

**We don't know why you're sick. It must be a virus...**

**Metagenomics may provide the answer**

**Dr Richard Hall**

**Clinical Virology (Virus Hunters)**

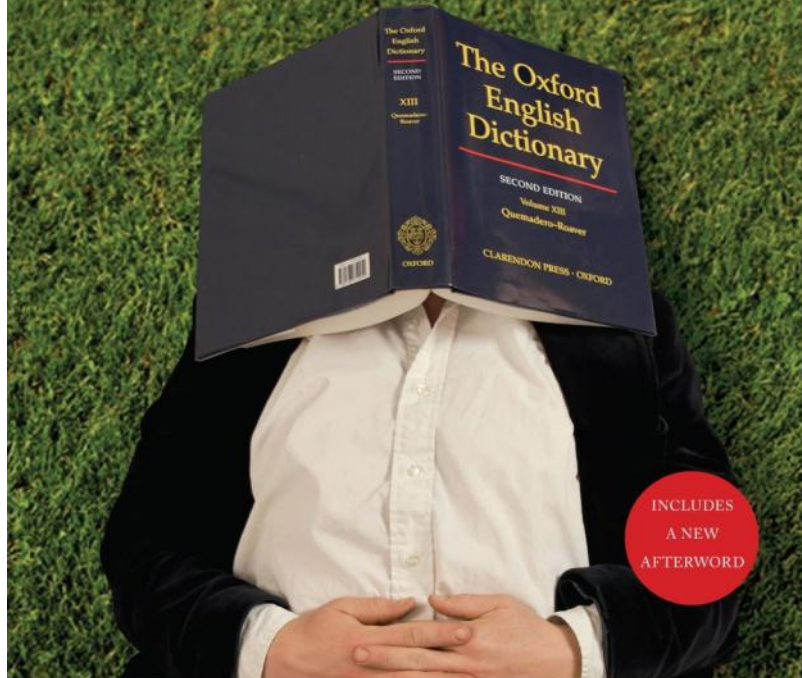
**Institute of Environmental Science & Research (ESR)  
at the National Centre for Biosecurity & Infectious Disease (NCBID), Upper Hutt, NZ.**

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# READING THE OED

ONE MAN, ONE YEAR,  
21,730 PAGES




"This is the *Super Size Me* of lexicography...Shea has walked the wildwood of our gnarled, ancient speech and returned singing incomprehensible sounds in a language that turns out to be our own." —Nicholson Baker, *The New York Times Book Review*

Ammon Shea

# apocryphal

Line breaks: apoc|ryph|al

Pronunciation: /ə'pɒkrɪf(ə)l  /

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## ADJECTIVE

- 1 (Of a story or statement) of doubtful authenticity, although widely circulated as being true:

*'an apocryphal story about a former president'*

*'his alleged description of opera as 'fat gits singing' is probably apocryphal'*

**abc 13 EYEWITNESS NEWS**

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NEWS

# MYSTERY RESPIRATORY VIRUS LIKELY TO HIT KIDS ACROSS COUNTRY

**3 NEWS** 16° 7° Auckland

HOME VIDEO NZ NEWS DECISION 14 WORLD

# Kiwifruit PSA virus spreads north

Wednesday 23 Nov 2011 10:08 p.m. Join the discussion



## Mystery virus ends Dobbo's boxing dream

BY NEIL REID

Last updated 05:00 13/06/2010

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TV star Howard Dobson has told how a double health check knocked him out of a fight for charity.

The TV3 sports presenter was having an MRI scan on his torn right bicep, suffered while training for a boxing match in Queenstown, when he was struck with a mystery virus.

"I knew I was in trouble. My blood tem

**The New Zealand Herald** Search keywords...

National Video Opinion Election 14 Business Tech World Sport Ent

Rugby refs, Warriors and female strikers. It's on The Vent with Tony Veitch...

# No need for panic over mystery virus, say officials

5:00 AM Thursday Apr 3, 2003

New Zealand

☆ f 0 t 0 in 0 g+ 0

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## Mystery virus sidelines Stoner

Updated at 7:42 am on 12 August 2009

The battle for motor cycling's MotoGP championship has been reduced to a two-man race between Australia's 2007 champion Casey Stoner to sit out the next three rounds of the

## Sir Paul McCartney in hospital battling serious mystery virus during tour in Japan

May 22, 2014 20:38 By Tom Bryant, Clemmie Moodie

His world tour gigs on May 18 and 19 were postponed as doctors ordered "complete rest" in his hotel room while he recuperated

