What do we know about potential waterborne pathogens in livestock and water in NZ?

Nigel French

Drinking Water Workshop, Hamilton, 19th Sept 2017

Outline

- Waterborne pathogens: *Campylobacter, Giardia, Cryptosporidium*, Shiga toxin-producing *E. coli*

- Prevalence in animals

- Prevalence in water

- Using molecular typing to link animals, water and human cases

- Havelock North application of whole genome sequencing
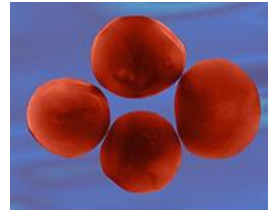
Waterborne zoonotic infections include:

Protozoa

- *Cryptosporidium*
- *Giardia*
- *Toxoplasma*
- …. others
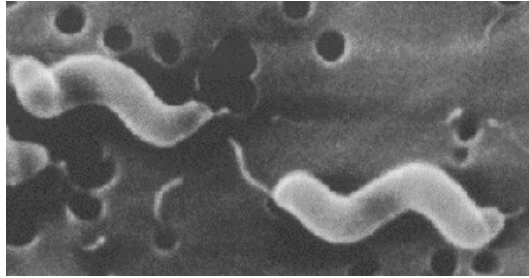
Bacteria

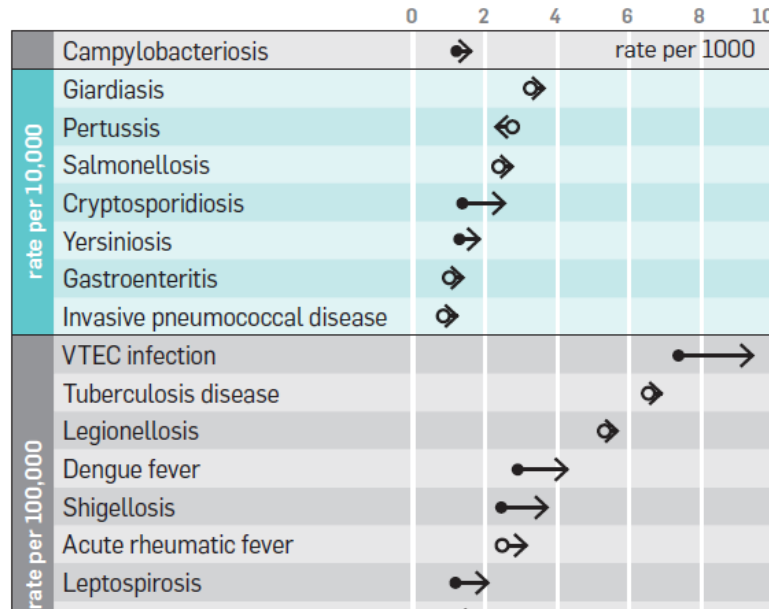- *Campylobacter*
- *Salmonella*
- *E. coli* O157
- *Leptospira*

© 2004 Dennis Kunkel Microscopy, Inc.

Water borne diseases high on the list of notifiable diseases in New Zealand

**National surveillance data 12-monthly notification rate changes[1]**

	0	2	4	6	8	10
Campylobacteriosis				rate per 1000		

rate per 10,000
- Giardiasis
- Pertussis
- Salmonellosis
- Cryptosporidiosis
- Yersiniosis
- Gastroenteritis
- Invasive pneumococcal disease

rate per 100,000
- VTEC infection
- Tuberculosis disease
- Legionellosis
- Dengue fever
- Shigellosis
- Acute rheumatic fever
- Leptospirosis

Many also foodborne

Source: ESR Ltd
Quarter to March 2017

Cryptosporidium and *Giardia*

Cryptosporidium and *Giardia*

Country	*Cryptosporidium* Rate of cryptosporidiosis per 100 000 population	*Giardia* Rate of giardiasis per 100 000 population
New Zealand	23·8	32·9
Australia	15·8	Not available
United Kingdom	8·5	5·5
United States	2·8	6·7
Germany	1·6	5·5
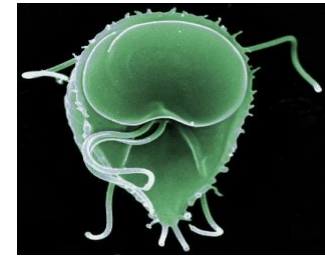
A tale of two parasites: the comparative epidemiology of cryptosporidiosis and giardiasis

S. J. SNEL[1], M. G. BAKER[1]*, V. KAMALESH[1], N. FRENCH[2] AND J. LEARMONTH[2]
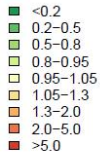
Cryptosporidium and *Giardia:* seasonal patterns
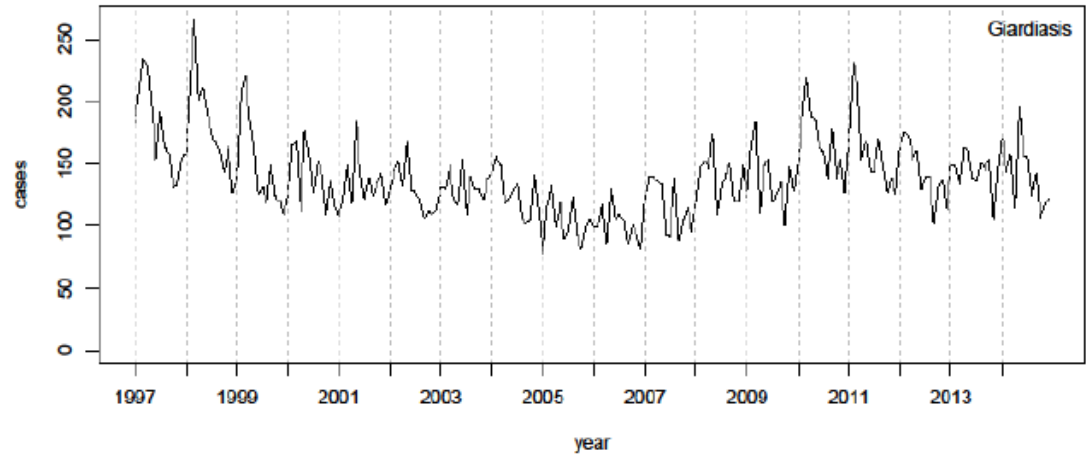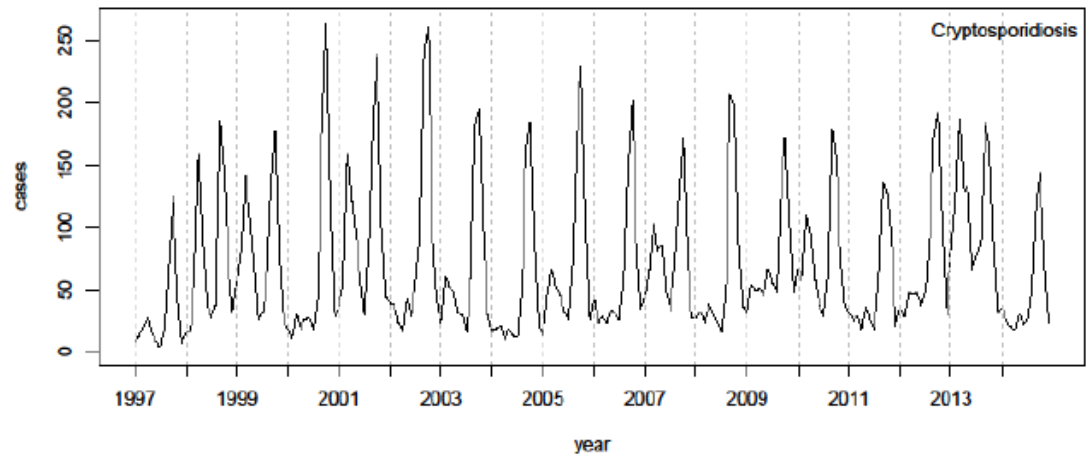
Cryptosporidium: two species
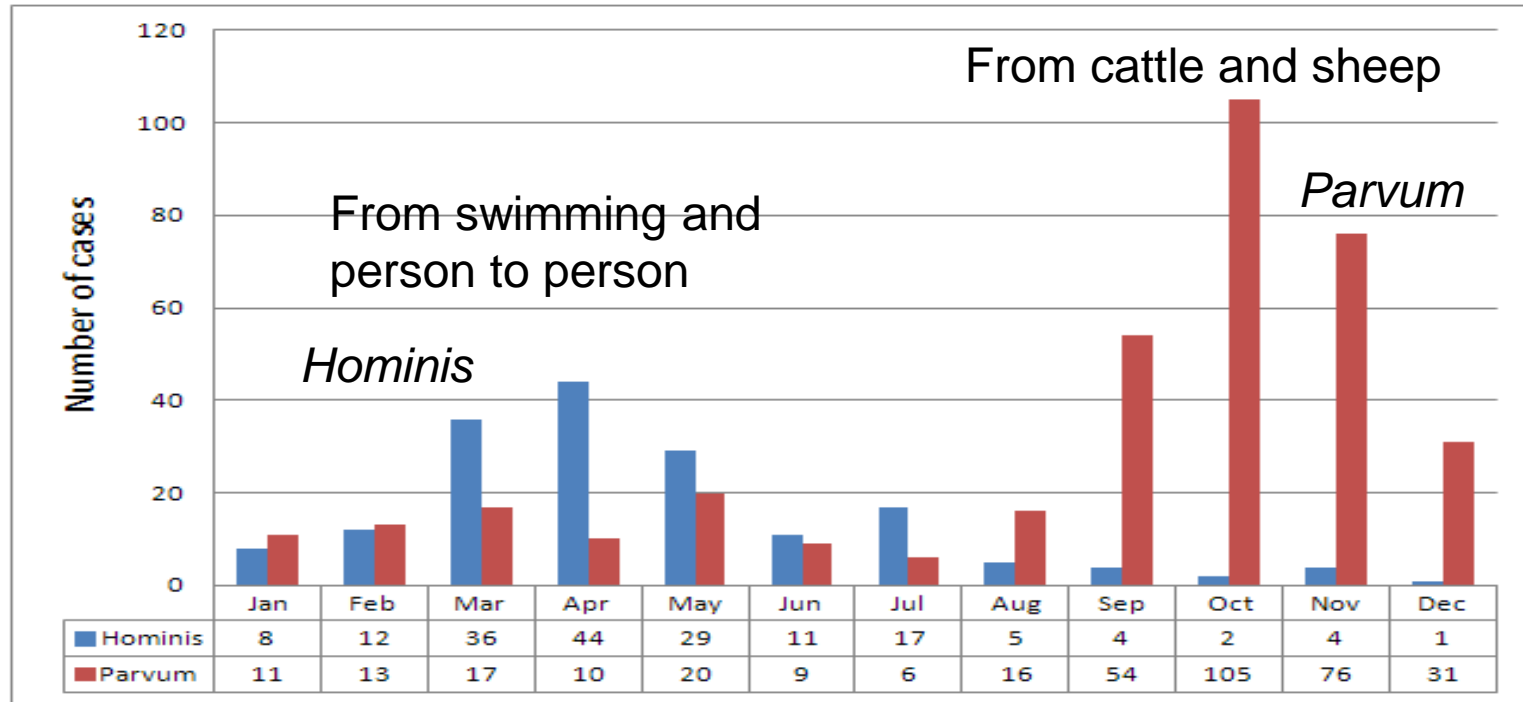
Cryptosporidium and *Giardia*

Giardia

Crypto

Legend (both maps):
- <0.2
- 0.2–0.5
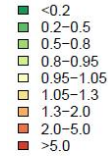- 0.5–0.8
- 0.8–0.95
- 0.95–1.05
- 1.05–1.3
- 1.3–2.0
- 2.0–5.0
- >5.0
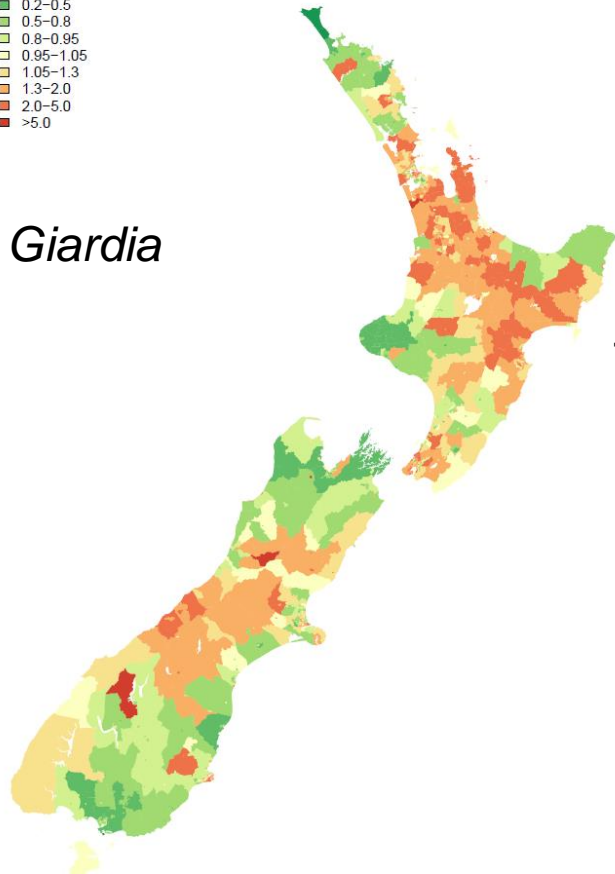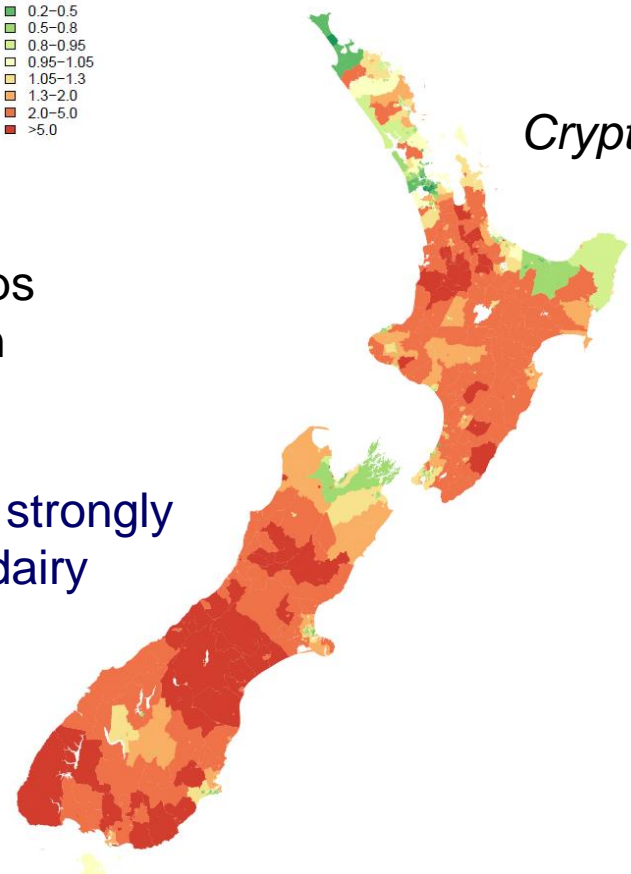
Relative risk maps – red high, green low

