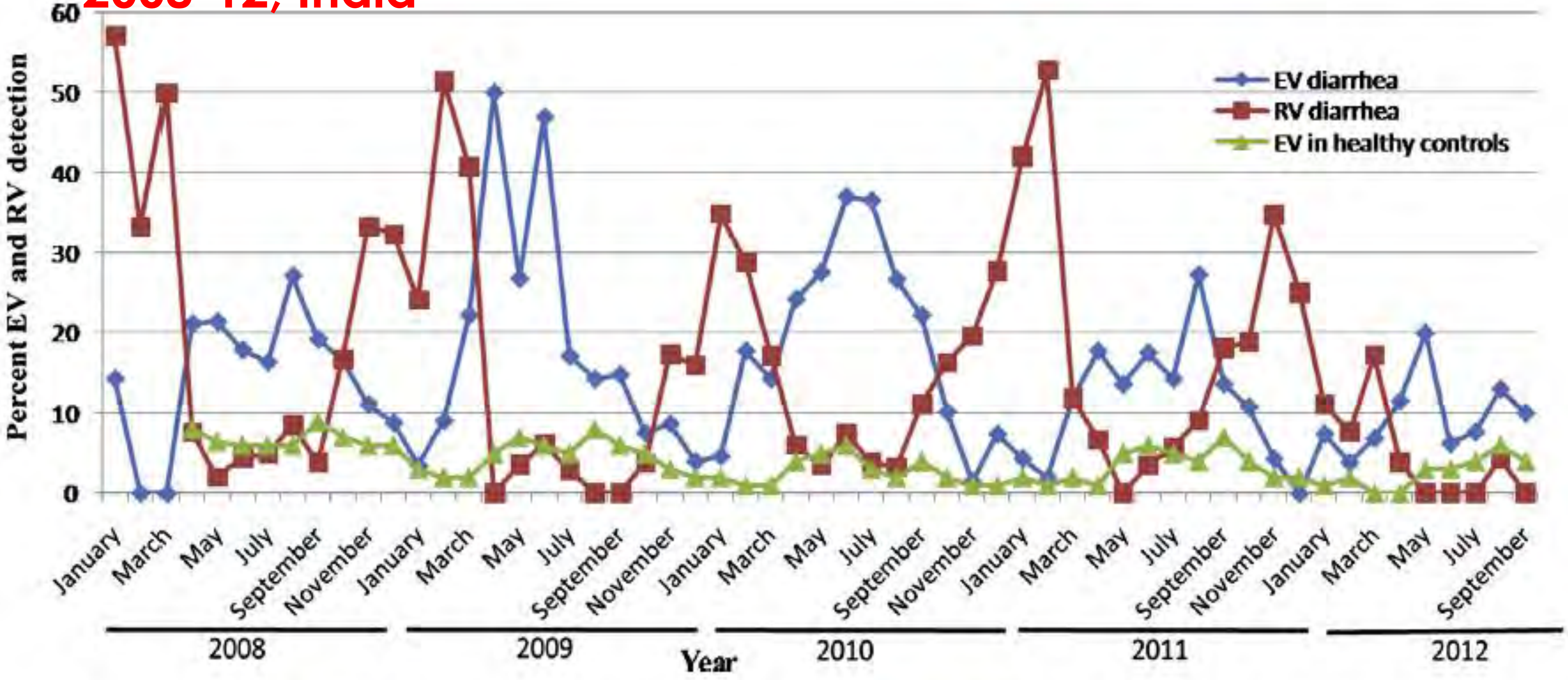


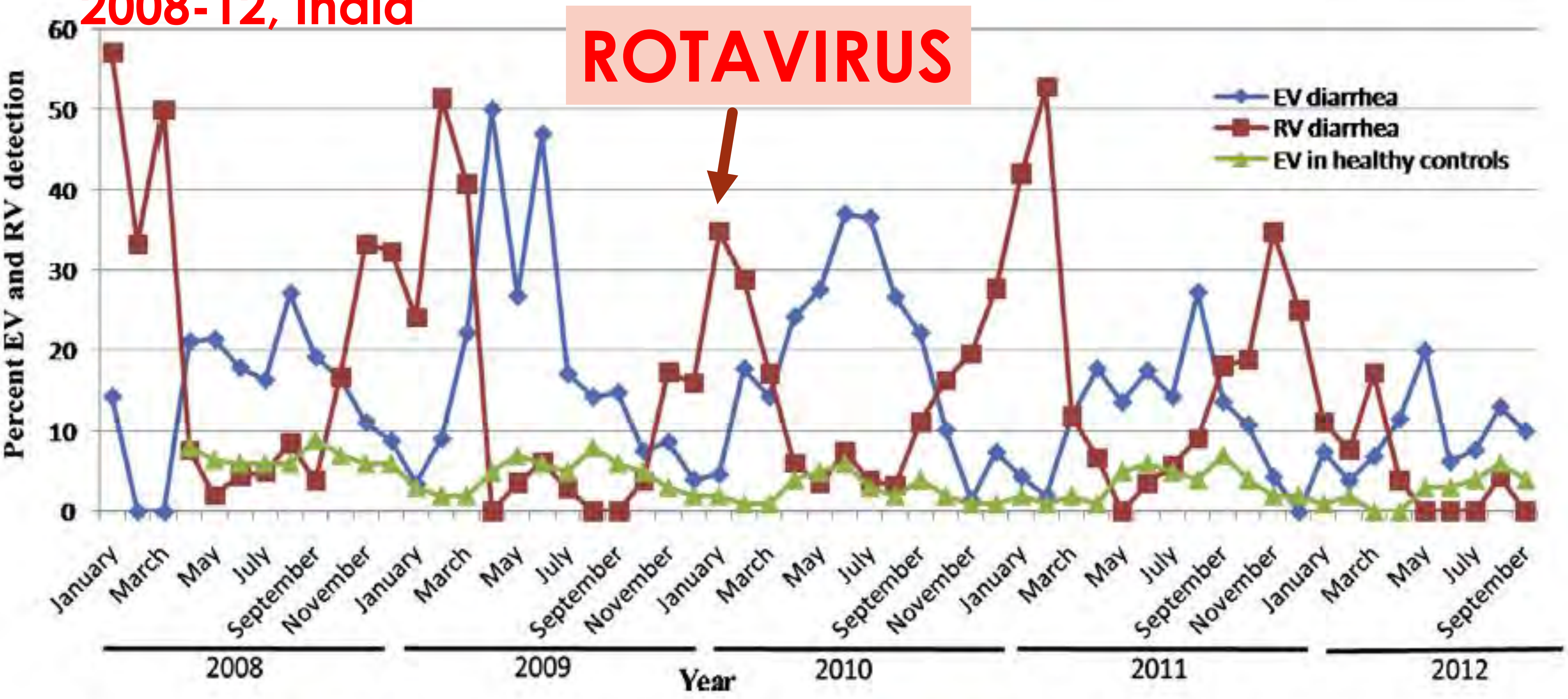
Fig. 2. Long-term analysis of the contrasting seasonal predominance of enterovirus and rotavirus diarrhea.