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## Coronation Street

Coming Next Story So Far News Pictures Cast

## Michelle signed off Corrie with mystery virus

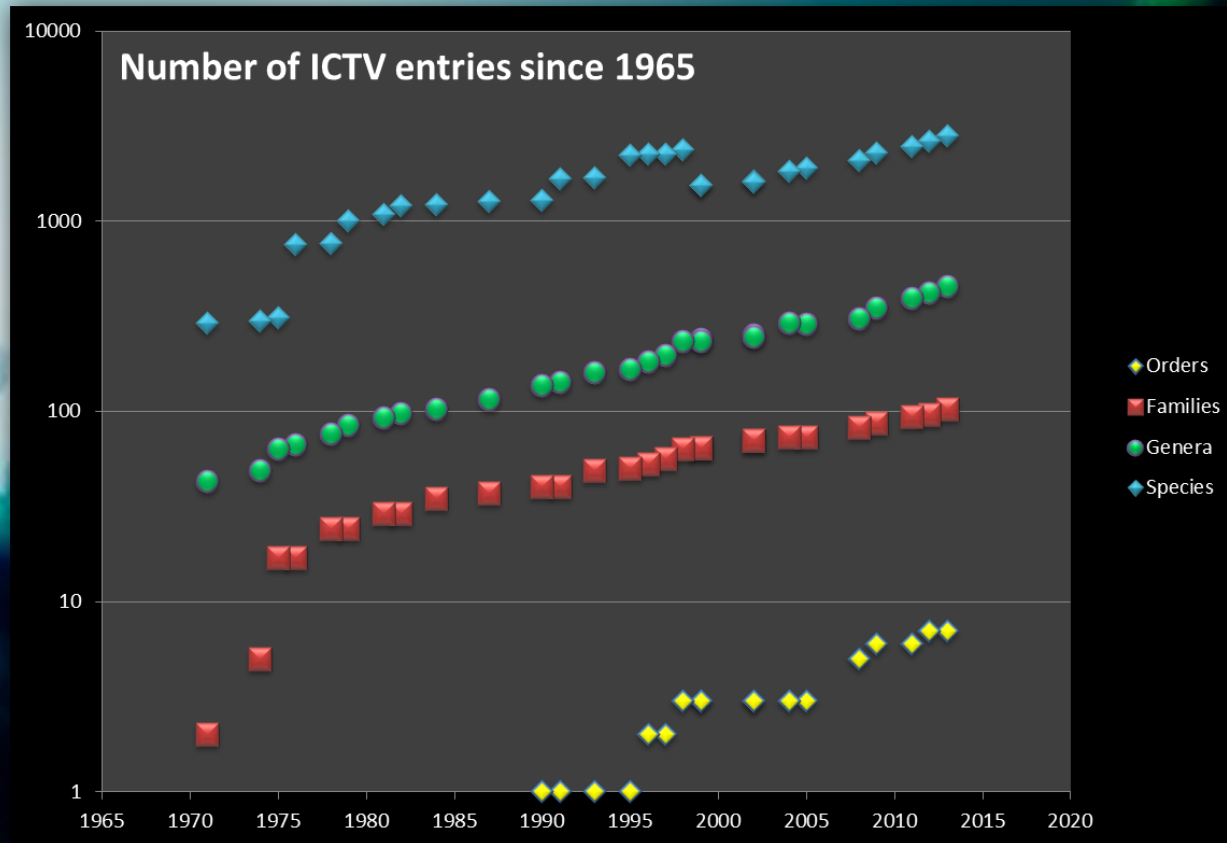
3:03pm, Friday, 19 April 2013



Coronation Street's Michelle Keegan has been signed off sick with a 'mystery' virus.



# Viruses : Discovery



“...estimate that there are a minimum of 320,000 mammalian viruses awaiting discovery...”

Anthony *et al.* (2013) *mBio* 4(5): e00598-13.

# Viruses : Detection

- Clinical acumen
- Pathology
- Epidemiology
- Viral culture
- Electron microscopy
- **qPCR**



# Metagenomic pipeline for virus discovery



## Submission to NGS platform



## Roche GS Junior



## Illumina MiSeq



## Oxford Nanopore (MinION)

**NCBI Genbank**

**171 million known sequences**

Mouse AGTCGIGTATATTAGATCTCTCGTCTATAGATCGCTGCTATC...  
Human GGTGCTCGCTATATAGCTCGCGCTCGCTCTGCT...  
E. Coli CCTCTGATGAGCTCTAGATATCGCTCGCGCGCTCGGCGCTATG...  
Bovine GGGTCGTATCCGCGCTCGCTATAGACTCTCGCGCGCGCT...  
Ovine GCTCGGCTATAGTTTTCGCTCTCTCGATAGCTCATCTGCATC...  
Ebola GGGTCTGCTCGCTAAATATATGATAATAGATATAGTAG...  
Giardia GCTCGCGATTATAGATAGGAGAGGGCTCTCGCTCGCT...