Cryptosporidium strongly associated with dairy cattle density

Strain types of *Crypto* and *Giardia*

RESEARCH ARTICLE

Local and global genetic diversity of protozoan parasites: Spatial distribution of *Cryptosporidium* and *Giardia* genotypes

Juan C. Garcia–R[1]*, Nigel French[1], Anthony Pita[1], Niluka Velathanthiri[1], Rima Shrestha[2], David Hayman[1]

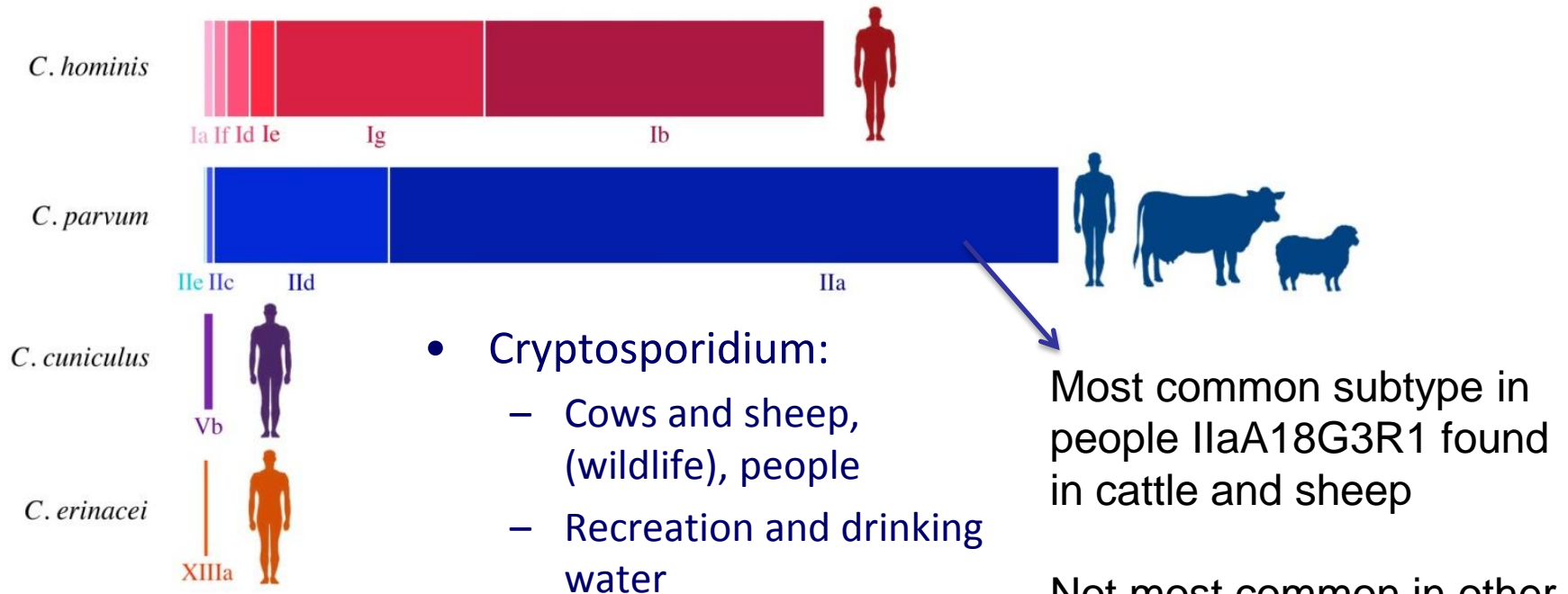Parasites & Vectors

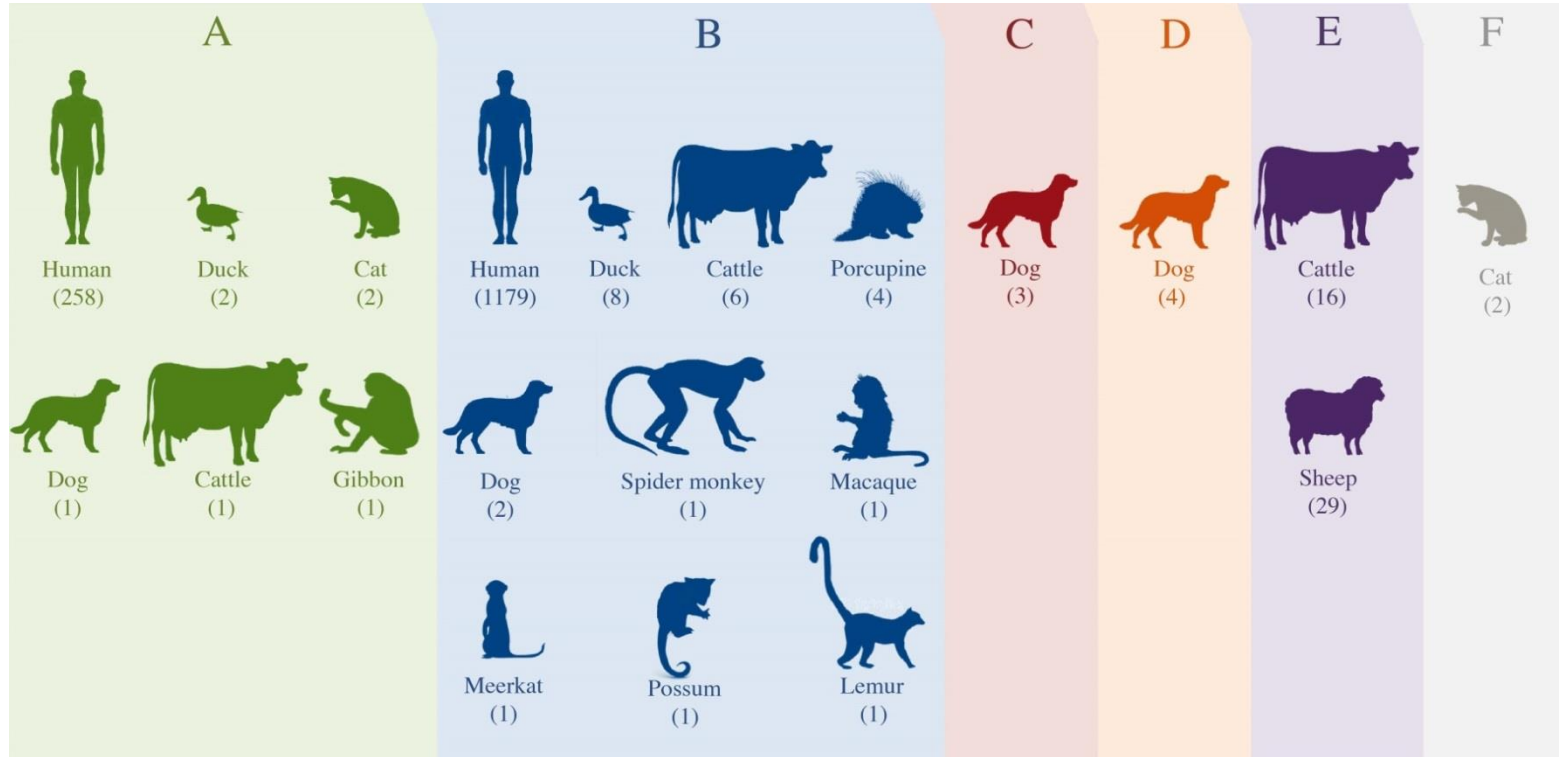**RESEARCH**   **Open Access**

Cross sectional study of prevalence, genetic diversity and zoonotic potential of *Cryptosporidium parvum* cycling in New Zealand dairy farms

Julanda Al Mawly[1], Alex Grinberg[2]*, Niluka Velathanthiri[2] and Nigel French[1]

Cryptosporidium

C. hominis
Ia If Id Ie   Ig   Ib

C. parvum
IIe IIc   IId   IIa

C. cuniculus
Vb

C. erinacei
XIIIa

- Cryptosporidium:
  - Cows and sheep, (wildlife), people
  - Recreation and drinking water

Most common subtype in people IIaA18G3R1 found in cattle and sheep

Not most common in other countries (except Ireland)

Giardia

Prevalence of *Cryptosporidium* and *Giardia* in cattle faeces

Risk factors for neonatal calf diarrhoea and enteropathogen shedding in New Zealand dairy farms

CrossMark

J. Al Mawly [a], A. Grinberg [b,*], D. Prattley [a], J. Moffat [c], J. Marshall [a], N. French [a]

[a] mEpiLab, Infectious Disease Research Centre, Hopkirk Research Institute, Massey University, Palmerston North, 4410, New Zealand
[b] Infectious Diseases Group, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, 4410, New Zealand
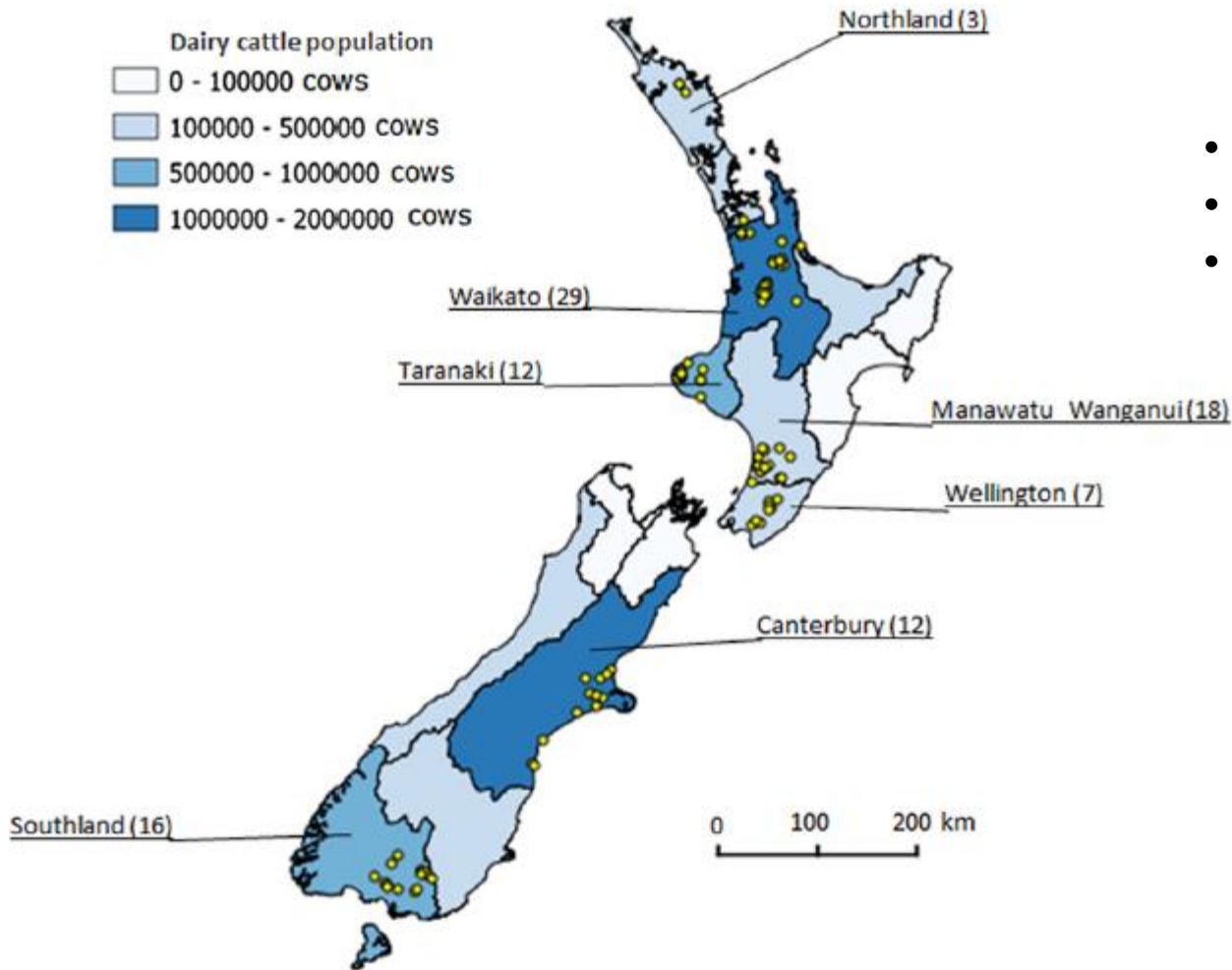[c] MSD Animal Health, 33 Whakatiki Street, Upper Hutt, Wellington 5018, New Zealand

Prevalence of endemic enteropathogens of calves in New Zealand dairy farms

J Al Mawly*, A Grinberg[†§], D Prattley*, J Moffat[‡] and N French*

Dairy cattle population
- 0 - 100000 cows
- 100000 - 500000 cows
- 500000 - 1000000 cows
- 1000000 - 2000000 cows

Northland (3)

Waikato (29)

Taranaki (12)

Manawatu Wanganui (18)

Wellington (7)

Canterbury (12)

Southland (16)

0   100   200 km

- Year of study: 2011
- 1,283 samples
- 97 dairy farms

Recent prevalence study in cattle: Farm level

	1-5 day-old calves	9-21 day-old calves
Rotavirus	46% (31-67)	57% (41-80)
Coronavirus	13.7% (7-28)	30.5% (20-48)
C. parvum	17.5% (8-35)	52% (36-75)
Salmonella spp.	3% (1-15)	4% (1-16)
K99 *E. coli*	11% (5-25)	Not tested
Giardia spp.	23% (13-41)	88% (67-116)

Both highly prevalent: Giardia mainly non-zoonotic strains

Recent prevalence study in cattle : Calf level

Enteropathogen	Number of calves positive for these enteropathogens	
	9 to 21-day-old calves	1 to 5-day-old calves
Rotavirus (total)	158/797 (19.8%)	86/429 (20%)
Coronavirus (total)	49/797 (6.1%)	23/429 (5.3%)
C. parvum (total)	126/797 (15.8%)	25/429 (5.8%)
Salmonella spp. (total)	4/797 (0.5%)	3/429 (0.6%)

Ecosystem health and farming: Faecal outputs of cattle...and humans

- Cattle: Number of defecations
  - 9 – 16, average 12 per day
  - Average 2kg per defecation
  - Total output of 25kg per cow per day.
- 9 million cattle in NZ
- 230,000 tonnes faecal material per day...
- 84 million tonnes per year
- Humans 800 tonnes per day

Source Dr Brent Gilpin ESR Ltd

Water sampling: abstraction points

- Location of 20 drinking water abstraction sites monitored for water quality, September 2009 to July 2016.