Enterovirus-positive diarrhea samples

- 0.64% co-infection with rotavirus
- Diarrheagenic *E. coli: 2%*

Rao et al. 2013. Infect., Genetics and Evol. 17:153

2008-12, India



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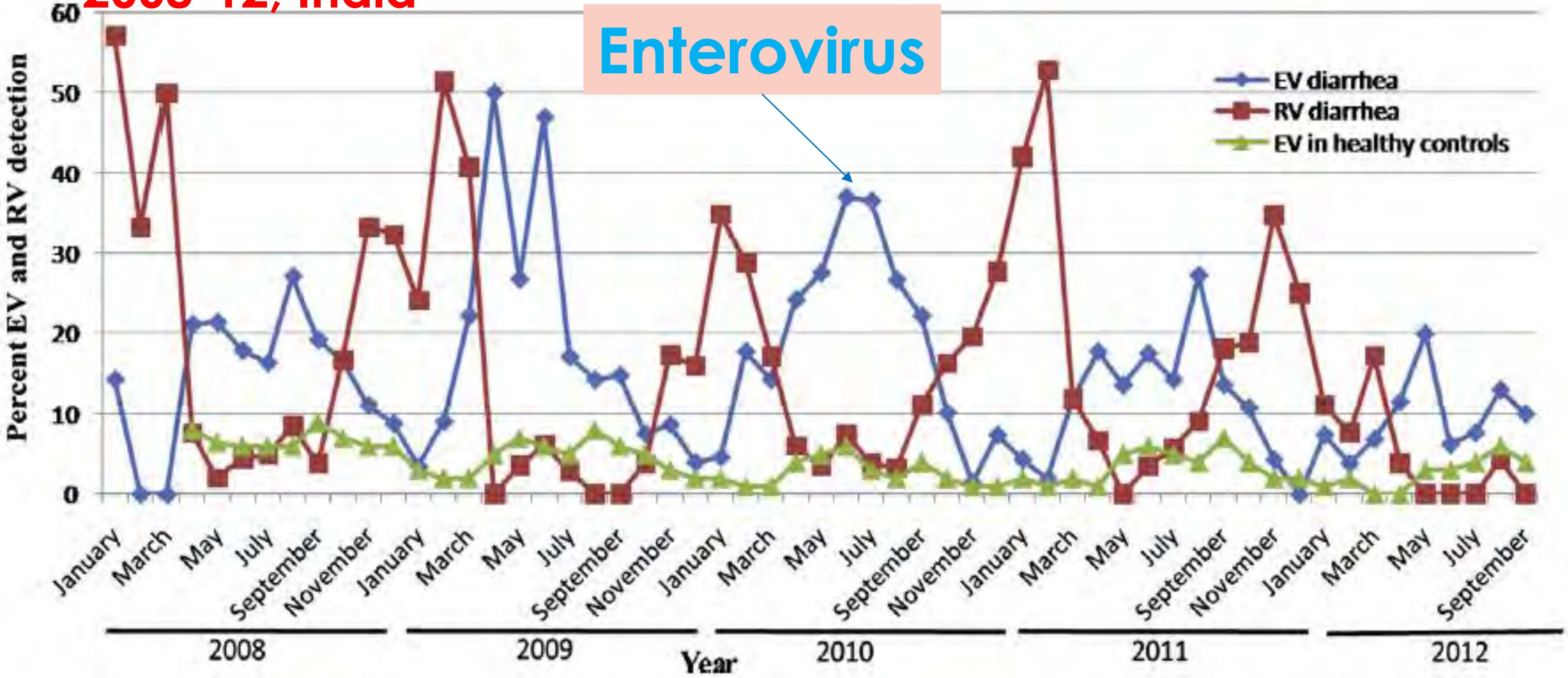

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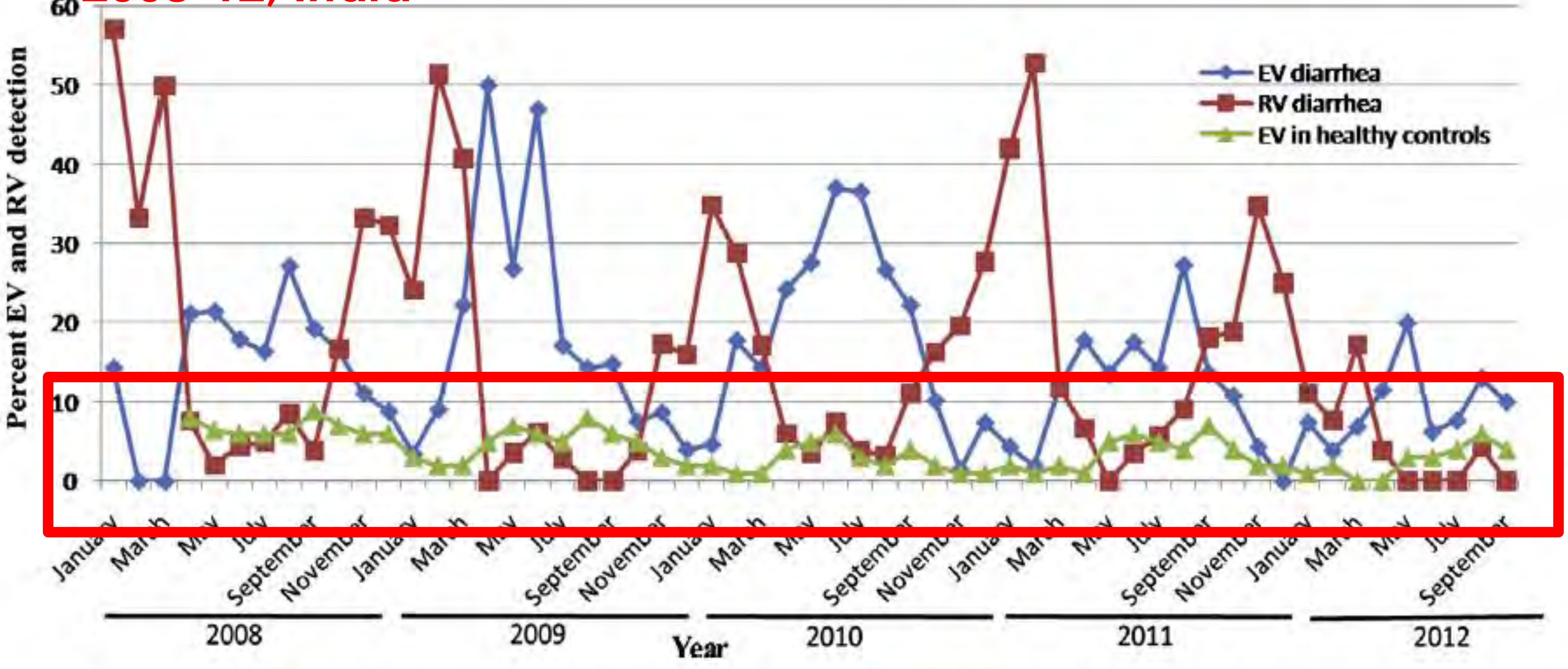

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Rao et al. 2013. Infect., Genetics and Evol. 17:153