## Pairwise Comparison

## Candidate Pathogen List

### Specific Assays for confirmation of identity of agent

## Sequence Data

**>5 million reads per sample**

```
Read 1 AGATAGGATGAGAGAT
Read 2 ATAGATGGATAGGAGAGGGATGAT
Read 3 GCTCGTAGATAGATTAGAGAT
Read 4 AGATAGAGNNANATTAT
Read 5 NNAGATAGGATAGGAT
Read 6 GGGATAGATATTTAAATTAGATATAG
Read 7 ACACACACACACACACACACACACACA
```







*“The FDA learned about the contamination after an academic research team using a novel technique to look for viruses in a range of vaccines found the material in GlaxoSmithKline's product and told the company” - CNN*

# Examples of viral metagenomics

- Detection of viruses in the air
- Application to unsolved outbreaks of gastroenteritis
- Trial on wastewater samples

# Metagenomic detection of viruses in aerosol samples from workers in animal slaughterhouses

Richard Hall<sup>1</sup>, Mily Leblanc-Maridor<sup>2</sup>, Jing Wang<sup>1</sup>, Una Ren<sup>1</sup>, Nicole Moore<sup>1</sup>  
Colin Brooks<sup>2</sup>, Matthew Peacey<sup>1</sup>, Jeroen Douwes<sup>2</sup> and David McLean<sup>2</sup>.



CENTRE FOR  
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**Massey University**



1. Institute of Environmental Science & Research, National Centre for Biosecurity & Infectious Disease, Wallaceville, Upper Hutt, New Zealand.
2. Centre for Public Health Research, Massey University, Wellington Campus, Wellington, New Zealand.

# Mortality and cancer incidence in NZ Meat Workers

McLean *et al.* Occup Environ Med 2004;61:541-7

Cause of death (ICD-9)	Observed	Expected	SMR	95% CI
All Causes	227	203.6	<b>1.12</b>	0.98 - 1.27
All Cancer (140-208)	69	61.4	<b>1.12</b>	0.88 - 1.42
Lung (162)	23	12.9	<b>1.79</b>	1.13 – 2.68
Haematologic (200-208)	6	6.3	<b>0.96</b>	0.35 – 2.09

(*n* = 6,647)



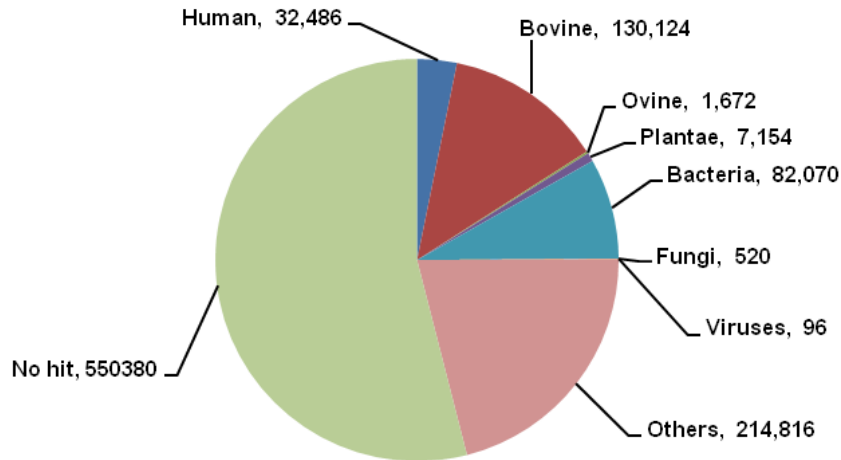


# Metagenome of an aerosol sample

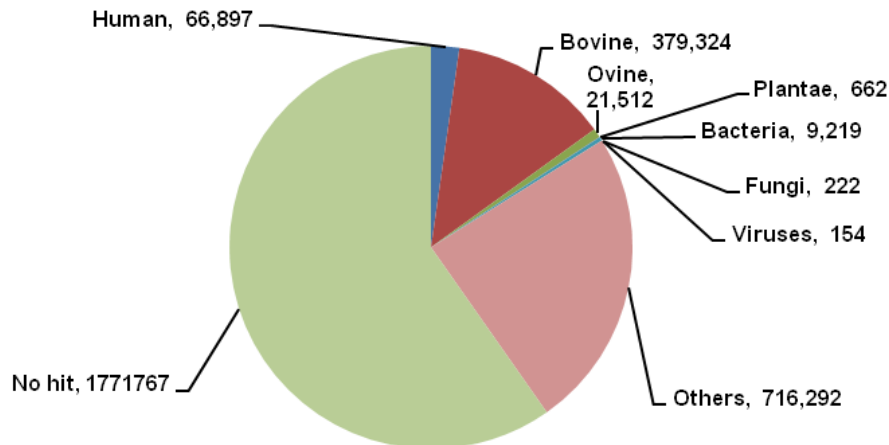
- PTFE aerosol filters
- Pooled samples (n = 9)
- Extracted DNA
  - Illumina HiSeq2000
  - 85bp reads (QC)
- Cattle area
  - 332 million reads
  - 145 million reads (collapsed)
- Sheep area
  - 250 million reads
  - 217 million reads (collapsed)



### Cattle Processing Area



### Sheep Processing Area



## Identified two viruses:

- **WU polyomavirus**

Suspected paediatric respiratory pathogen

- **Human papillomavirus 120**

Recently discovered virus found at various sites on human skin. Role in any disease is unclear.