- Letters ground water
- Numbers surface water

- Selected to represent different catchment types
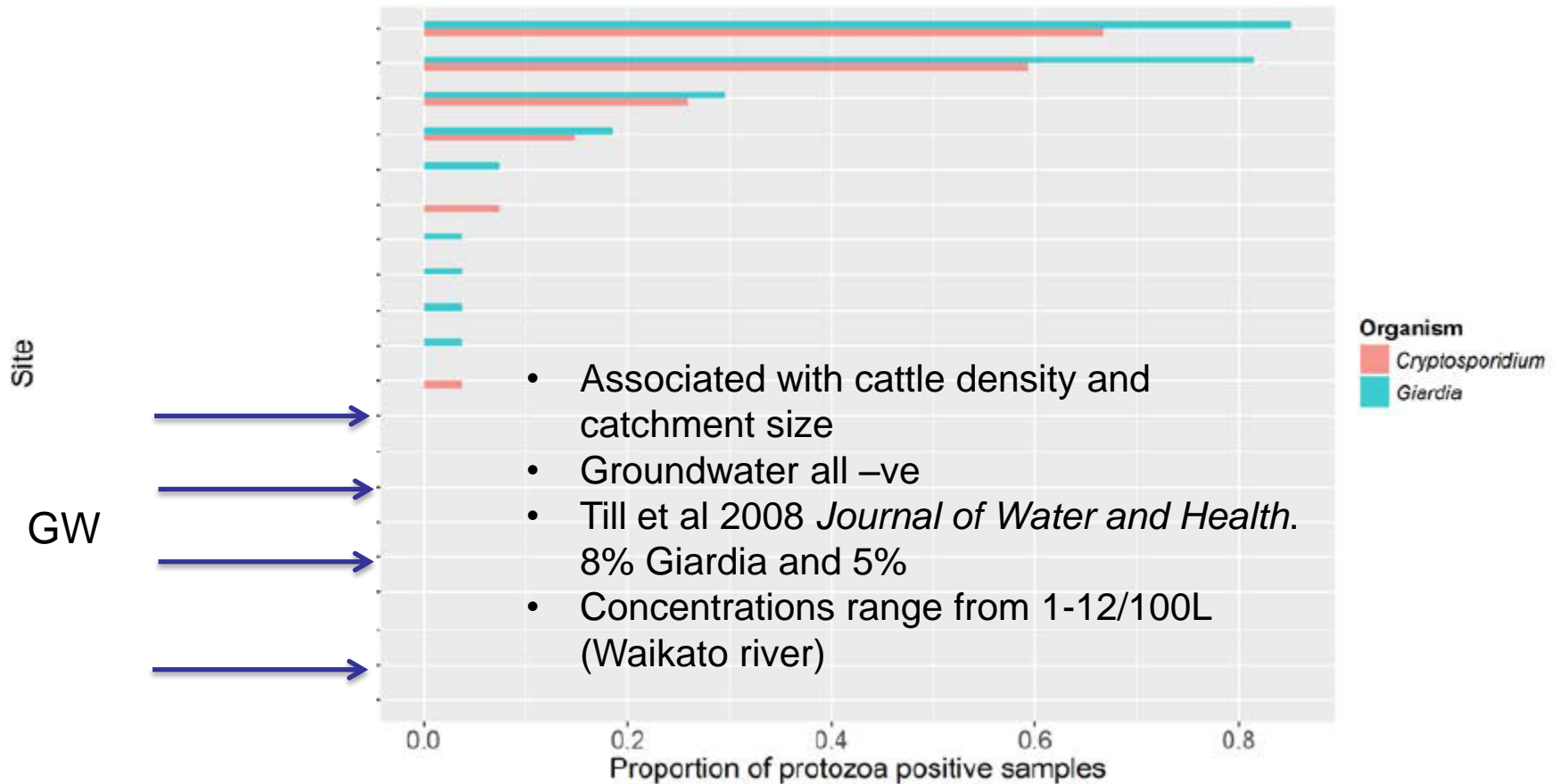
- 4 samples per site per year, N=540

D. Hayman, N French, A. Pita J-C Garcia et al

- Associated with cattle density and catchment size
- Groundwater all –ve
- Till et al 2008 *Journal of Water and Health*. 8% Giardia and 5%
- Concentrations range from 1-12/100L (Waikato river)

GW

Site

Proportion of protozoa positive samples

Organism
Cryptosporidium
Giardia

E. coli O157:H7 (and other STEC)

E. coli O157:H7 (and other STEC)

- Bacterial infection associated with transmission from ruminants (mainly cattle)

- 80% bloody diarrhoea

- Highest prevalence in young children

- ~8% develop complications
  - HUS

Highest prevalence in dairy farming areas
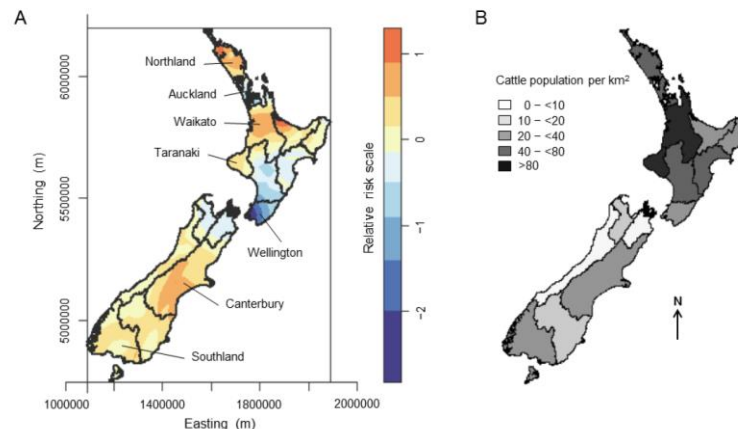
Recent trends in STEC in New Zealand

Number of cases / million

E. coli, mainly STEC O157 until recently
e.g. spike in Feb 2016 30/86 were O157

First case in 1993

"The increase in notifications for DHBs in the Auckland region may be due to a recent change in laboratory methods; all faecal specimens are now screened for VTEC/STEC using PCR"
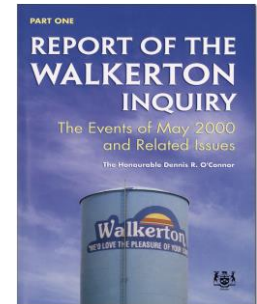
Date

Rate in 2014 = 2.3 x rate in 2004
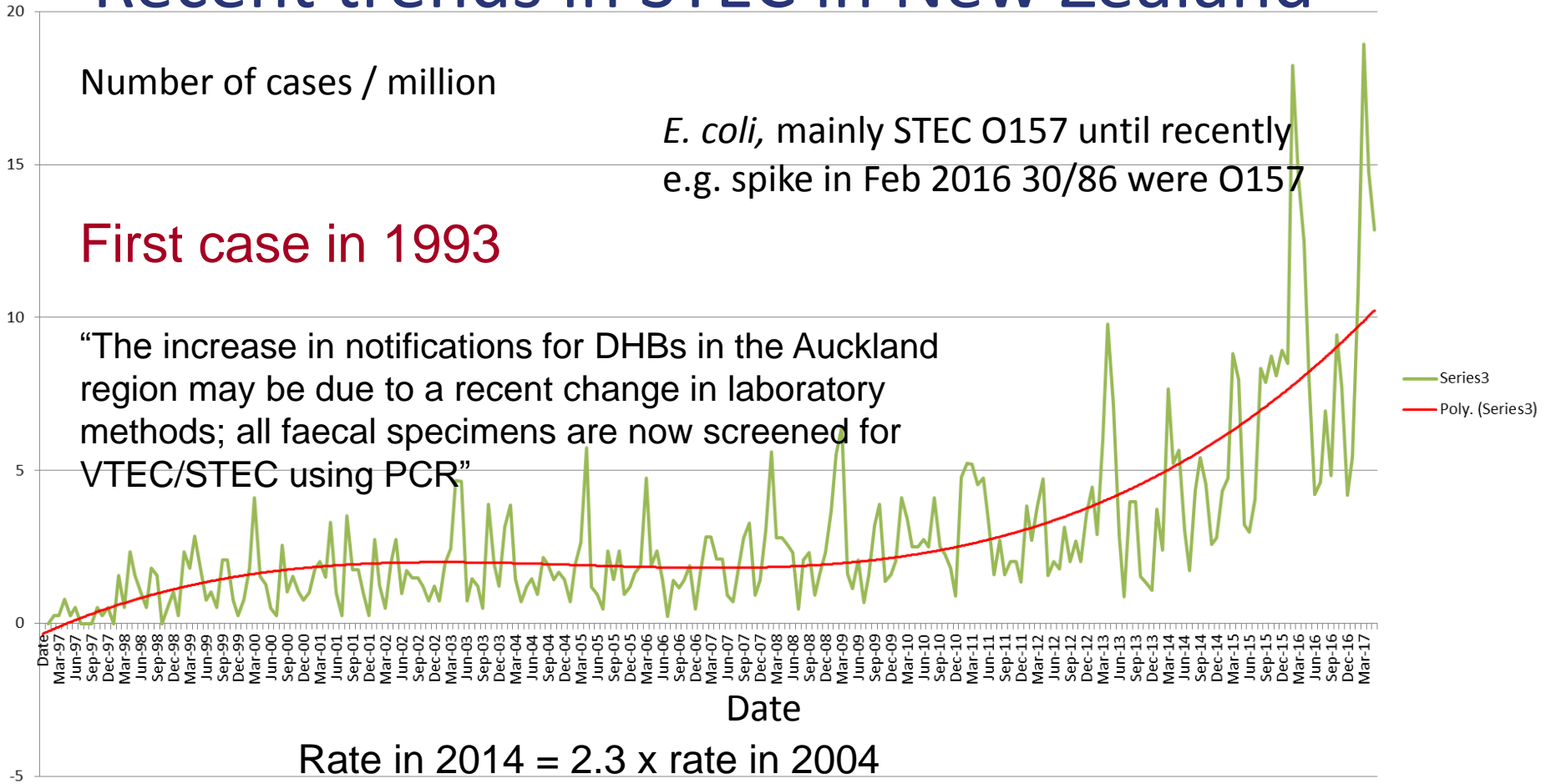
RESEARCH ARTICLE   **Open Access**

A prospective case–control and molecular epidemiological study of human cases of Shiga toxin-producing *Escherichia coli* in New Zealand

Patricia Jaros[1*], Adrian L Cookson[2], Donald M Campbell[3], Thomas E Besser[4], Smriti Shringi[4], Graham F Mackereth[5], Esther Lim[5], Liza Lopez[6], Muriel Dufour[5], Jonathan C Marshall[1], Michael G Baker[7], Steve Hathaway[3], Deborah J Prattley[1] and Nigel P French[1,8]

- Significant environmental risk factors included:
  - Children <5 contact with farm animals
  - Water – recreation and drinking
  - Contact with cattle faeces
- No food-related risk factors

PART ONE
REPORT OF THE
WALKERTON
INQUIRY
The Events of May 2000
and Related Issues
The Honourable Dennis R. O'Connor

Walkerton
WE'D LOVE THE PLEASURE OF YOUR

STEC prevalence on dairy farms (S. Browne)

- Study of 102 farms, 1508 calves

- Year of study: 2014

- MPI/MIA funded study

- Validated culture independent assay (NeoSEEK)

- Simultaneously detects serotype and *stx/eae* genes (i.e. in same bacterium)
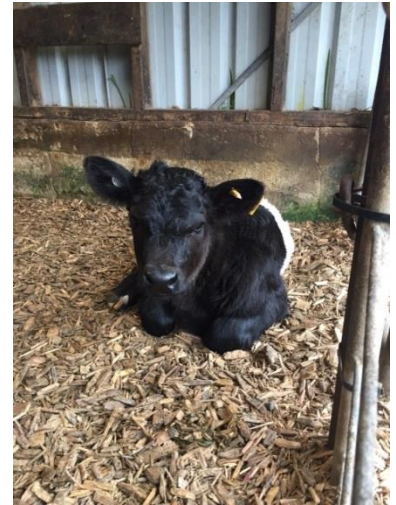
Any Top 7 STEC

Any Top 7 STEC

Northland
Farm 100%
Calf 43.5%

Waikato
Farm 66%
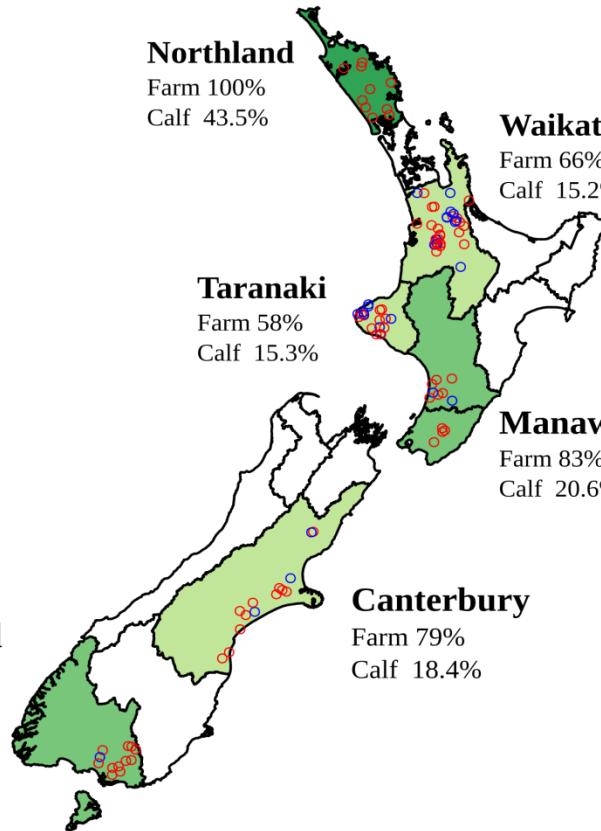Calf 15.2%

Taranaki
Farm 58%
Calf 15.3%

Manawatu-Wellington
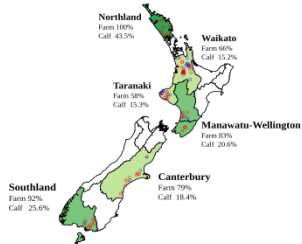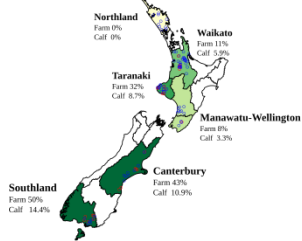Farm 83%
Calf 20.6%