Guangdong, China 2009-2012

1778 enterovirus-related hand, foot, mouth disease/ herpangia

Serotypes identified in 763 cases (with and without diarrhea)

Serotypes tend to reflect circulating strains

No strong association of diarrhea
with specific strains

Zhou *et al.* 2016. BMC Infect Dis, 16:128

Molecular detection of enteroviruses

VP1 Target

Study by Rao et al. 2012
4 sets of species-specific degenerate primers

Rao et al. 2013:
Used VP1 region (above)and 5'UTR using 2 sets of species-specific degenerate primers:

3 degenerate primers

- Forward: Specific for 4 enterovirus species
- Reverse primer for species A and B
- Reverse primer for species D

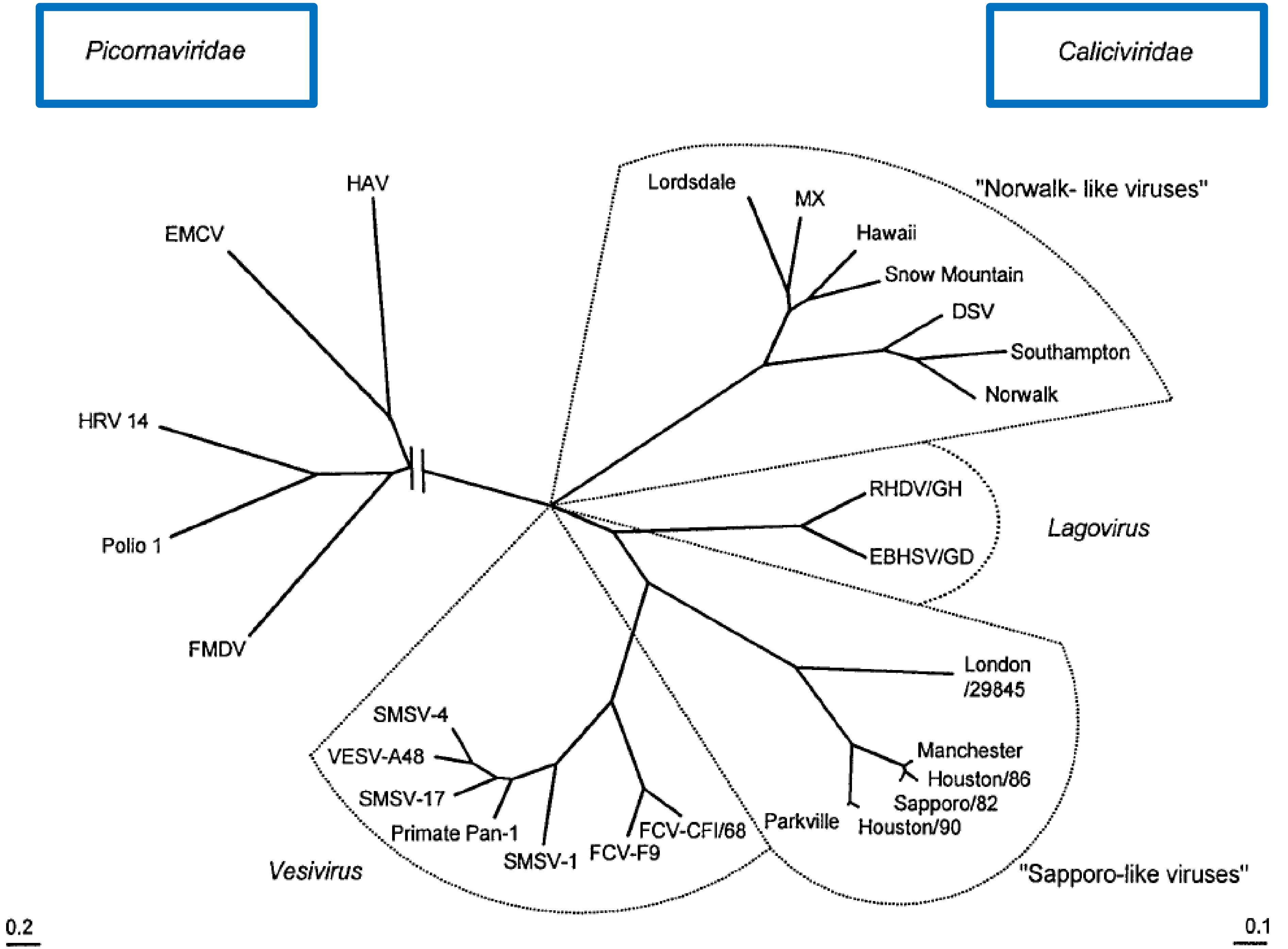
Sapovirus

First identified in outbreak in Sapporo, Japan in 1977

Responsible for gastroenteritis in all ages in both outbreaks and sporadic cases world-wide

Clinical symptoms are indistinguishable from noroviruses

Increasing reports of infections (particularly in SE Asia) suggest a rise in prevalence and virulence