Hall *et al.* (2013) *PLOS ONE* 8(8): e72226

# Bulk sampling

- **Portable**
  - 9 hour battery life
  - “Ruggedized”
- **Increased sample size**
  - Up to 310 litres per minute
  - 8 hour shift = 144,000 L
  - 150-fold greater than personal (960L)
- **Sampled seven work areas**
  - Sticking, Gutting, Boning room...
  - 24 x 8 hour shifts
  - n = 16 MiSeq, 250bp length, 170 million reads
- **Metagenomic detection of veterinary pathogens**
  - Adenoviruses
  - Bovine rotavirus
  - Bovine coronavirus



# Enterovirus 74 Infection in Children

Matthew Peacey<sup>1\*</sup>, Richard J. Hall<sup>1</sup>, Jing Wang<sup>1</sup>, Angela K. Todd<sup>1</sup>, Seiha Yen<sup>1</sup>, Jasmine Chan-Hyams<sup>1</sup>, Christy J. Rand<sup>2</sup>, Jo-Ann Stanton<sup>2</sup>, Q. Sue Huang<sup>1</sup>

<sup>1</sup> Clinical Virology, The Institute of Environmental Science and Research, National Centre for Biosecurity and Infectious Disease, Wellington, New Zealand, <sup>2</sup> Department of Anatomy, University of Otago, Dunedin, New Zealand

Todd et al. *Virology Journal* 2013, **10**:103  
http://www.virologyjournal.com/content/10/1/103



VIROLOGY JOURNAL

RESEARCH

Open Access

## Detection and whole genome sequence analysis of an enterovirus 68 cluster

Angela K Todd<sup>1\*</sup>, Richard J Hall<sup>1</sup>, Jing Wang<sup>1</sup>, Mathew Peacey<sup>1</sup>, Sharla McTavish<sup>1</sup>, Christy J Rand<sup>2</sup>, Jo-Ann Stanton<sup>2</sup>, Susan Taylor<sup>3</sup> and Q Sue Huang<sup>1</sup>

Journal of Virological Methods 195 (2014) 194–204



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Journal of Virological Methods

journal homepage: [www.elsevier.com/locate/jviromet](http://www.elsevier.com/locate/jviromet)



## Evaluation of rapid and simple techniques for the enrichment of viruses prior to metagenomic virus discovery<sup>☆</sup>

Richard J. Hall<sup>\*</sup>, Jing Wang, Angela K. Todd, Ange B. Bissielo, Seiha Yen, Hugo Strydom, Nicole E. Moore, Xiaoyun Ren, Q. Sue Huang, Philip E. Carter, Matthew Peacey

Institute of Environmental Science and Research, at the National Centre for Biosecurity & Infectious Disease, 66 Ward Street, Wallaceville, Upper Hutt 5018, New Zealand



# New Alphacoronavirus in *Mystacina tuberculata* Bats, New Zealand

Richard J. Hall, Jing Wang, Matthew Peacey, Nicole E. Moore, Kate McInnes, and Daniel M. Tompkins

Because of recent interest in bats as reservoirs of emerging diseases, we investigated the presence of viruses in *Mystacina tuberculata* bats in New Zealand. A novel alphacoronavirus sequence was detected in guano from roosts of *M. tuberculata* bats in pristine indigenous forest on a remote offshore island (Codfish Island).



# Metagenomic analysis of unsolved outbreaks of gastroenteritis in New Zealand

Nicole Moore<sup>1</sup>, Jing Wang<sup>1</sup>, Joanne Hewitt<sup>2</sup>, Dawn Croucher<sup>2</sup>,  
Deborah Williamson<sup>1</sup>, Shevaun Paine<sup>1</sup>, Seiha Yen<sup>1</sup>, Gail Greening<sup>2</sup>, Richard Hall<sup>1</sup>.

1. ESR, at National Centre for Biosecurity & Infectious Disease (NCBID), Upper Hutt, NZ.

2. Norovirus Reference Laboratory, ESR, Porirua, NZ.



# Summary of outbreaks in New Zealand

2012

Prepared as part of a Ministry of Health contract for scientific services by the  
Health Intelligence Team, Institute of Environmental Science and Research Limited

May 2013

ISSN: 1176-3485

[www.surv.esr.cri.nz](http://www.surv.esr.cri.nz)

*“No specific pathogen or condition was identified in the remaining 25.1% (180/716) of outbreaks, all of which were recorded as gastroenteritis outbreaks.”*