Canterbury
Farm 79%
Calf 18.4%

Southland
Farm 92%
Calf 25.6%

STEC O157

Northland
Farm 10%
Calf 1.4%

Waikato
Farm 17%
Calf 1.5%

Taranaki
Farm 0%
Calf 0%

Manawatu-Wellington
Farm 50%
Calf 8.9%

Canterbury
Farm 7%
Calf 1.0%

Southland
Farm 8%
Calf 0.6%

STEC O26

Northland
Farm 0%
Calf 0%

Waikato
Farm 9%
Calf 5.9%

Taranaki
Farm 32%
Calf 8.7%

Manawatu-Wellington
Farm 8%
Calf 3.3%

Canterbury
Farm 43%
Calf 10.9%

Southland
Farm 50%
Calf 14.4%

STEC O45

Northland
Farm 60%
Calf 17.7%

Waikato
Farm 9%
Calf 1.3%

Taranaki
Farm 0%
Calf 0%

Manawatu-Wellington
Farm 33%
Calf 2.2%

Canterbury
Farm 14%
Calf 1.5%

Southland
Farm 25%
Calf 2.2%

STEC O103

Northland
Farm 50%
Calf 8.2%

Waikato
Farm 40%
Calf 5.5%

Taranaki
Farm 16%
Calf 1.5%

Manawatu-Wellington
Farm 42%
Calf 7.2%

Canterbury
Farm 29%
Calf 2.5%

Southland
Farm 42%
Calf 6.7%

STEC O145

Northland
Farm 80%
Calf 34.7%

Waikato
Farm 37%
Calf 6.1%

Taranaki
Farm 32%
Calf 8.4%

Manawatu-Wellington
Farm 42%
Calf 7.2%

Canterbury
Farm 43%
Calf 6.5%

Southland
Farm 50%
Calf 8.9%

Legend

Low — High
Calf Level STEC Prevalence

Negative   Positive
Farm Presence

Northland
Farm 100%
Calf 43.5%

Waikato
Farm 66%
Calf 15.2%

Taranaki
Farm 58%
Calf 15.3%

Manawatu-Wellington
Farm 83%
Calf 20.6%

Canterbury
Farm 79%
Calf 18.4%

Southland
Farm 92%
Calf 25.6%

STEC O157

Northland
Farm 10%
Calf 1.4%

Waikato
Farm 17%
Calf 1.5%

Taranaki
Farm 0%
Calf 0%

Manawatu-Wellington
Farm 50%
Calf 8.9%

Canterbury
Farm 7%
Calf 1.0%

Southland
Farm 8%
Calf 0.6%

STEC O26

Northland
Farm 0%
Calf 0%

Waikato
Farm 11%
Calf 5.9%

Taranaki
Farm 32%
Calf 8.7%

Manawatu-Wellington
Farm 8%
Calf 3.3%

Canterbury
Farm 43%
Calf 10.9%

Southland
Farm 50%
Calf 14.4%

STEC O157 relatively recently introduced into New Zealand

- At least 3 introductions in last 40-50 years

- Population of STEC O157 grew rapidly after 1985

- First human case in 1993
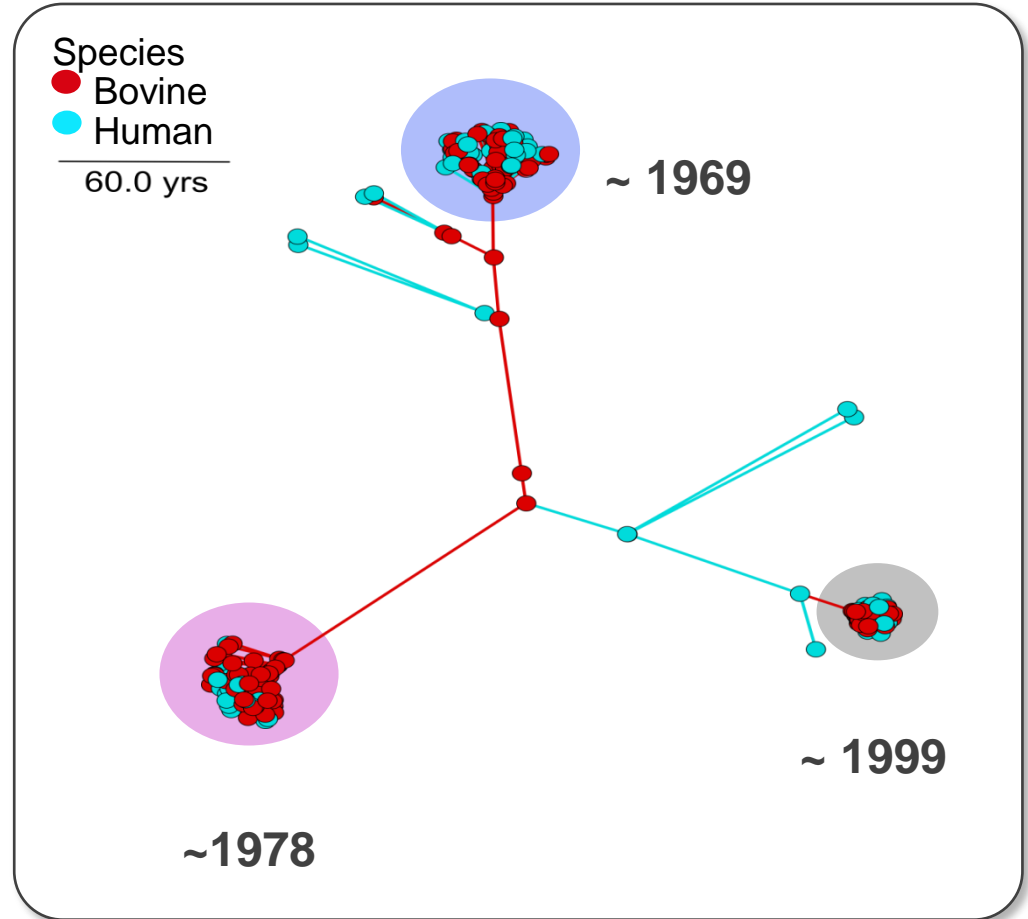
Livestock importations into New Zealand
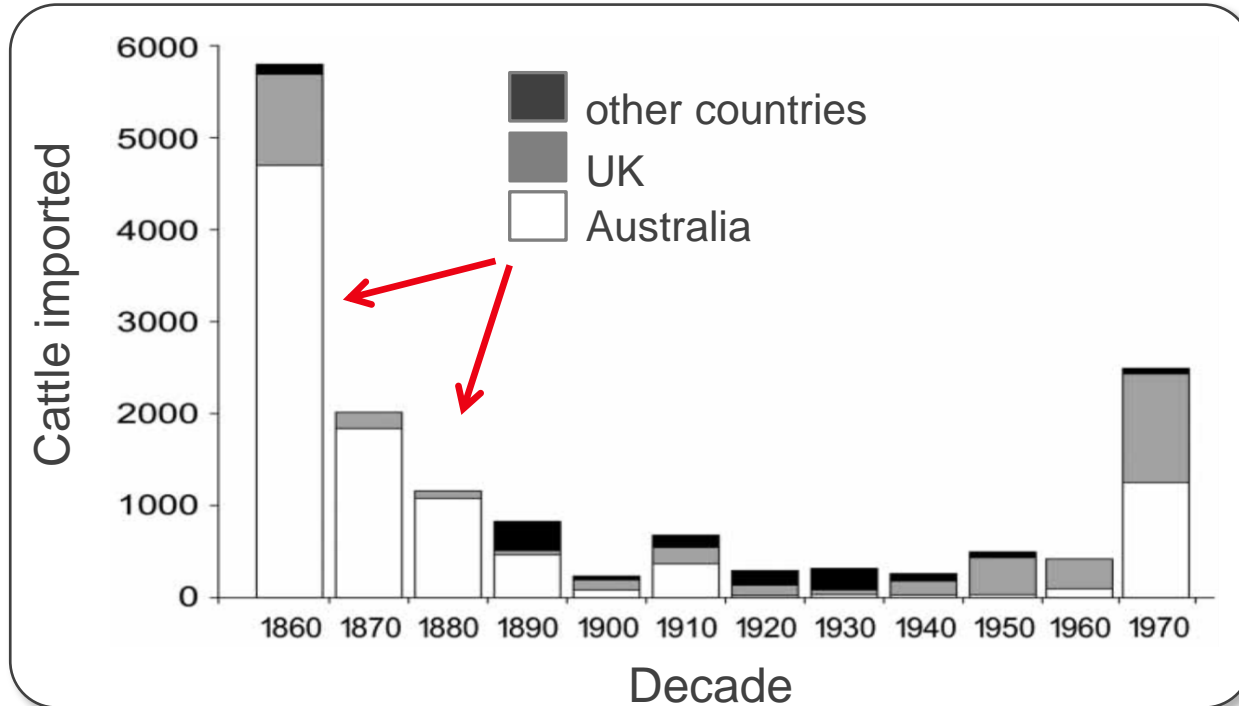
Population sizes of STEC O157 and rise in dairying

Number of dairy cows

STEC O157 case
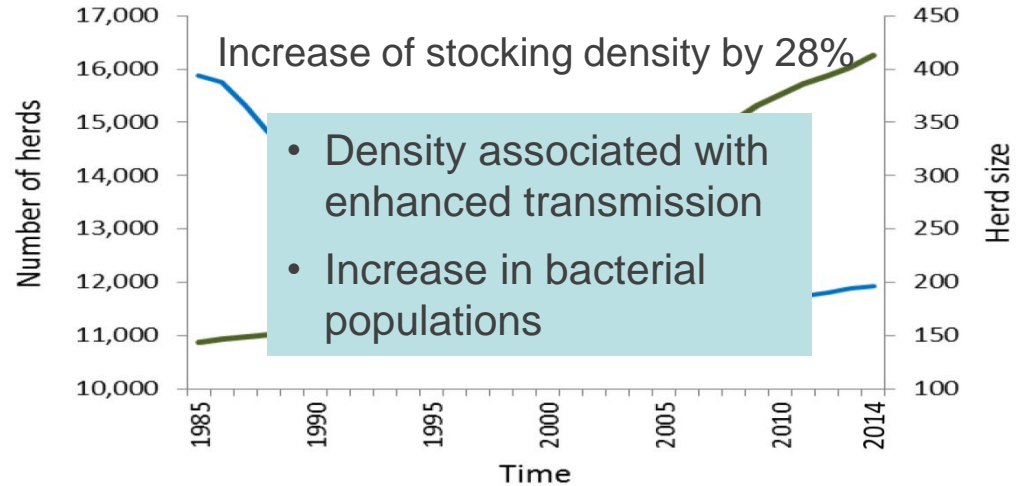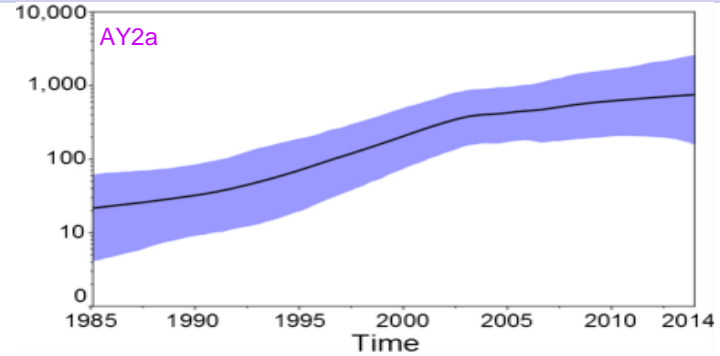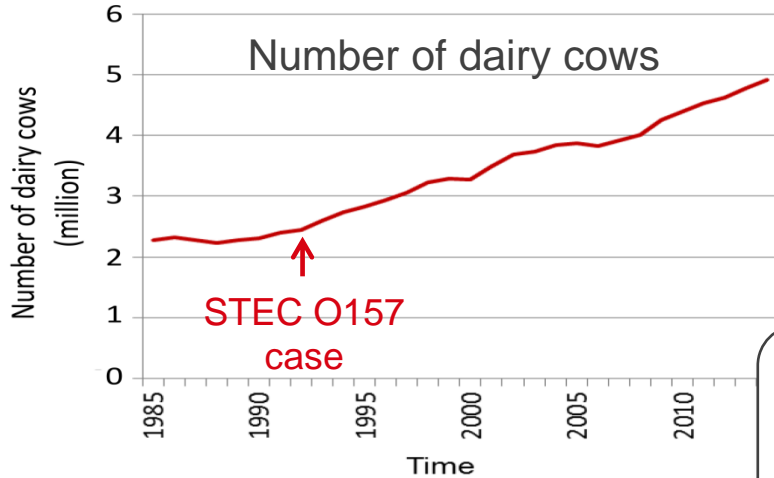
Source: NZ Dairy Statistics 2013/14, LIC & Dairy NZ, Hamilton, NZ

AY2a

Increase of stocking density by 28%

- Density associated with enhanced transmission
- Increase in bacterial populations

Campylobacter (jejuni and coli)

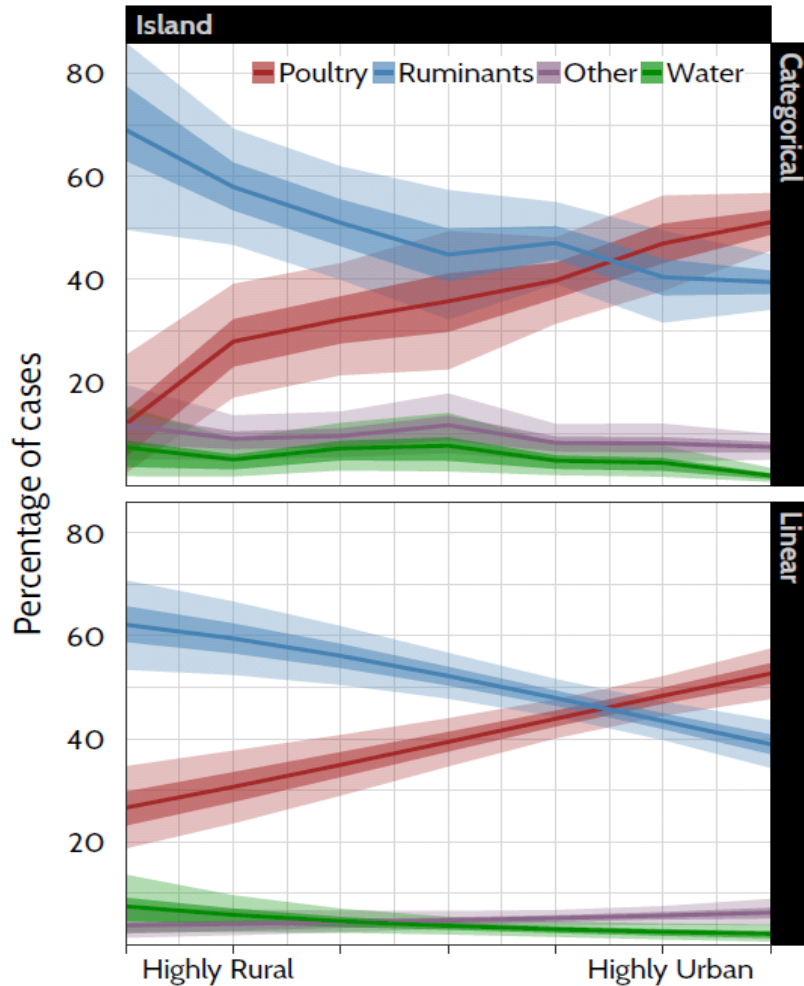# Marked Campylobacteriosis Decline after Interventions Aimed at Poultry, New Zealand

Ann Sears, Michael G. Baker, Nick Wilson, Jonathan Marshall, Petra Muellner, Donald M. Campbell, Robin J. Lake, and Nigel P. French