Picornaviridae
Caliciviridae
HAV
EMCV
HRV 14
Polio 1
FMDV
Lordsdale
MX
Hawaii
Snow Mountain
DSV
Southampton
Norwalk
"Norwalk- like viruses"
RHDV/GH
EBHSV/GD
Lagovirus
London
/29845
Manchester
Houston/86
Sapporo/82
Houston/90
Parkville
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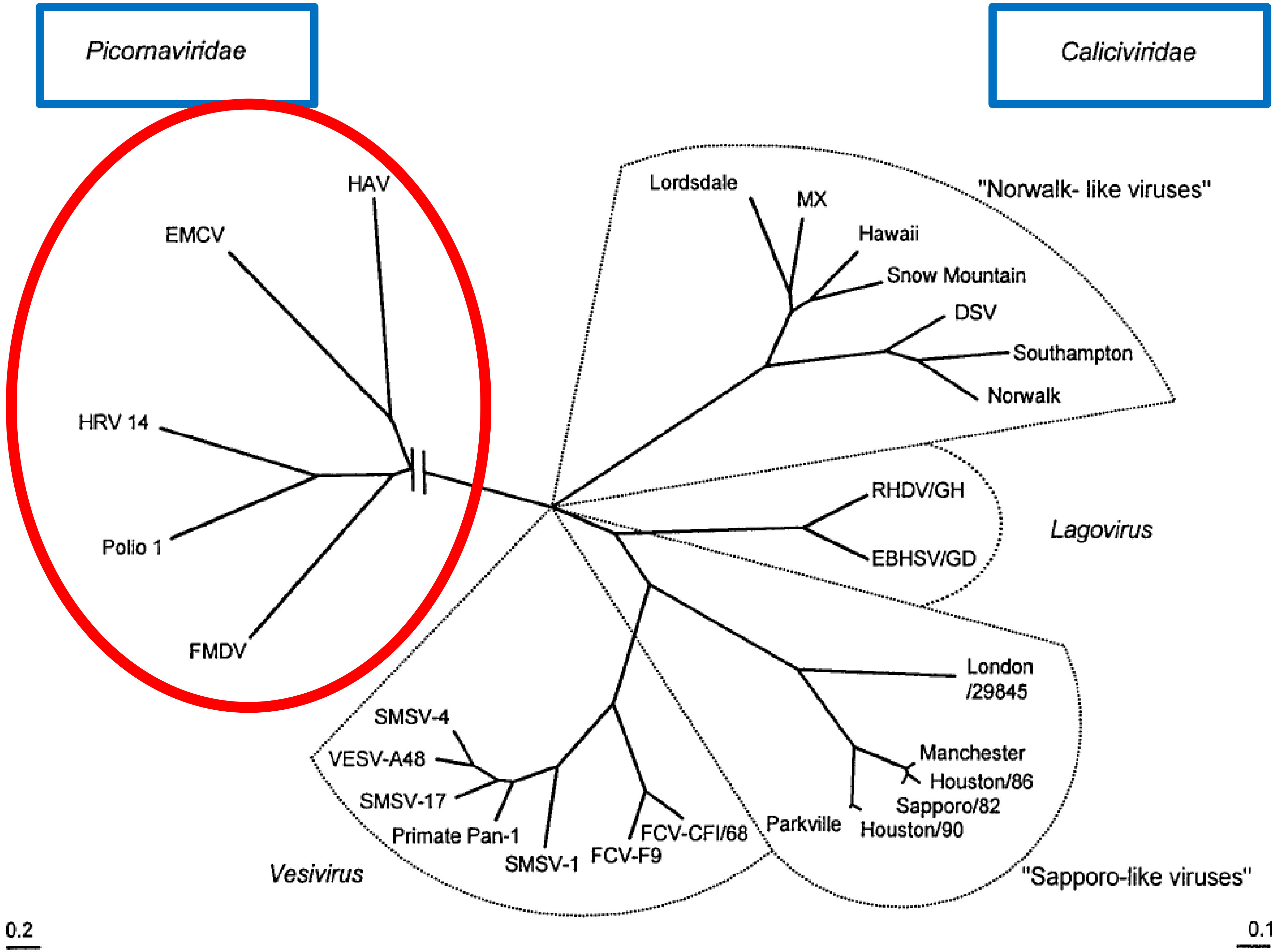
SMSV-4
VESV-A48
SMSV-17
Primate Pan-1
SMSV-1
FCV-F9
FCV-CFI/68
Vesivirus
0.2
0.1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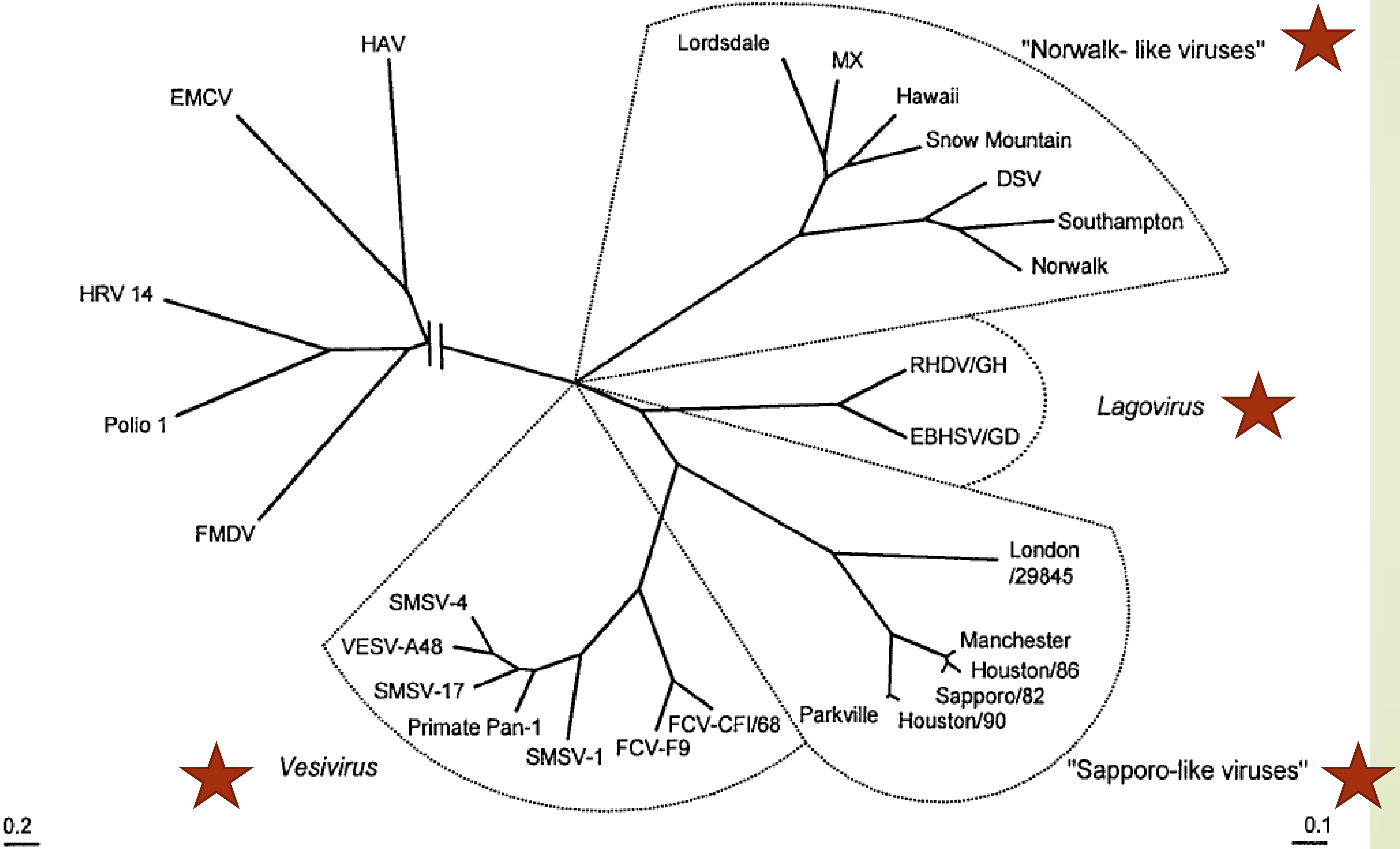