*Annual Summary of outbreaks in New Zealand 2012, ESR, Page 16*

Table 2. Outbreaks and associated cases by pathogen, 2012

Pathogen or condition	Outbreaks <sup>1</sup>			Cases <sup>1</sup>	
	Total	% of outbreaks (n=716)	Median cases per outbreak	Total	% of cases (n=10 491)
<b>Enteric<sup>1</sup></b>	<b>656</b>	<b>91.6</b>	<b>7.0</b>	<b>9474</b>	<b>90.3</b>
Norovirus	249	34.8	19.0	6097	58.1
<i>Giardia</i> spp.	69	9.6	3.0	284	2.7
<i>Cryptosporidium</i> spp.	47	6.6	3.0	164	1.6
<i>Campylobacter</i> spp.	32	4.5	3.0	282	2.7
<i>Salmonella</i> spp.	27	3.8	3.0	149	1.4
Rotavirus	23	3.2	15.0	360	3.4
<i>Shigella</i> spp.	12	1.7	2.5	43	0.4
<i>Clostridium difficile</i>	6	0.8	14.5	107	1.0
<i>Yersinia</i> spp.	5	0.7	3.0	14	0.1
<i>Clostridium perfringens</i>	4	0.6	4.5	18	0.2
<i>Escherichia coli</i> (EPEC)	3	0.4	23.0	63	0.6
Sapovirus	3	0.4	4.0	18	0.2
Astrovirus	2	0.3	2.5	5	0.0
<i>Aeromonas</i> spp.	1	0.1	8.0	8	0.1
Hepatitis A	1	0.1	30.0	30	0.3
Histamine fish poisoning	1	0.1	2.0	2	0.0
<i>Plesiomonas shigelloides</i>	1	0.1	3.0	3	0.0
<i>Salmonella</i> Paratyphi	1	0.1	2.0	2	0.0
<i>Salmonella</i> Typhi	1	0.1	2.0	2	0.0
<i>Staphylococcus aureus</i>	1	0.1	3.0	3	0.0
Toxic shellfish poisoning	1	0.1	29.0	29	0.3
VTEC/STEC infection	1	0.1	3.0	3	0.0
Pathogen not identified <sup>2</sup>	180	25.1	6.0	1996	19.0

# Metagenomic Analysis of Human Diarrhea: Viral Detection and Discovery

Stacy R. Finkbeiner<sup>1,2\*</sup>, Adam F. Allred<sup>1,2\*</sup>, Phillip I. Tarr<sup>3</sup>, Eileen J. Klein<sup>4</sup>, Carl D. Kirkwood<sup>5</sup>, David Wang<sup>1,2\*</sup>

**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

JOURNAL OF VIROLOGY, Oct. 2009, p. 10836–10839  
0022-538X/09/\$08.00+0 doi:10.1128/JVI.00998-09

Vol. 83, No. 20

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## Identification of a Novel Astrovirus (Astrovirus VA1) Associated with an Outbreak of Acute Gastroenteritis<sup>▽</sup>

Stacy R. Finkbeiner,<sup>1†</sup> Yan Li,<sup>2†</sup> Susan Ruone,<sup>2</sup> Christina Conrardy,<sup>2</sup> Nicole Gregoricus,<sup>2</sup> Denise Toney,<sup>3</sup> Herbert W. Virgin,<sup>1</sup> Larry J. Anderson,<sup>2</sup> Jan Vinjé,<sup>2</sup> David Wang,<sup>1\*</sup> and Suxiang Tong<sup>2\*</sup>

JOURNAL OF VIROLOGY, June 2007, p. 5978–5984  
0022-538X/07/\$08.00+0 doi:10.1128/JVI.02650-06

Vol. 81, No. 11

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## New Adenovirus Species Found in a Patient Presenting with Gastroenteritis<sup>▽</sup>

Morris Saffold Jones II,<sup>1\*</sup> Balázs Harrach,<sup>5</sup> Robert D. Ganac,<sup>1</sup> Mary M. A. Gozum,<sup>1</sup> Wilfred P. dela Cruz,<sup>1</sup> Brian Riedel,<sup>2</sup> Chao Pan,<sup>2</sup> Eric L. Delwart,<sup>3,4</sup> and David P. Schnurr<sup>2</sup>

### DISPATCHES

## New Viruses in Idiopathic Human Diarrhea Cases, the Netherlands

Saskia L. Smits, Claudia M.E. Schapendonk, Janko van Beek, Harry Vennema, Anita C. Schürch, Debby Schipper, Rogier Bodewes, Bart L. Haagmans, Albert D. M. E. Osterhaus, and Marion P. Koopmans

Emerging viral infections can be identified by using a viral metagenomics approach for clinical human material. Diarrhea samples of patients with unexplained gastroenteritis from the Netherlands were analyzed by using viral metagenomics. Novel circular DNA viruses, bufaviruses, and genogroup III picobirnaviruses were identified. These data expand our knowledge of the human virome.