50% decline in notifications and hospitalisations
Est $50-70M saving per annum

5-year target achieved within 12 -24 months

Poultry interventions

Number of cases per month

Date
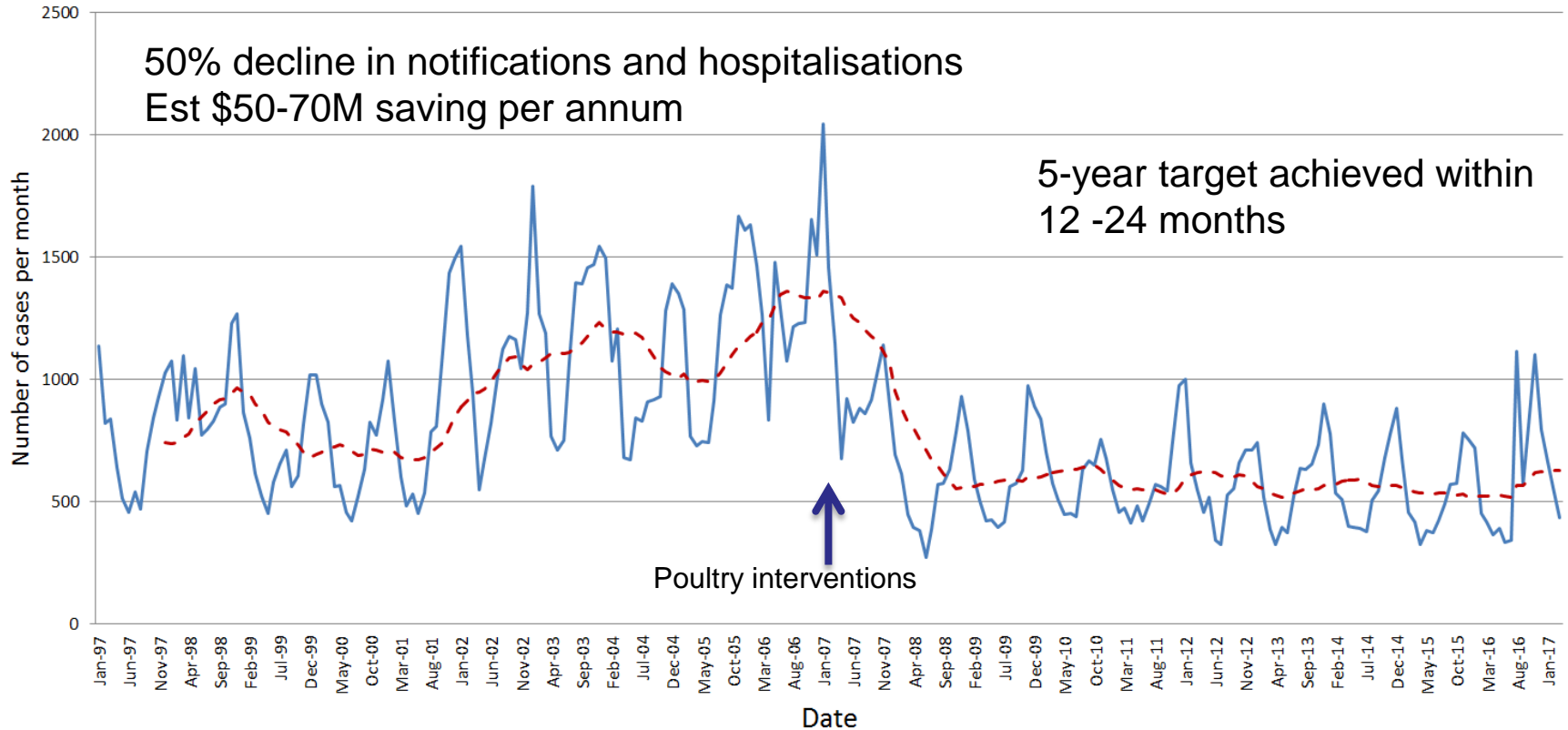
Spatial and temporal modelling of cases

Poultry intervention

— Urban
— Rural no cattle
— Rural high cattle
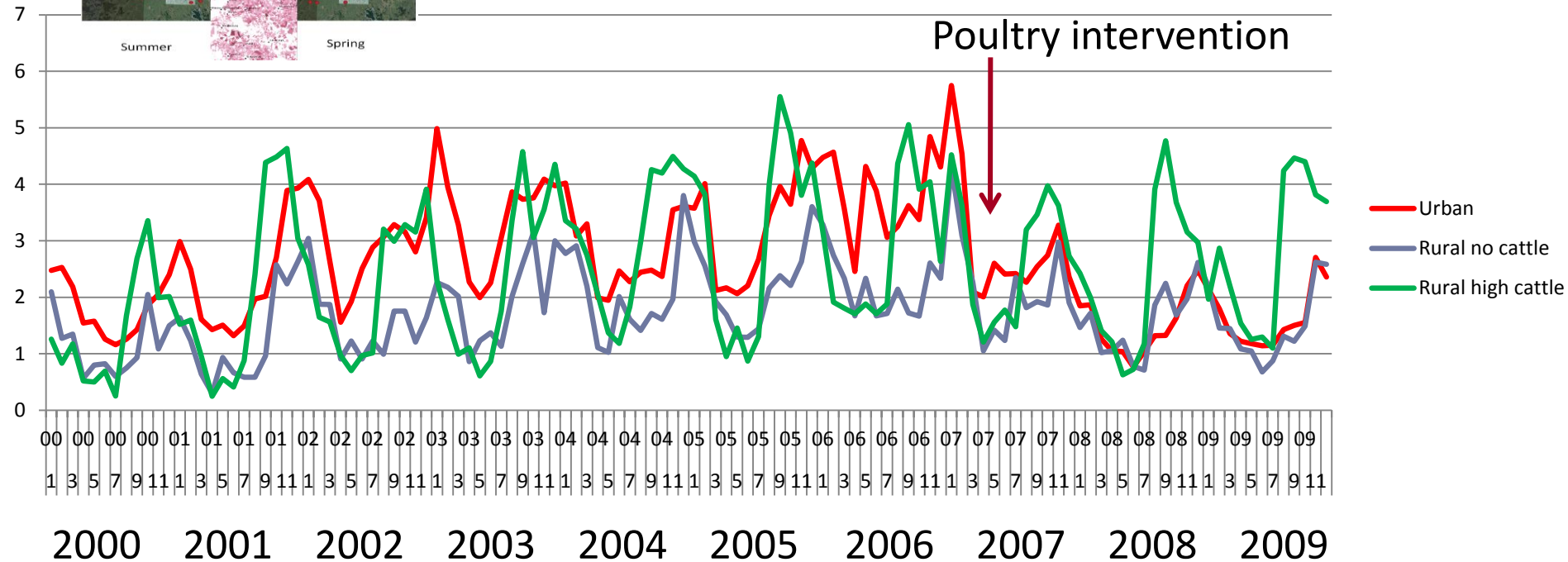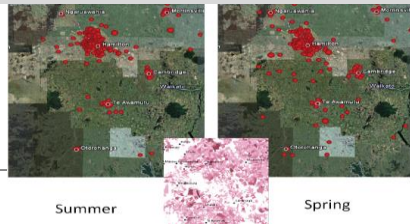
Different sources for urban and rural dwellers

- Using genotyping and modelling
- Jonathan Marshall, Jing Liao and Martin Hazelton

Distinguishing between ruminant sources: sporadic cases source attribution using genotyping
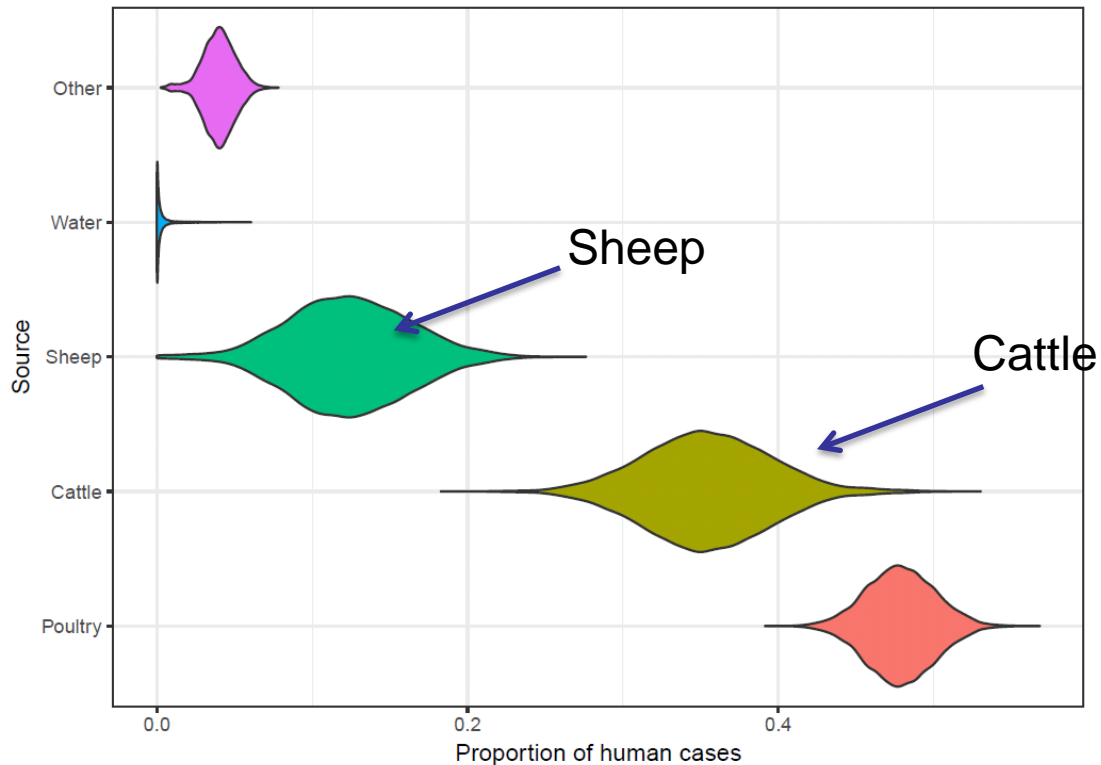
Campylobacter: prevalence in animals and water

Assigning the source of human campylobacteriosis in New Zealand: A comparative genetic and epidemiological approach

Petra Mullner [a,b,*], Simon E.F. Spencer [a,1], Daniel J. Wilson [c], Geoff Jones [d], Alasdair D. Noble [d], Anne C. Midwinter [a], Julie M. Collins-Emerson [a], Philip Carter [e], Steve Hathaway [b], Nigel P. French [a]

Campylobacter: prevalence in animals and water

Table 1

Overview of samples collected in the Manawatu, New Zealand, 2005–2008.

Source	Samples collected	Samples culture positive[a]	Samples confirmed *C. jejuni*[b]	
Human cases	773	661	584	
Fresh chicken carcasses	562	454	415	
Retail meats beef	400	44	33	
Retail meats lamb	418	112	103	
On-farm cattle	145	119	80	} 40-50%
On-farm sheep	133	92	61	
Environmental water	335	140	82	~25%
Total samples	2766	1622	1215	

- More recent study 32 cattle/sheep farms 2015-2016 >80% prev
- Allowing for imperfect test sensitivity: ~100% carriage in all ruminants?
- Similar water estimate to Till et al 2008, JWH (*C. jejuni* in ~30% samples)
- Waikato abstraction points >50% +ve (groundwater –ve)

Importance of water borne *Campylobacter* for public health?

250
40

Chicken
Human
Other Animal
Ruminant
Water
Wild Bird

C. coli

C. jejuni

48
42
2381
61
474
45
53
50
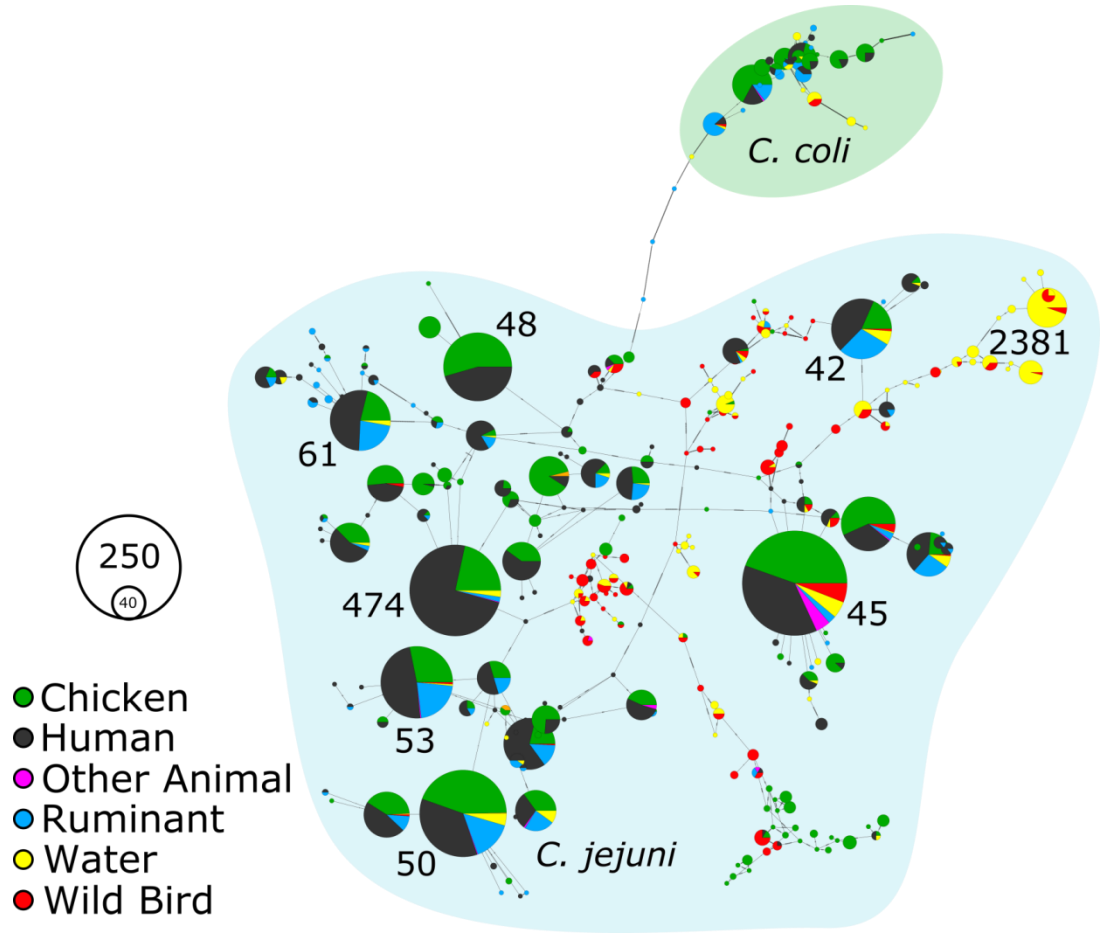
Novel Clonal Complexes with an Unknown Animal Reservoir Dominate *Campylobacter jejuni* Isolates from River Water in New Zealand[▽]