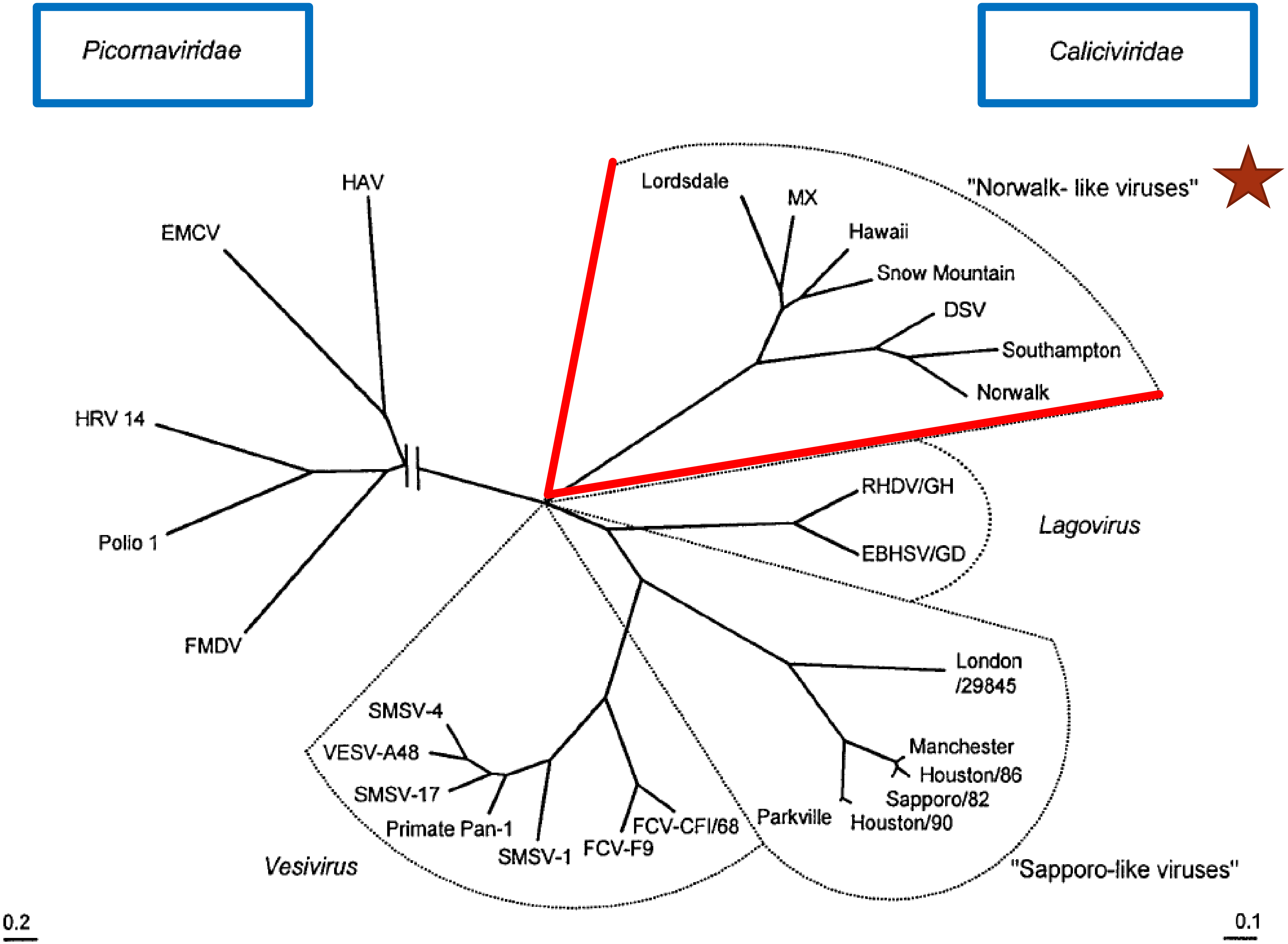

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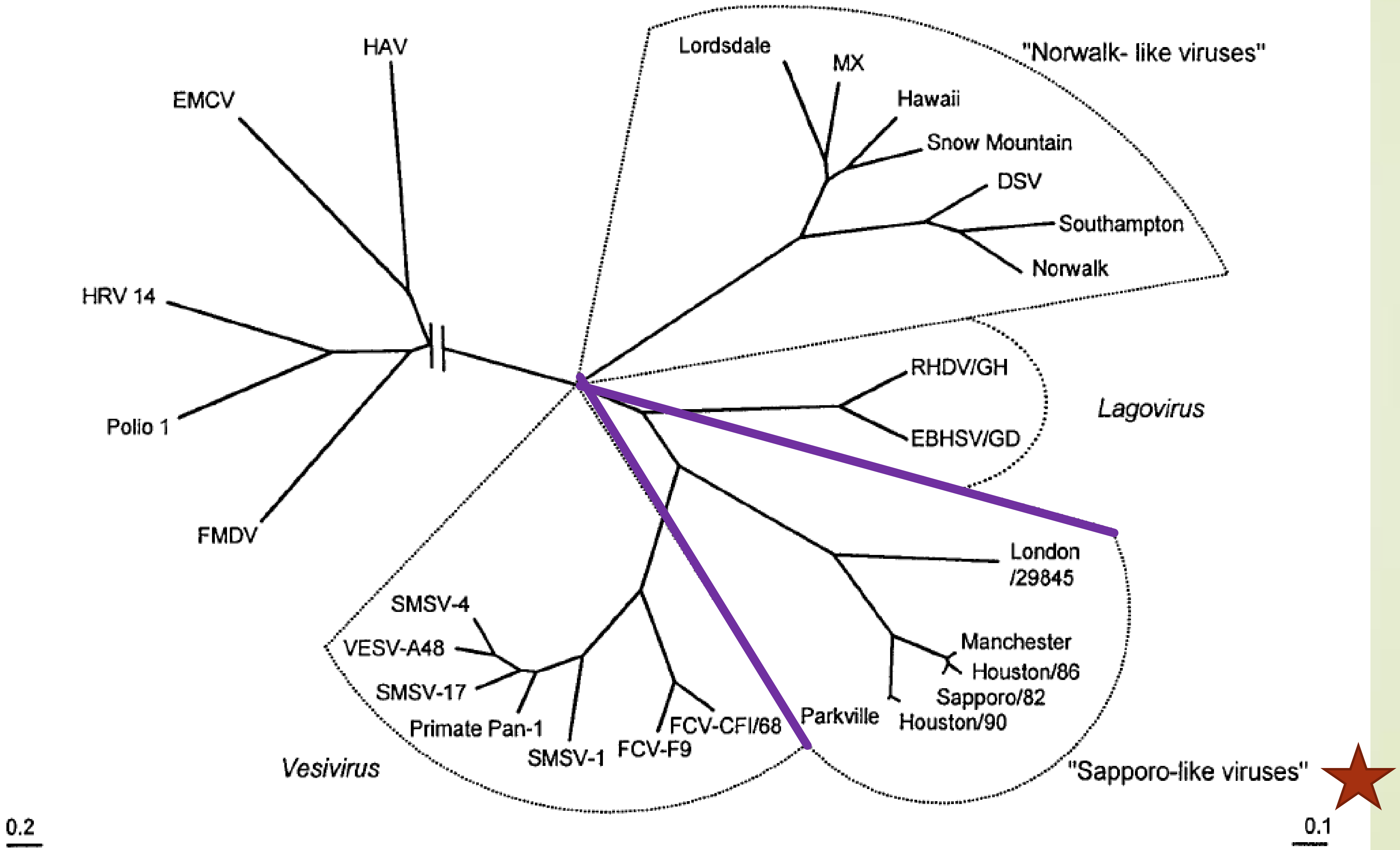
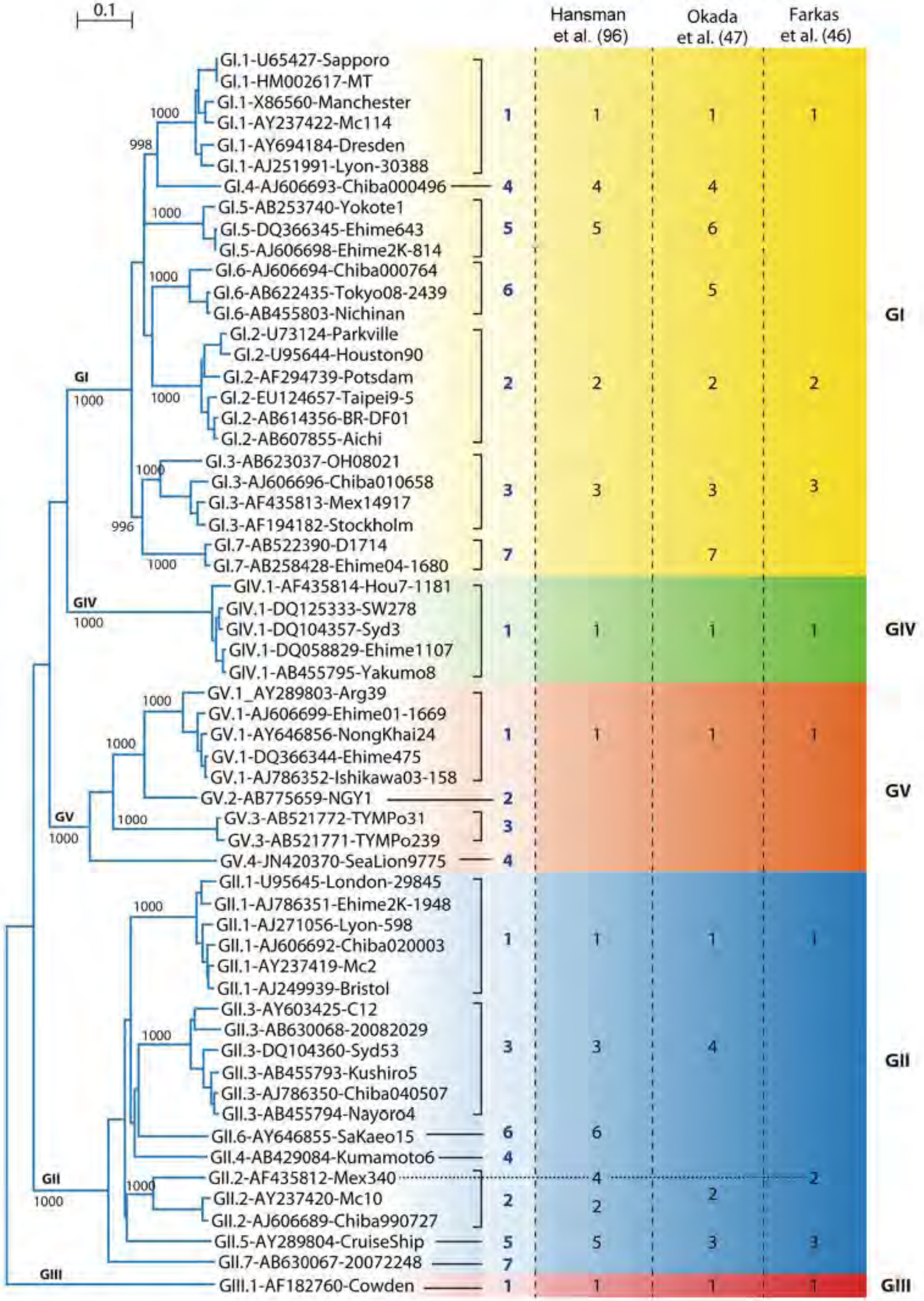