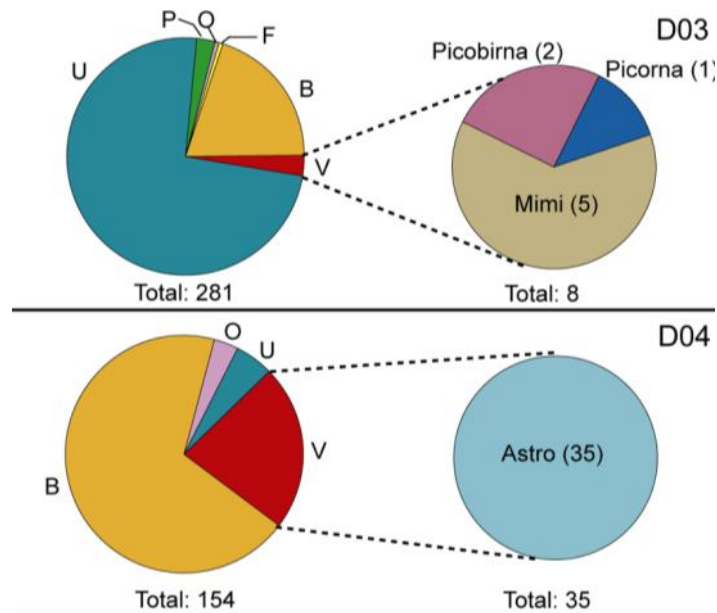
Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 20, No. 7, July 2014



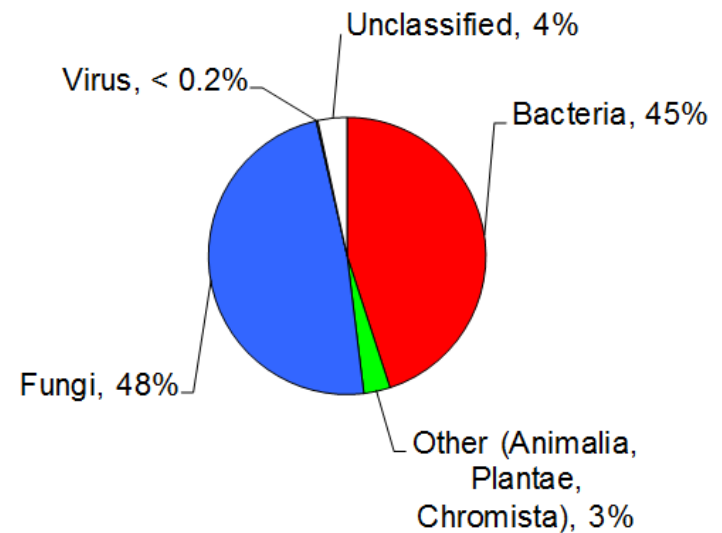
# Metagenomic analysis of unsolved gastroenteritis outbreaks in New Zealand

- 31 anonymous faecal samples from unsolved outbreaks
- Taken from 26 outbreaks occurring in 2011 / 2012
  - 5 outbreaks were subsequently resolved by public health investigations
- Illumina MiSeq
  - Pre-processing (spin, filter, nuclease)
  - NZGL / Massey Genome Service
  - Total of 193 million reads
  - Average of 3.1 million reads per sample
- BLASTN / BLASTX comparison to Genbank
- Eight viruses and one parasite identified





Adapted from FIGURE 1 in Finkbeiner et al. (2008) PLOS Pathogens 4(2):e1000011.



Hall et al. 2010. unpublished data

## Candidate pathogenic organisms detected in metagenomic data from unsolved outbreaks of gastroenteritis, and results of independent confirmatory testing.

Metagenomic data			Independent test		Diseases already known to be associated with the candidate pathogen
Candidate pathogen	Number of samples	% of total number of samples	Method	Result	
Adenovirus	1	3%	qPCR	Confirmed	Gastroenteritis, respiratory illness
Human enterovirus B	2	7%	qPCR	Confirmed <sup>1</sup>	Paralysis; gastrointestinal symptoms <sup>2</sup> ; HFMD <sup>3</sup>
Human parechovirus type 3	1	3%	RT-PCR	Confirmed	Neonatal sepsis, encephalitis, paralysis
Human picobirnavirus	10	33%	-	-	Gastroenteritis
Influenza A virus	1	3%	RT-PCR	Not confirmed	Respiratory illness, gastrointestinal symptoms <sup>1</sup>
Pepper mild mottle virus	3	10%	-	-	Plant virus; link to abdominal pain in humans <sup>4</sup>
Rotavirus	1	3%	Immunoassay, qPCR	Confirmed	Gastroenteritis
Sapovirus	1	3%	qPCR	Confirmed	Gastroenteritis
<i>Dientamoeba fragilis</i>	4	13%	qPCR	Confirmed	Possible link to gastroenteritis
No candidates	11	37%	-	-	-

<sup>1</sup> Confirmed in one sample only.

<sup>2</sup> Gastrointestinal symptoms are classified as vomiting and diarrhea

<sup>3</sup> HFMD: Hand, foot and mouth disease

<sup>4</sup> Colson *et al.* (2010) PLOS ONE 5(4):e10041

# Conclusions

- **No evidence for novel viruses**
- **Human parechovirus 3**
  - Unknown role in outbreak
  - Significant pathogen causing neonatal sepsis, encephalitis, paralysis
  - First reported detection in Australasia
- **Use for metagenomics as a diagnostic audit tool**



# Viruses in wastewater

Richard Hall<sup>1</sup>, Jing Wang<sup>1</sup>, Nicole Moore<sup>1</sup>, Gail Greening<sup>2</sup>, Dawn Croucher<sup>2</sup>, Joanne Hewitt<sup>2</sup>.