P. E. Carter,[1]* S. M. McTavish,[1]† H. J. L. Brooks,[2] D. Campbell,[3] J. M. Collins-Emerson,[4] A. C. Midwinter,[4] and N. P. French[4]
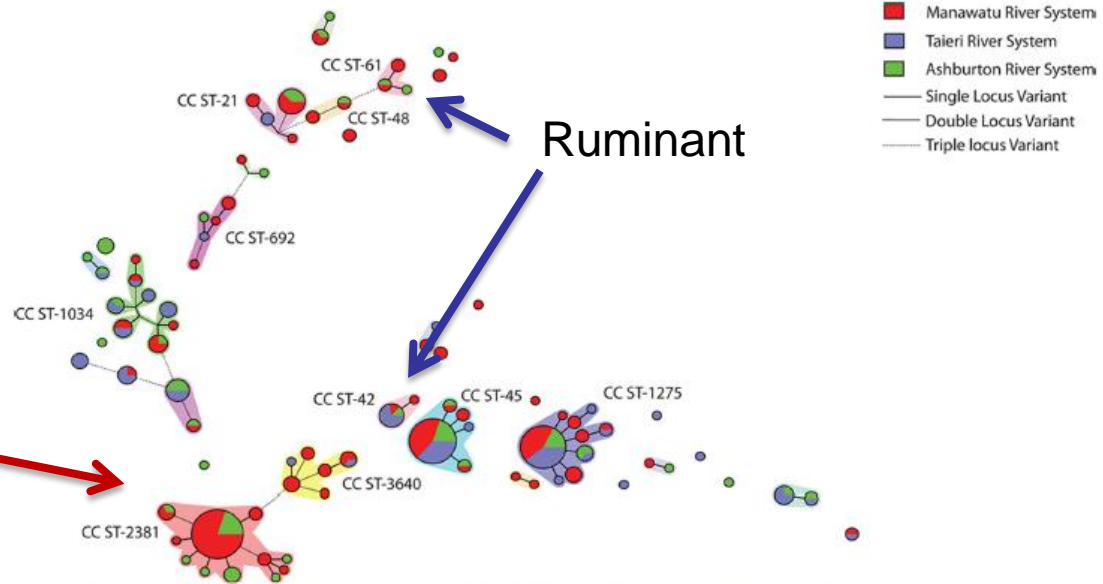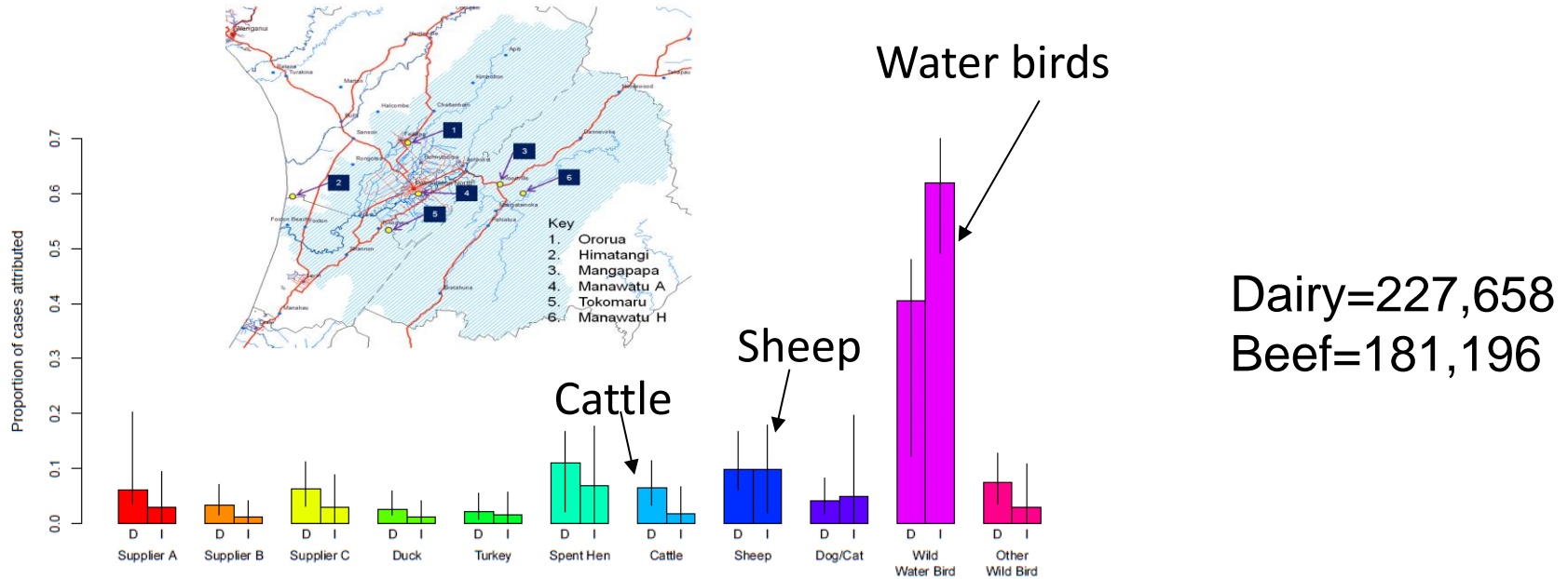
FIG. 1. Minimum spanning tree of New Zealand water isolates based on sequence types. Clusters are identified by the clonal complex number [as]sociated with the sequence types in the cluster.

Campylobacter sources in water

Most *Campylobacter* in water in Manawatu from wildlife – even in dairy catchments

Water birds

Sheep

Cattle

Dairy=227,658
Beef=181,196

Important:

Strains found in pukeko have not been found in humans
But cattle and sheep ones have….

Water low attribution to <u>sporadic</u> cases of campylobacteriosis (<5%)

NZ drinking water outbreaks: *Campylobacter*

- 1986
  - Ashburton, 19 cases in two weeks attributed to poorly chlorinated drinking water drawn from river
- 1990 –
  - 44 cases linked to livestock contaminating camp water supply
- 1997 –
  - 67 cases linked to drinking water contaminated with river water
- 2008 –
  - Pahiatua, Manawatu 9 cases linked to rainfall and river flow
- 2012 –
  - Darfield incident 110 cases
- 2016 –
  - Havelock North 5000 cases

Manawatu drinking water outbreak in 2008

Water flow
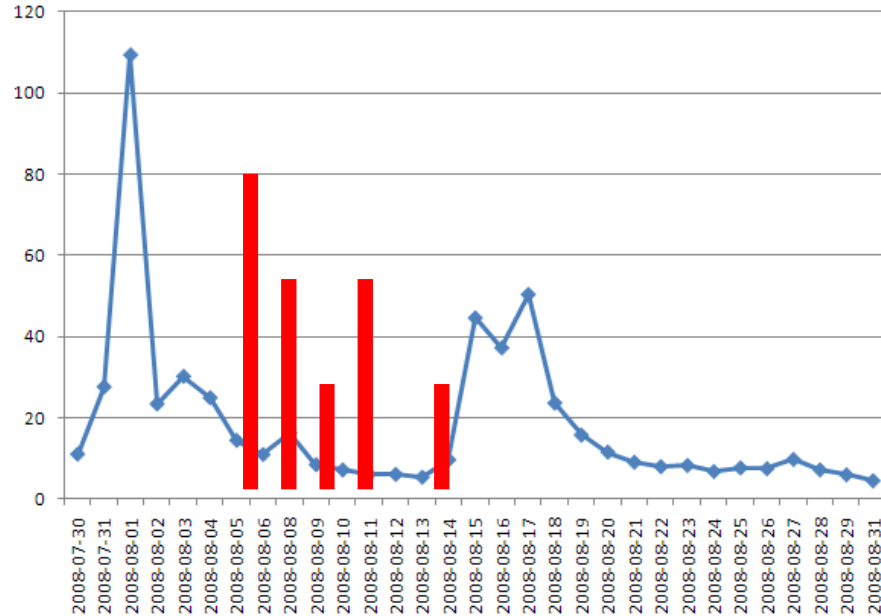
Comments on drinking water → Muddier than usual"toxic in it's self"
smells of dirt, brown
water been brown, "crap"

Attribution of Pahiatua 2008 human *C. jejuni* isolates
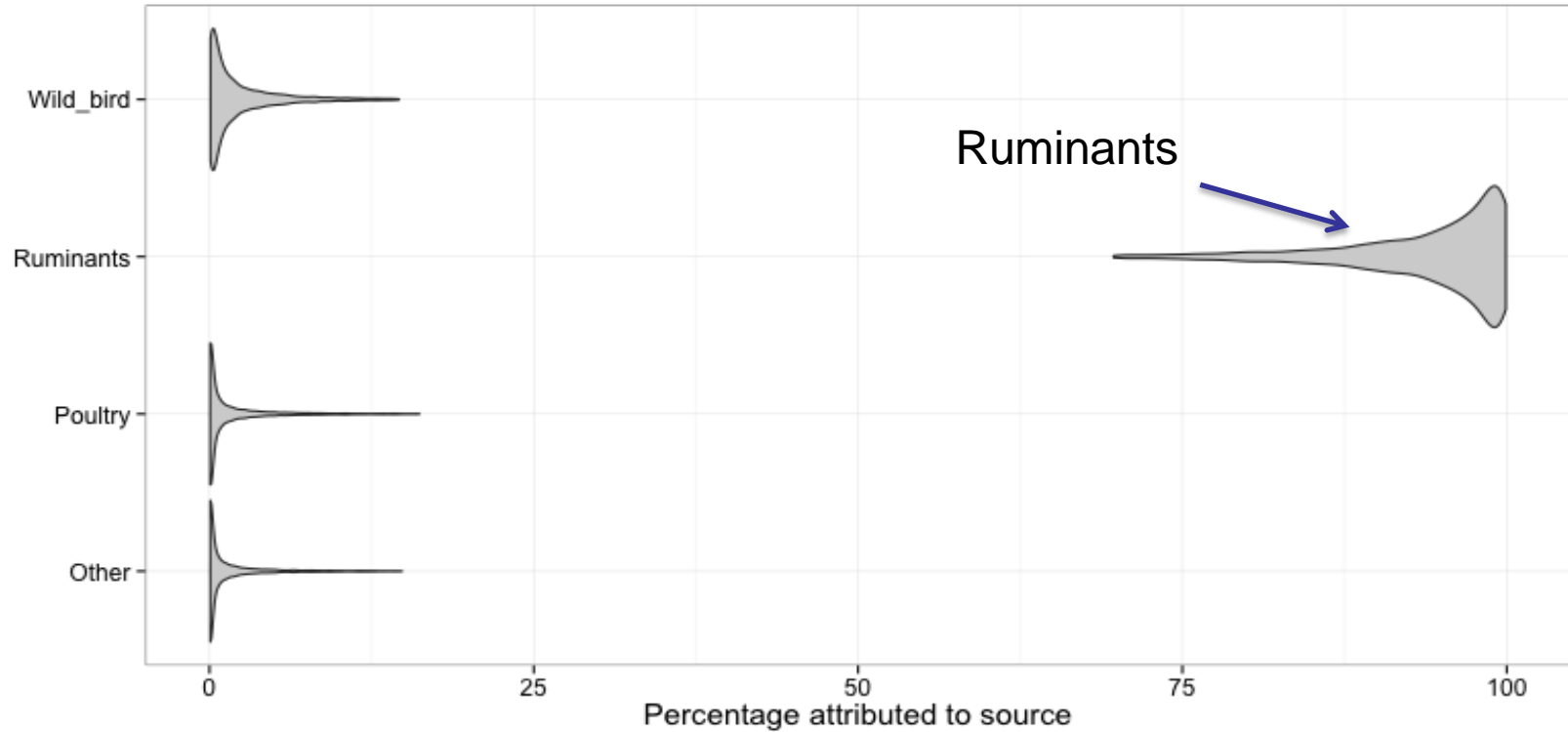
Use of molecular typing to inform the source of the Havelock North outbreak

- Two methods used
  - Mbit
  - Whole genome sequencing
- Isolates compared
  - Animals
  - Water
  - Human cases

Sampling and screening for outbreak relevant isolates: MBiT

- 100s of samples were taken from patients, environmental water samples and animal faecal from neighboring paddocks;

- MBiT analysis used as cheap rapid screen to exclude outbreak irrelevant isolates.

MBiT (Multiplex Ligation-Dependent Probe Amplification–Binary Typing) analysis for presence/absence of 18 genes

Isolates from **human, reticulated water** and **ovine** shared identical type

Legend:
- Human
- Surface water
- Ovine
- Reticulated Water
- Pit
- Bovine
- Bore water
- Animal

Several types of human isolates.
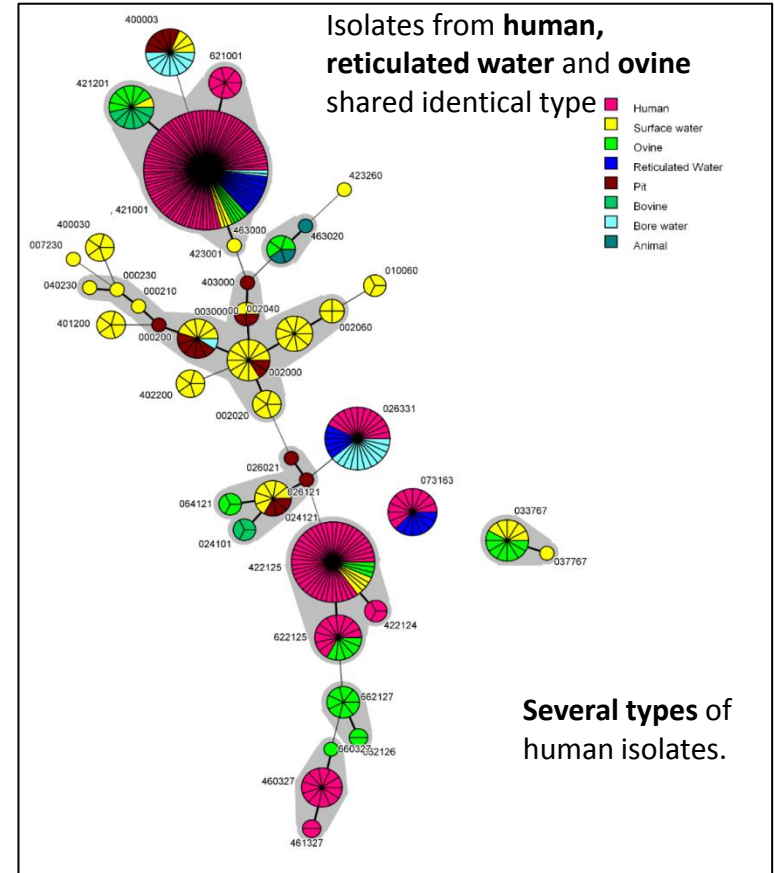
Multiple types found in water but not all in humans