Genogroups



Genotypes

GI.1 – GI.7

GIV.1

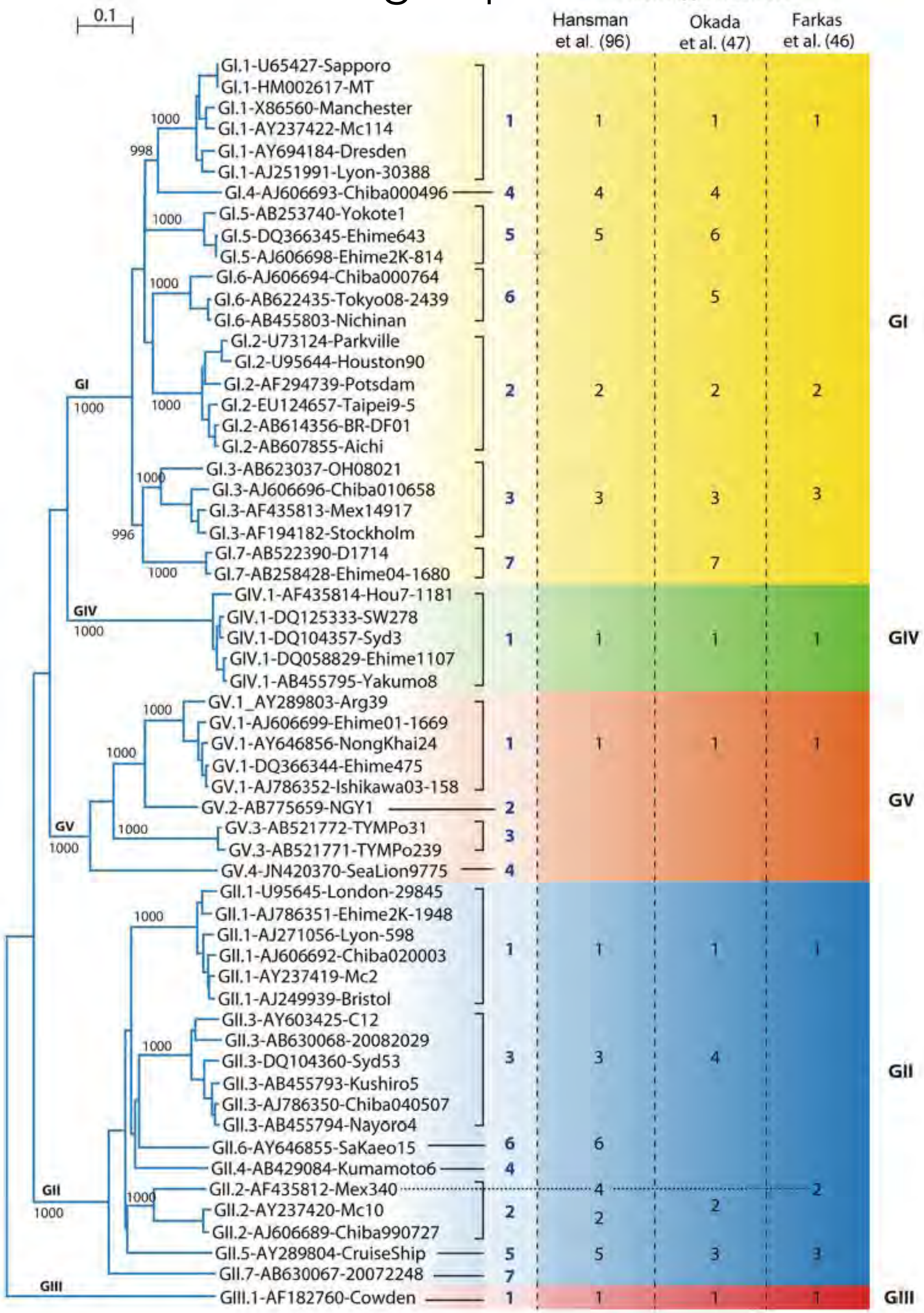
GV.1 and GV.2

GII.1 – GII.7

Porcine

Oka *et al.* 2015. CMR, 28:32

Genogroups



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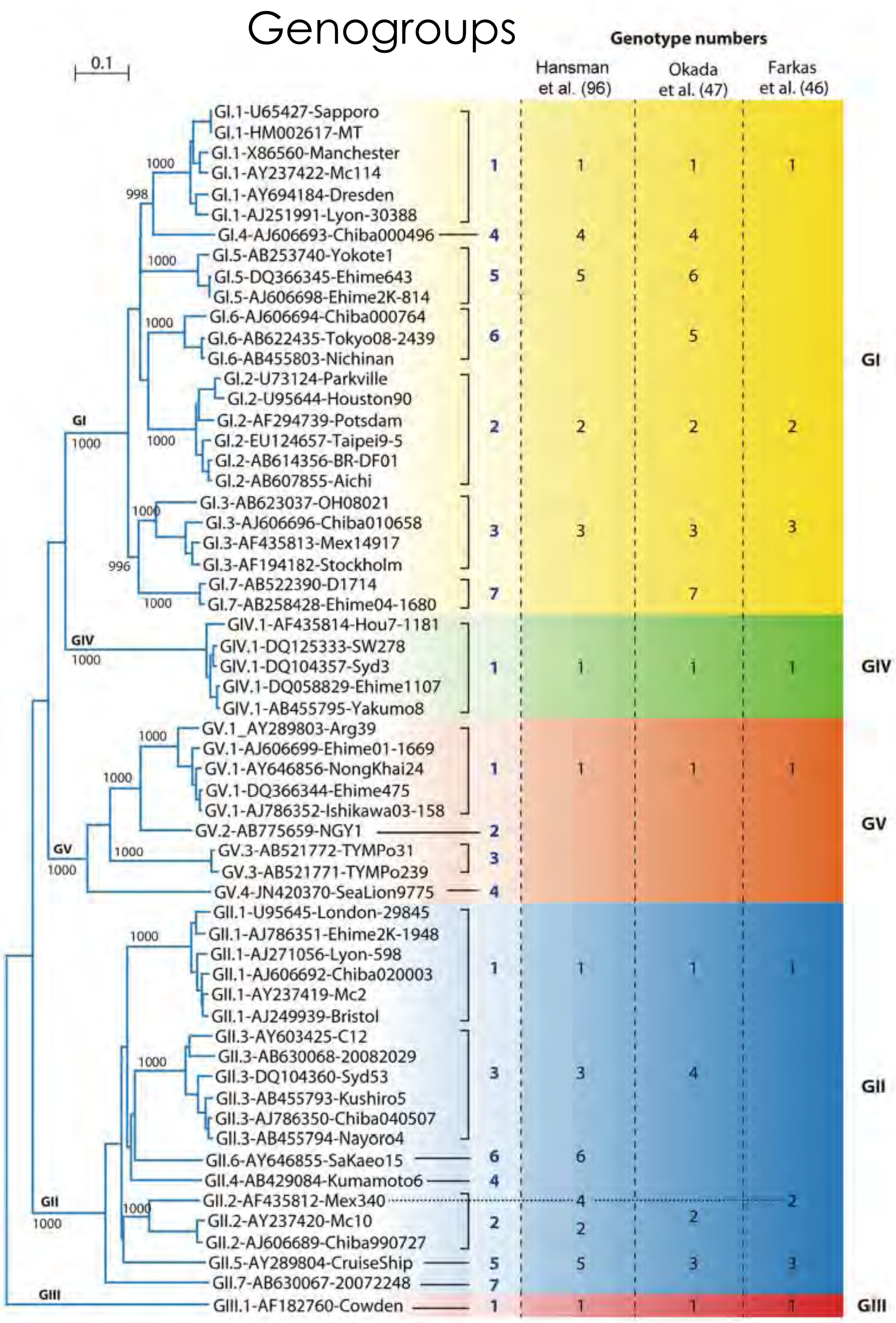

Genotypes