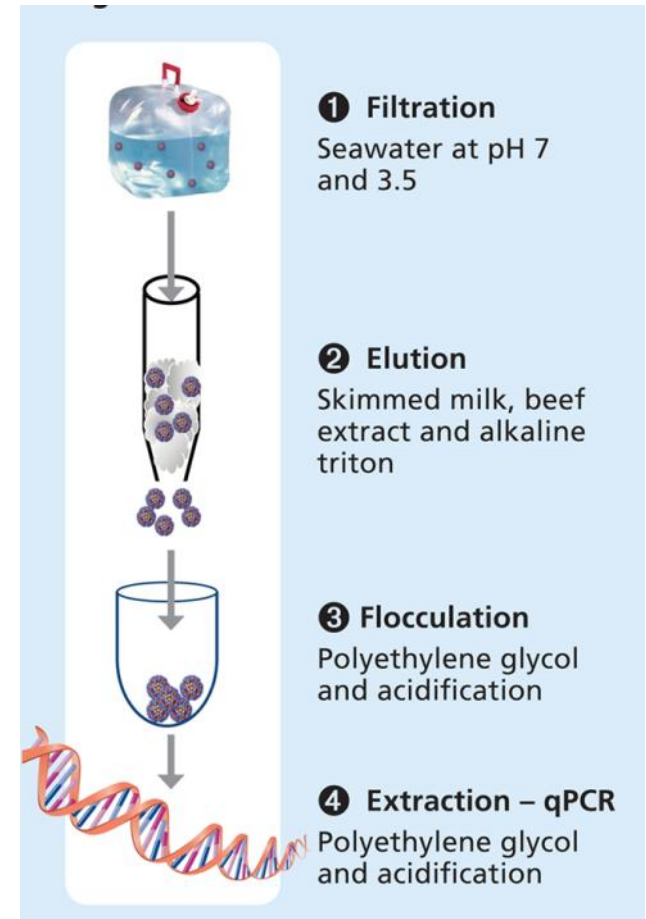
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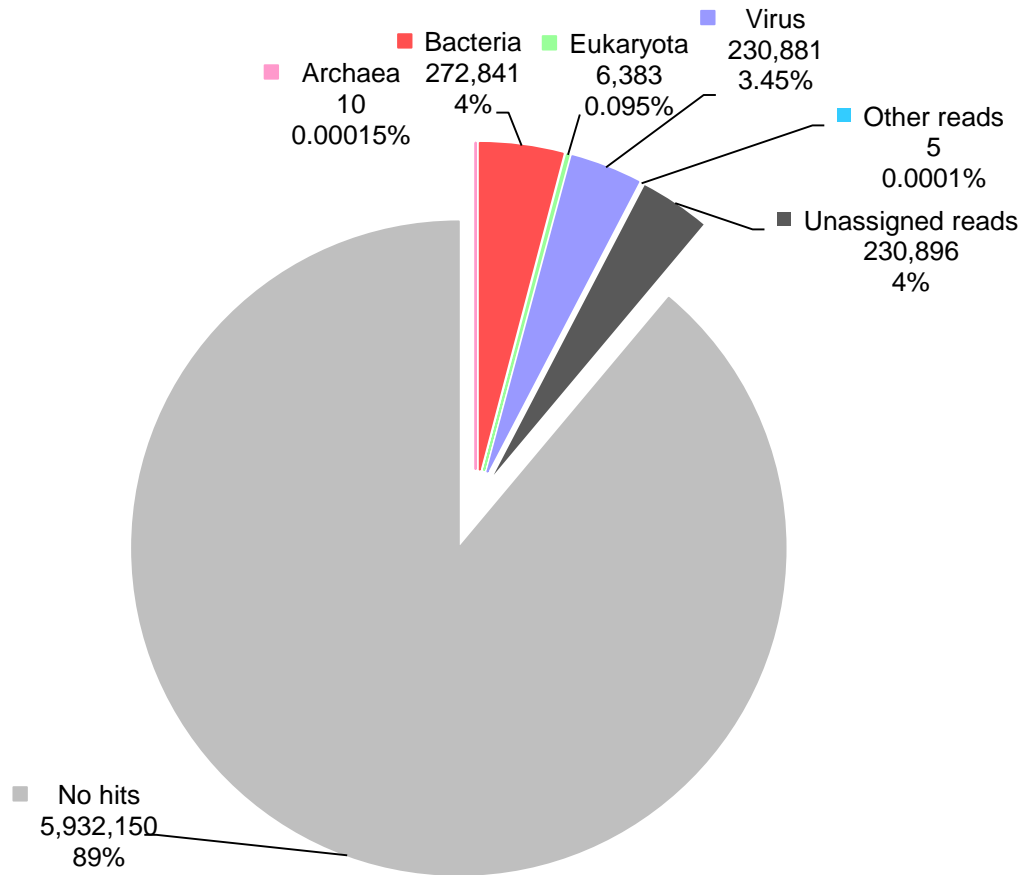
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protecting people and their environment through science