Whole-genome sequencing for the outbreak isolates

To test hypotheses at a higher resolution **120 isolates** sent for whole-genome sequencing.
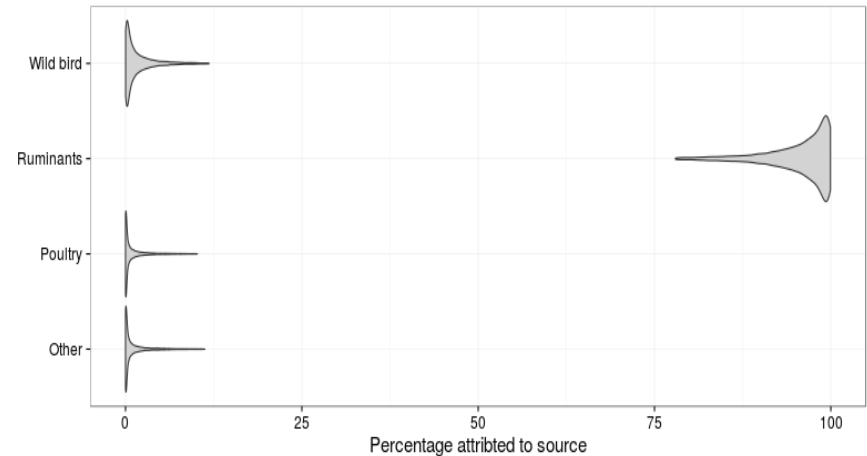
- human (patient) isolates, n = 80;

- animal (sheep and cow) isolates: n = 16;

- environmental isolates: n = 24;
    - reticulated water
    - bore water
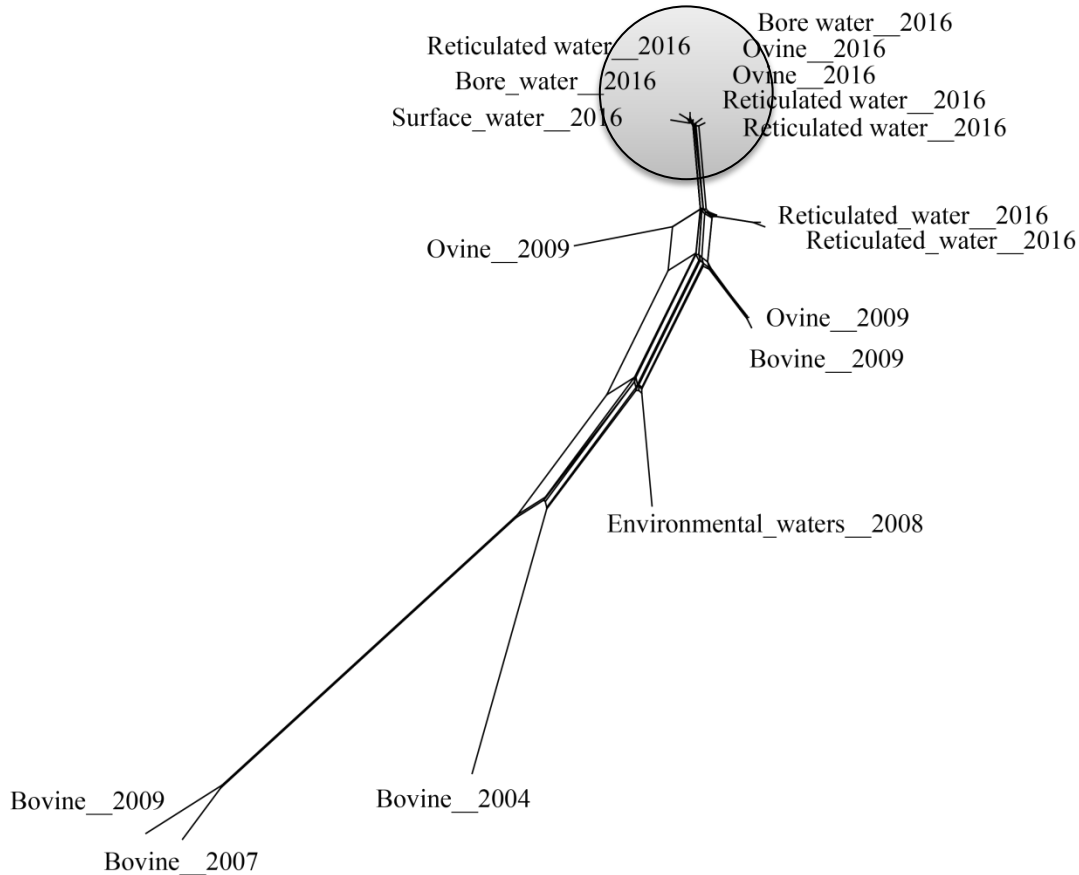   - river water
    - pit water
    - surface water
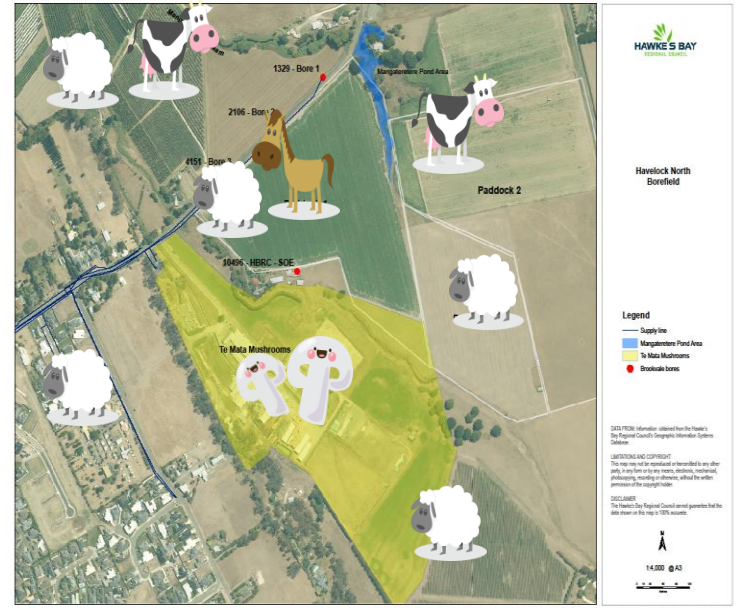
Initial 7 gene MLST analysis: compositions were similar
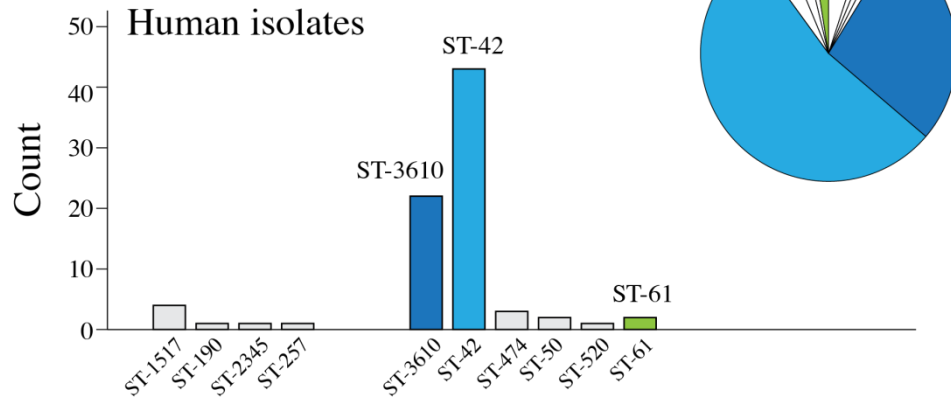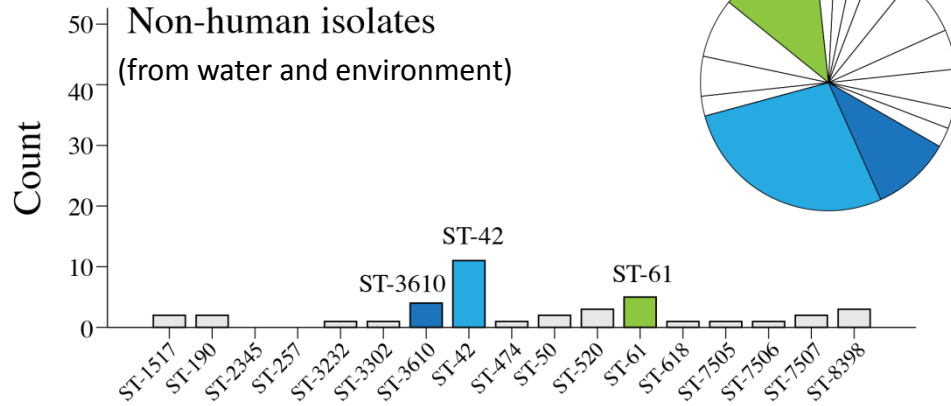
- ST42 and ST61 internationally ruminant strains
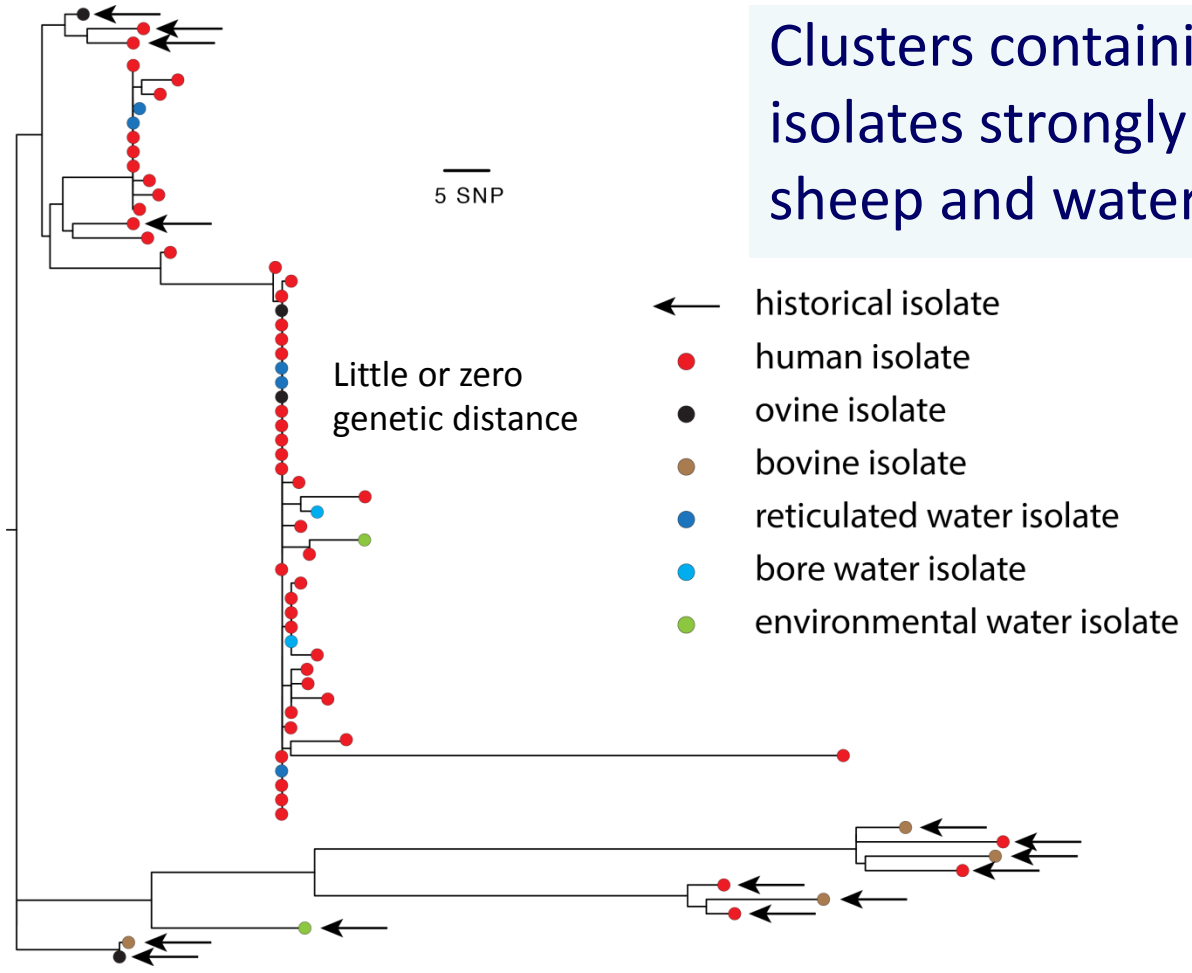- Next step to use all WGS data
- Applied to ST42 and ST3610

Relationship of ST-42 **<u>non-human</u>** isolates by *ad hoc* wgMLST

Isolates from **ovine**, **reticulated water**, **bore (well) water** and surface water clustered together.

New Zealand historical ST42 isolates were used for comparison.

Clusters containing patient isolates strongly related to sheep and water isolates

Legend:
← historical isolate
● human isolate
● ovine isolate
● bovine isolate
● reticulated water isolate
● bore water isolate
● environmental water isolate

5 SNP

Little or zero genetic distance
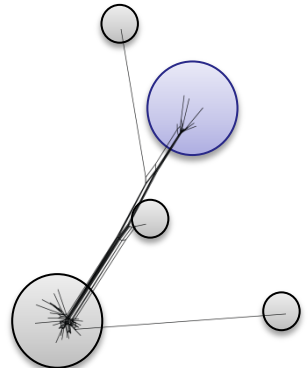
Phylogenetic tree inferred from the sequences of 1075 shared-loci found in *ad hoc* wgMLST of 66 ST-42 isolates.

ST-3610: similar findings

- 🔴 human isolate
- ⚫ ovine isolate
- 🔵 surface water isolate

Little or zero genetic distance

5 SNP
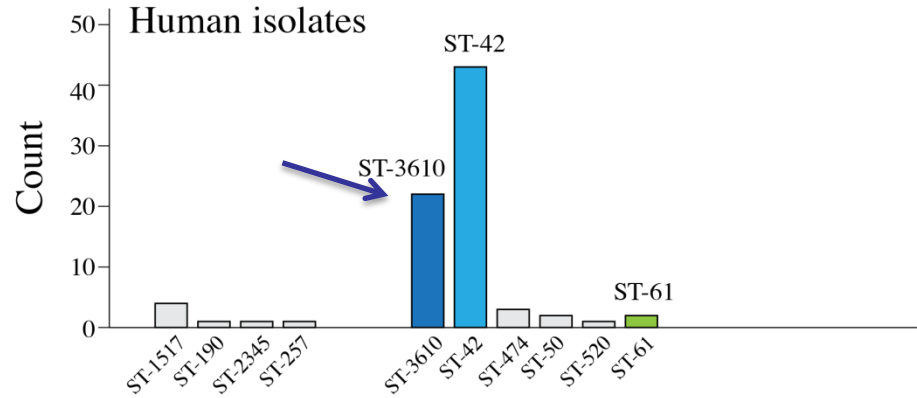
Phylogenetic tree inferred from the sequences of 1390 shared-loci found in *ad hoc* wgMLST analysis of the 26 ST-3610 isolates.
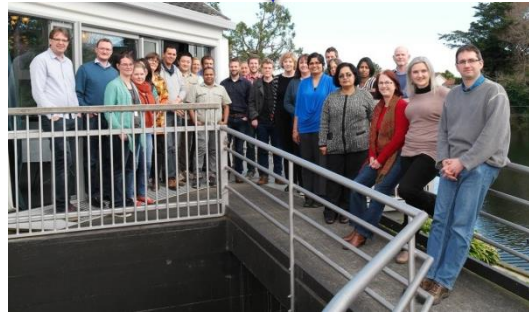
Summary of Havelock North analysis

- Using *ad hoc* **wgMLST** and phylogenetic analysis evidence of **clonal expansion** in branches of ST42 and ST3610.