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Sapovirus: local prevalence study

95 stool samples < 18 years (Sydney Children's Hospital)

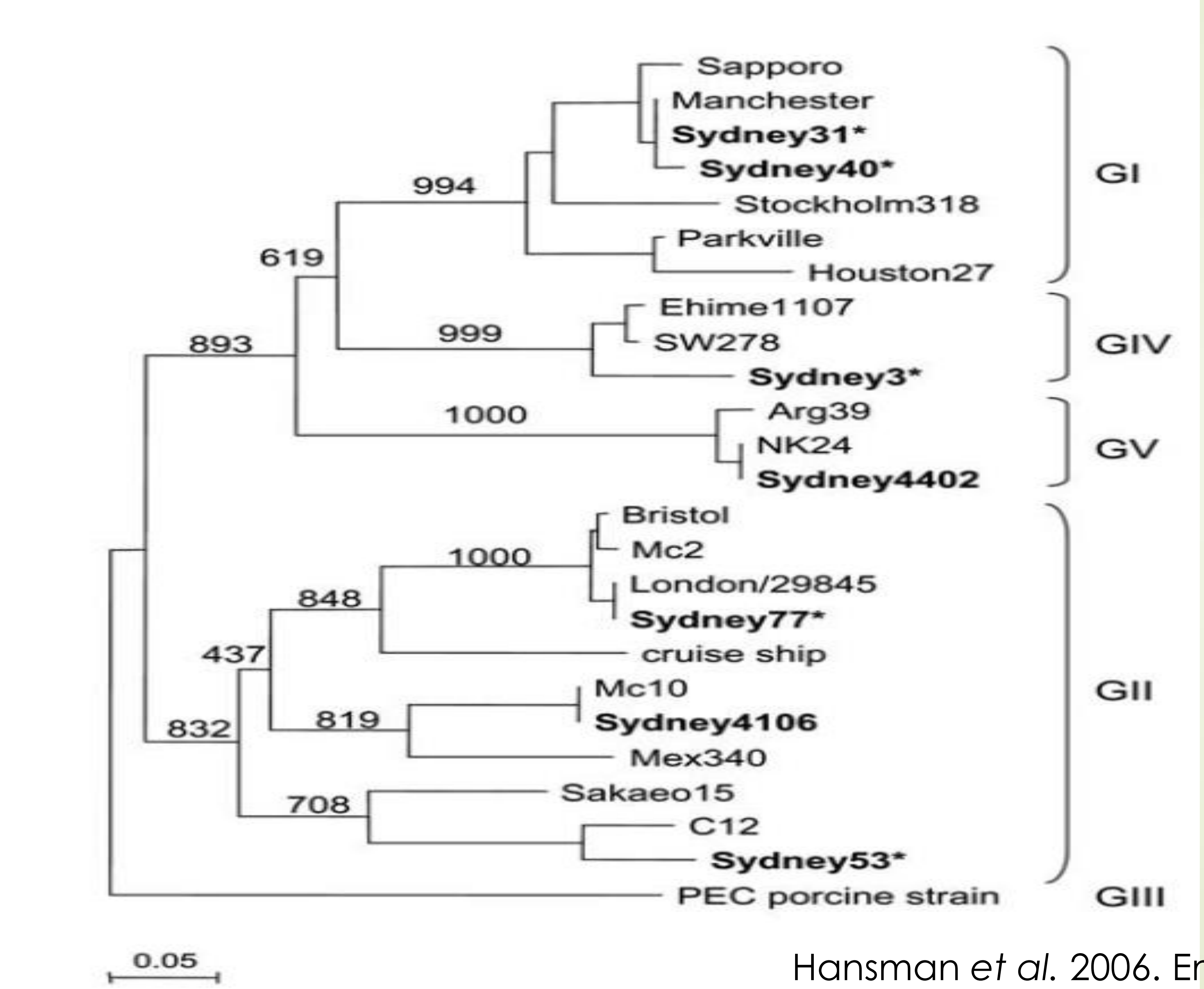
Negative: Salmonella, Shigella and Campylobacter,Rotavirus, Adenovirus, Astrovirus and Norovirus