# Metagenomic analysis of New Zealand wastewater

- One site (anonymous)
- Sampled 1 litre of wastewater (influent)
- 6,688,368 sequence reads x 250 bp PE
- BLASTX comparison to Genbank



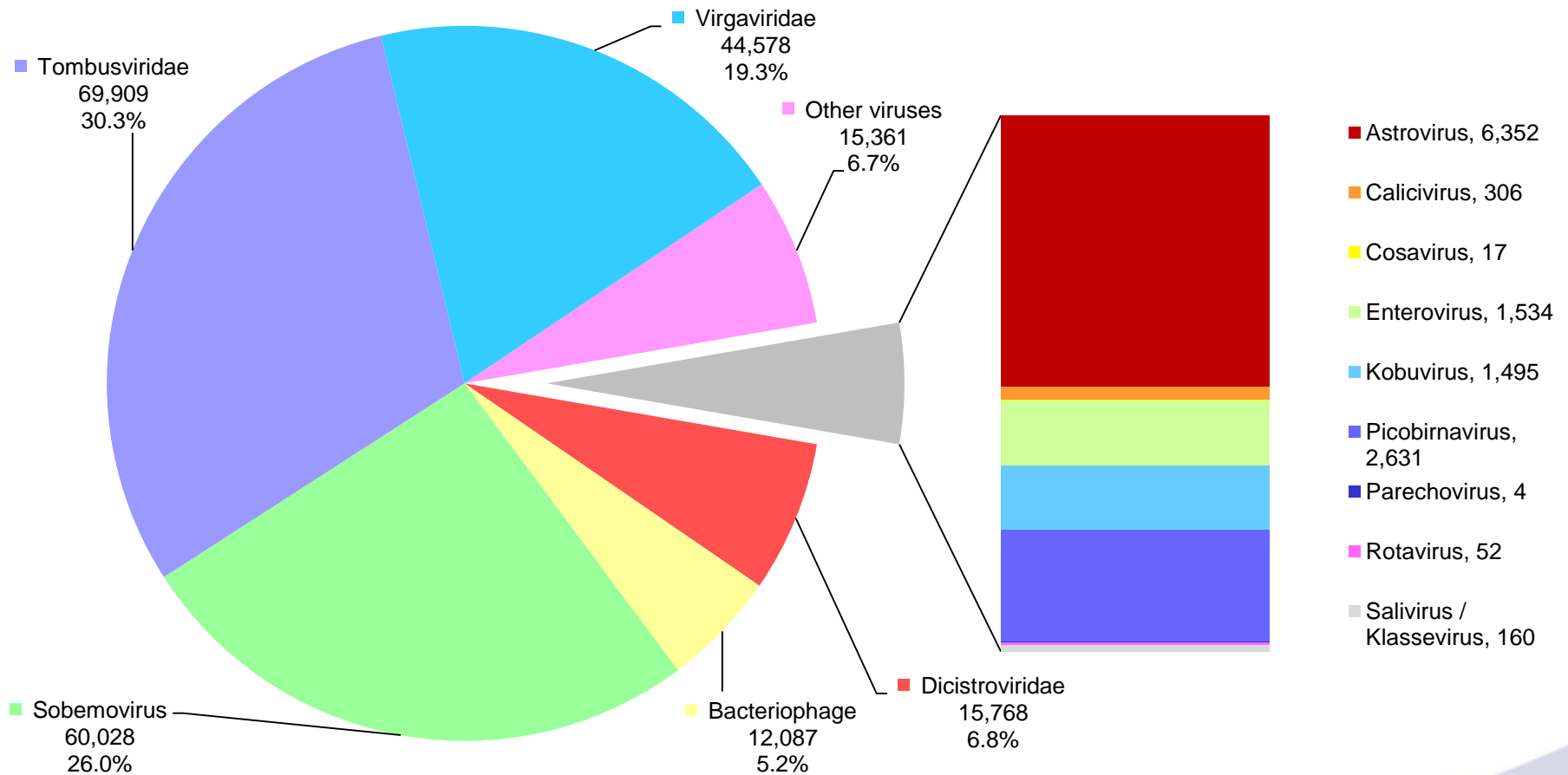
# Metagenomic composition of wastewater sample



Hall et al. (2014) Unpublished

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# Subset of viruses present within metagenome



Hall et al. (2014) Unpublished

© ESR 2014



# Acknowledgements

- **Centre for Public Health Research, Massey University**
  - Dave McLean, Jeroen Douwes, Mily Leblanc-Maridor
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- **New Zealand e-Science Infrastructure**
- **Ministry for Business, Innovation and Employment**
  - CRI Capability Fund
- **Health Research Council of New Zealand**
- **Ministry of Health**



**Ministry of Business,  
Innovation & Employment**