- Branches contained **patient isolates** mixed with **water** and **ovine isolates** from fields adjacent to the bores (wells) supplying the reticulated water, **strongly** suggesting their close relationship and a **likely origin of contamination of the water supply**.

- Distantly related to historical isolates

- The outbreak was caused by multiple clones of *C. jejuni* of different lineages.

Conclusions

- Waterborne pathogens highly prevalent in livestock in New Zealand.

- Surface waters frequently contaminated with ruminant-associated pathogens.

- STEC are biggest concern due to severe health impacts.

- Increasing stock numbers and densities likely to increase transmission between animals, and from animals to people.

- Adverse weather events (climate change) also likely to exacerbate transmission unless extra measures in place.

- New tools such as Whole Genome Sequencing and modelling enable robust inference to be made, linking animal sources to water and human health.
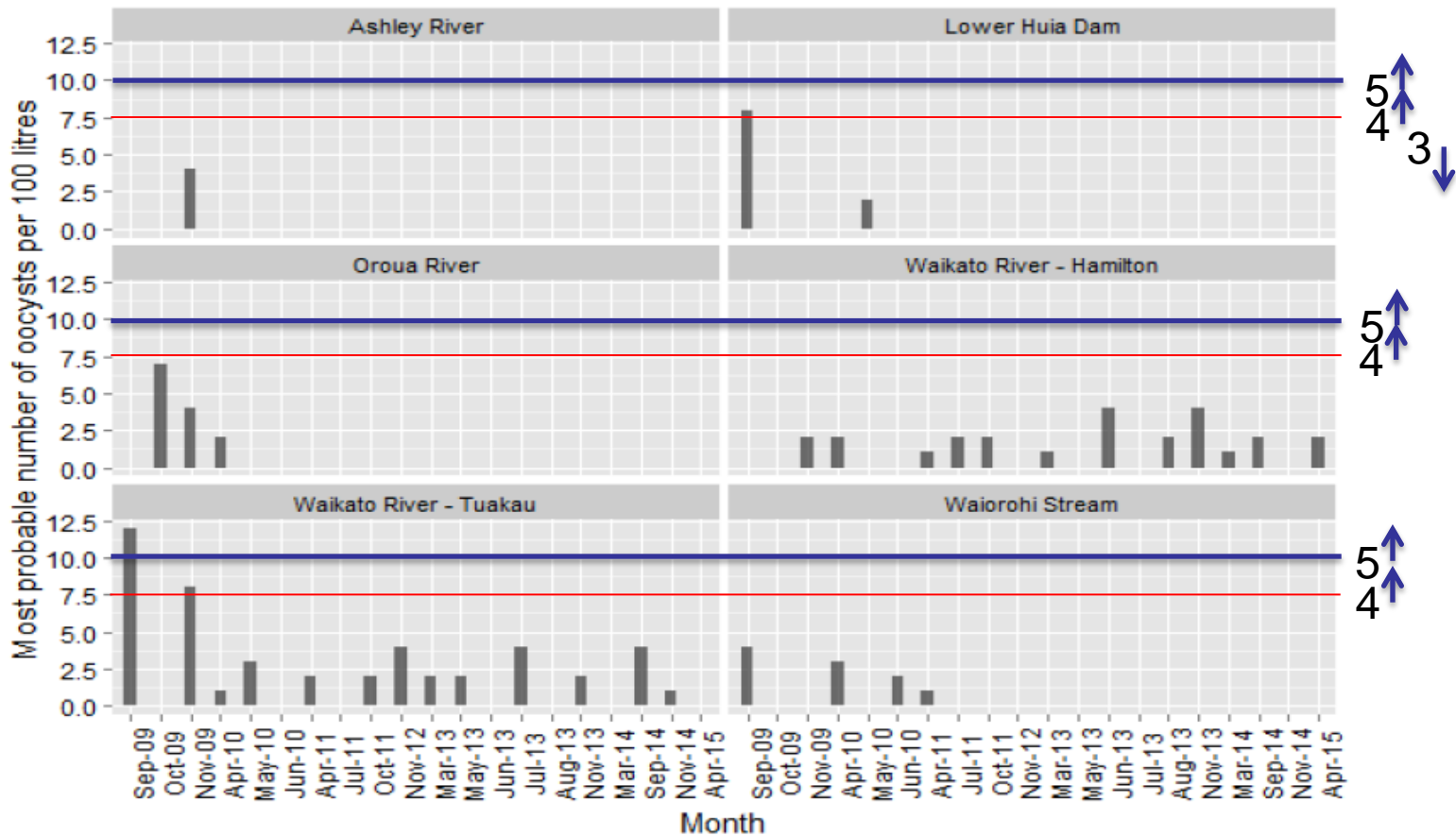
Acknowledgements
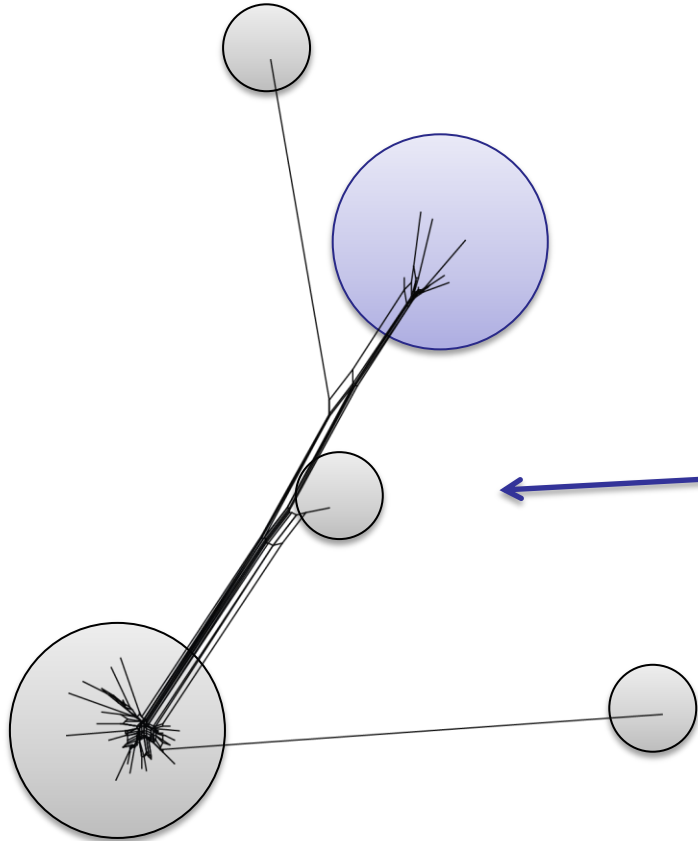
- Human isolates formed **several distinct** clusters.
- Expected given diversity in source
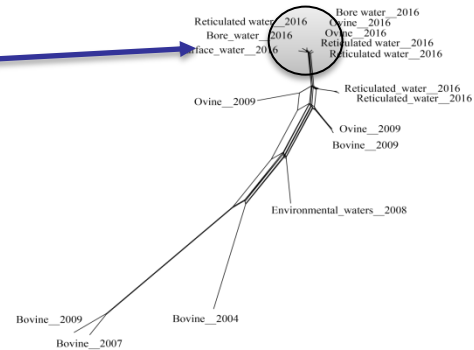
Water- low attribution for sporadic cases (<5%)
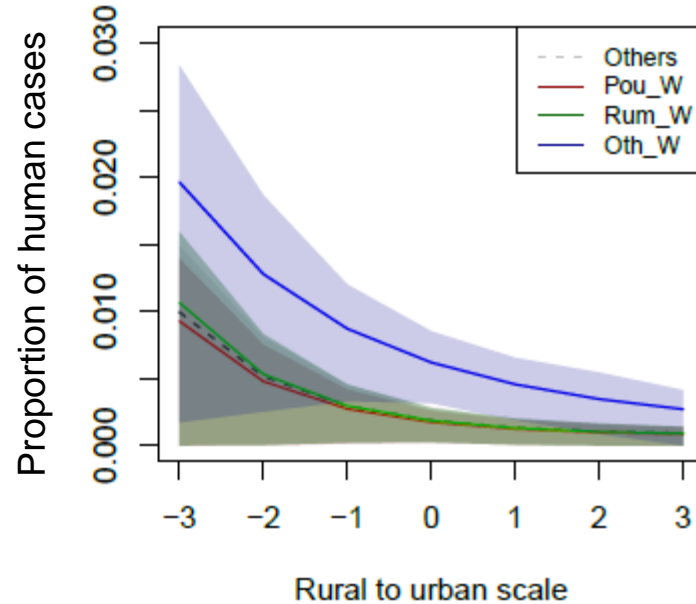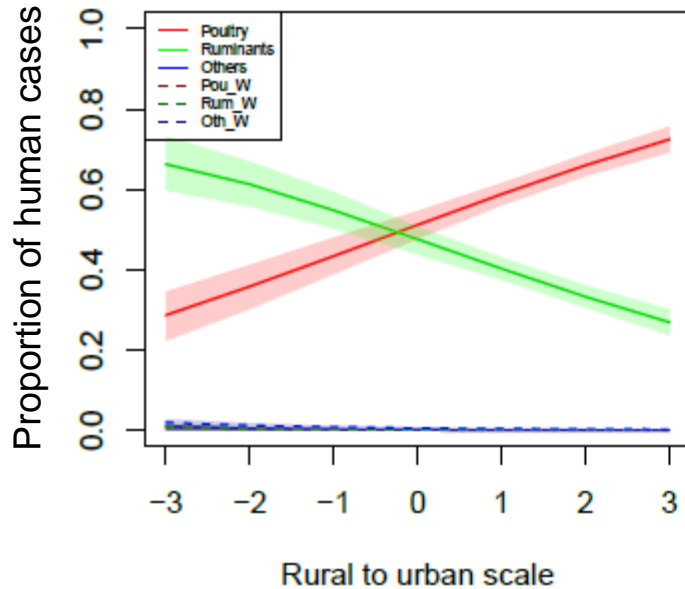
Concentration of crypto oocysts per 100L

Abstraction points

E. coli > 200/ml
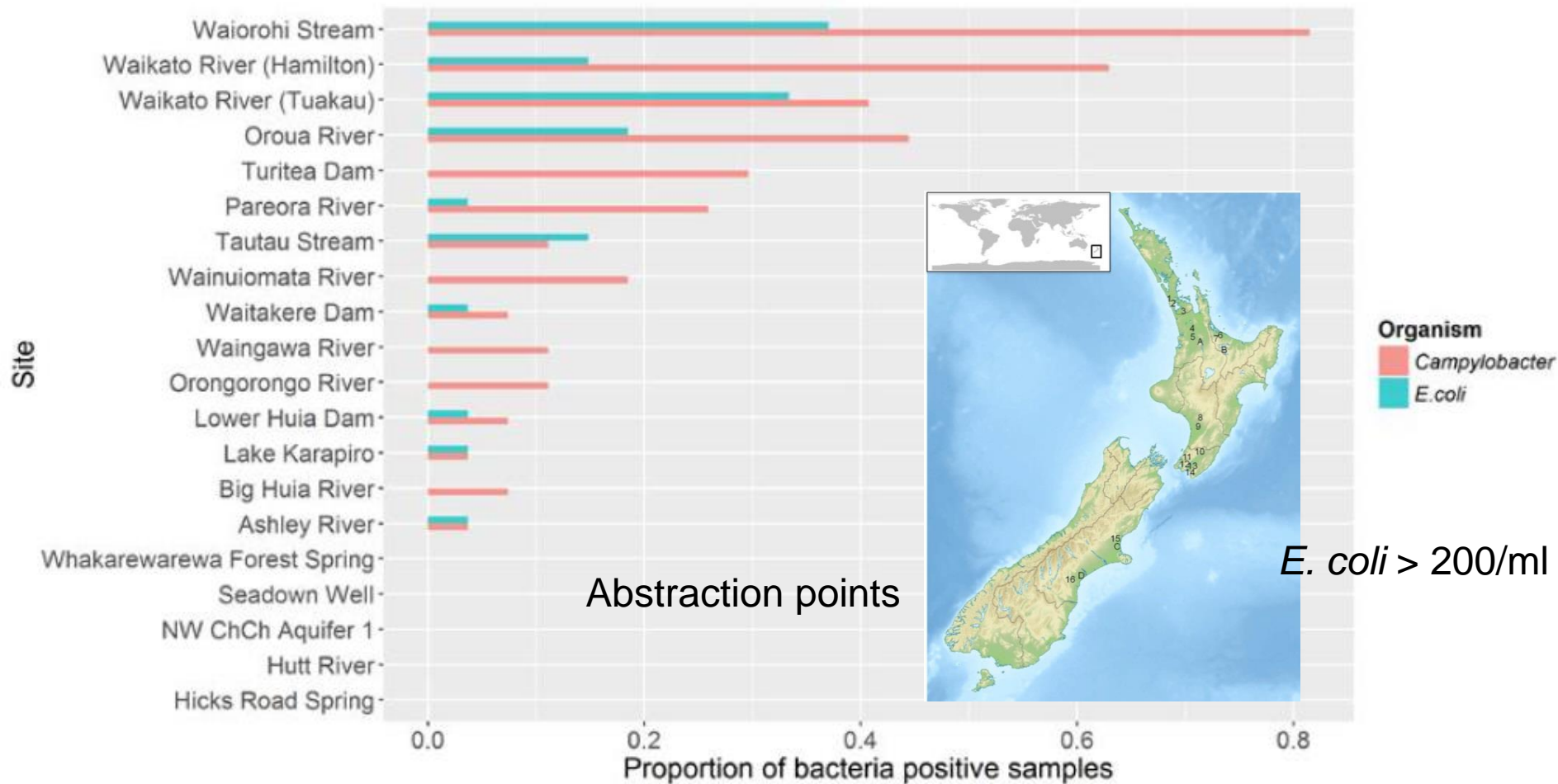
Climate and land use changes

Table 2. Influence of environmental changes on *Cryptosporidium* and *Giardia*

Expected environmental change	Effect on pathogen survival/transmission[a]	Change in risk[b]
Increasing rainfall	↑ Runoff	+
	↑ Resuspension of oocysts in water courses	+
	↑ Flooding	+
	↑ Contamination of water courses by sewage effluent	+
Increasing livestock intensification	↑ Pathogen sources/reservoirs	+
	↑ Contamination of water courses	+
	↑ Contamination of food sources	+
	↑ Population connectivity	+
	↓ Population connectivity	−
	↑ Ecological connectivity	+
	↓ Ecological connectivity	−

[a]↑, increase; ↓, decrease.

[b]+, increase in disease risk; −, decrease in disease risk.

Lal, Baker, Hales and French 2013