Sapovirus detected in **7/95 (7.4%)** stool samples 9mths - 7 years

Other studies in Australia in 1998, 2001, and 2005:
0.32-0.56% of the total caliciviruses detected

Hansman *et al.* 2006. Emerg Infect Dis., 12:141

Sapovirus: local prevalence study



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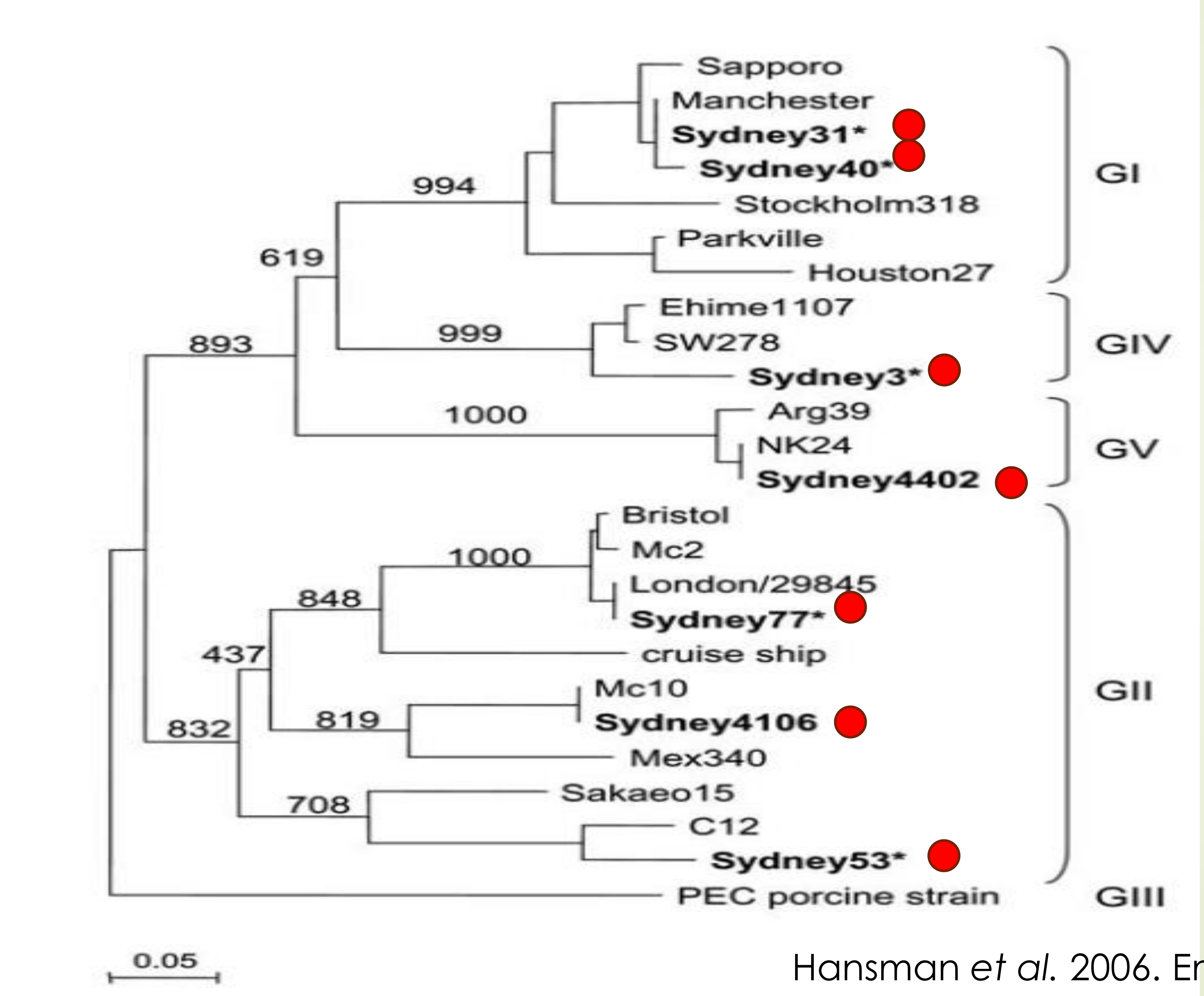


Hansman *et al.* 2006. Emerg Infect Dis., 12:141

Molecular detection of sapovius

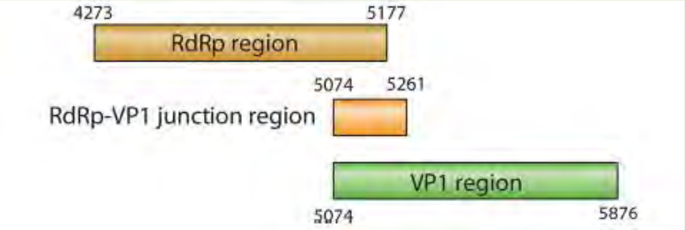
Reverse transcription-PCR is a major and routine method for detection in clinical specimens

Due to high diversity of sapoviruses, most assays include multiple or degenerate primers

Currently, limited primer sets have demonstrated the ability to detect all genogroups of human sapoviruses

*Oka *et al.* 2015. CMR, 28:32

Molecular detection of sapovius



Target	Number of methods cited*	
Partial RdRP	**11**	*Cross-react with norovirus, rotavirus and astrovirus*
RdRp-VP1 junction	**11**	*Have the highest detection rate, recommended for clinical testing*
Partial VP1	**9**	*Contains capsid coding region (preferred method, products can be sequenced for genotyping)*

*Oka *et al.* 2015. CMR, 28:32

Concluding remarks

Astrovirus, adenovirus, sapovirus, and enterovirus are common causes of gastroenteritis in Australia.

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These enteric viruses (exception of sapovirus)are now routinely screened in cases of gastroenteritis using capture enzyme immunoassays and molecular techniques including multiplex PCRs and allow epidemiologic surveillance of these putative agents.

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Established methods need to be updated periodically to ensure coverage of newly identified types.

Acknowledgements

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