A genomic and modelling approach to understanding the evolution, source and transmission of a recently emerged antimicrobial resistant lineage of *Campylobacter*

Nigel French, Sabrina Greening, Ji Zhang, Anne Midwinter, David Wilkinson, Ahmed Fayaz, Marti Anderson, Jonathan Marshall, Patrick Biggs, Lynn Rogers, Kristen Dyet, Brent Gilpin, Danielle Ingle, Glenn Carter, Kerry Mulqueen, Carolyn Gates, Debbie Williamson.

OHA 2020

One Health AOTEAROA

New Zealand FOOD SAFETY SCIENCE & RESEARCH CENTRE

mEpiLab

IDReC Infectious Disease Research Centre

Te Kunenga ki Pūrehuroa MASSEY UNIVERSITY

THE UNIVERSITY OF MELBOURNE

E/S/R Science for Communities

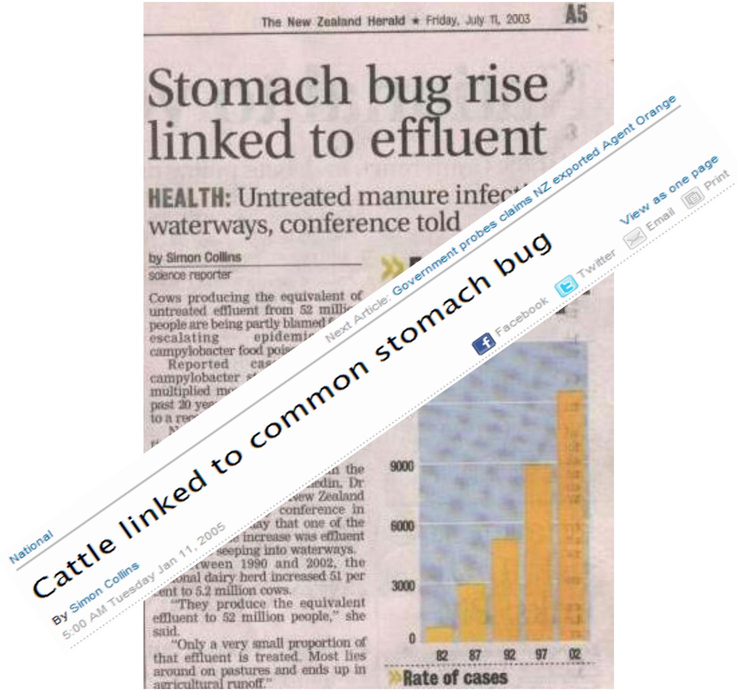
New Zealand: Food and waterborne diseases high on the list of notifiable diseases

Number of notifications by disease, 2019

Disease	Total
Campylobacteriosis	6202
Measles	2213
Giardiasis	1749
Pertussis	1206
Salmonellosis	1188
Yersiniosis	1186
Shiga toxin-producing *Escherichia coli* infection	1101
Cryptosporidiosis	1035
Invasive pneumococcal disease	497
Acute gastroenteritis	489

Most zoonotic, many food and waterborne

Campylobacter in NZ*:* 1980-2006



The New Zealand Herald ★ Friday, July 11, 2003 A5

Stomach bug rise linked to effluent

HEALTH: Untreated manure infect… waterways, conference told

by Simon Collins
science reporter

Cows producing the equivalent of untreated effluent from 52 milli… people are being partly blamed f… escalating epidemi… campylobacter food pois…
Reported ca… campylobacter … multiplied mo… past 20 yea… to a rec…

…n the …edin, Dr …New Zealand … conference in …ay that one of the …e increase was effluent …seeping into waterways.
…tween 1990 and 2002, the …onal dairy herd increased 51 per …ent to 5.2 million cows.

"They produce the equivalent effluent to 52 million people," she said.

"Only a very small proportion of that effluent is treated. Most lies around on pastures and ends up in agricultural runoff."

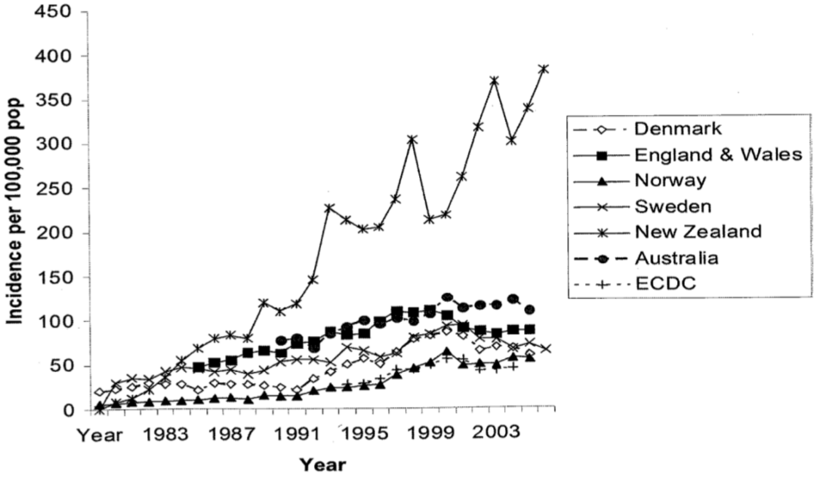
Rate of cases

National

Cattle linked to common stomach bug

By Simon Collins
5:00 AM Tuesday Jan 11, 2005

Next Article: Government probes claims NZ exported Agent Orange



THE NEW ZEALAND MEDICAL JOURNAL
Vol 119 No 1243 ISSN 1175 8716

2006

Regulation of chicken contamination urgently needed to control New Zealand's serious campylobacteriosis epidemic

Michael Baker, Nick Wilson, Rosemary Ikram, Steve Chambers, Phil Shoemack, Gregory Cook

Manawatū sentinel site 2005-2018

- ‘One health’ approach: human, animal and environment
- Identify Multilocus Sequence Types common to particular sources
- Modelling (reservoir attribution)

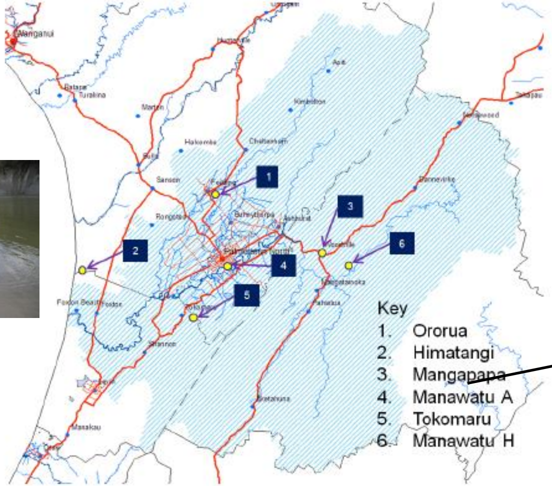
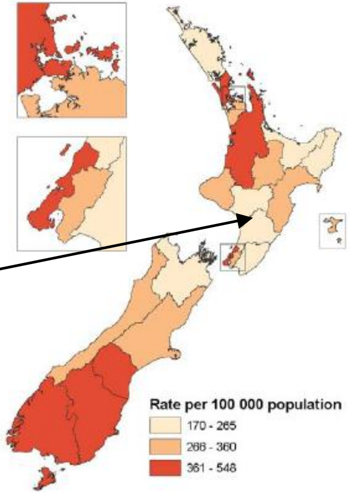
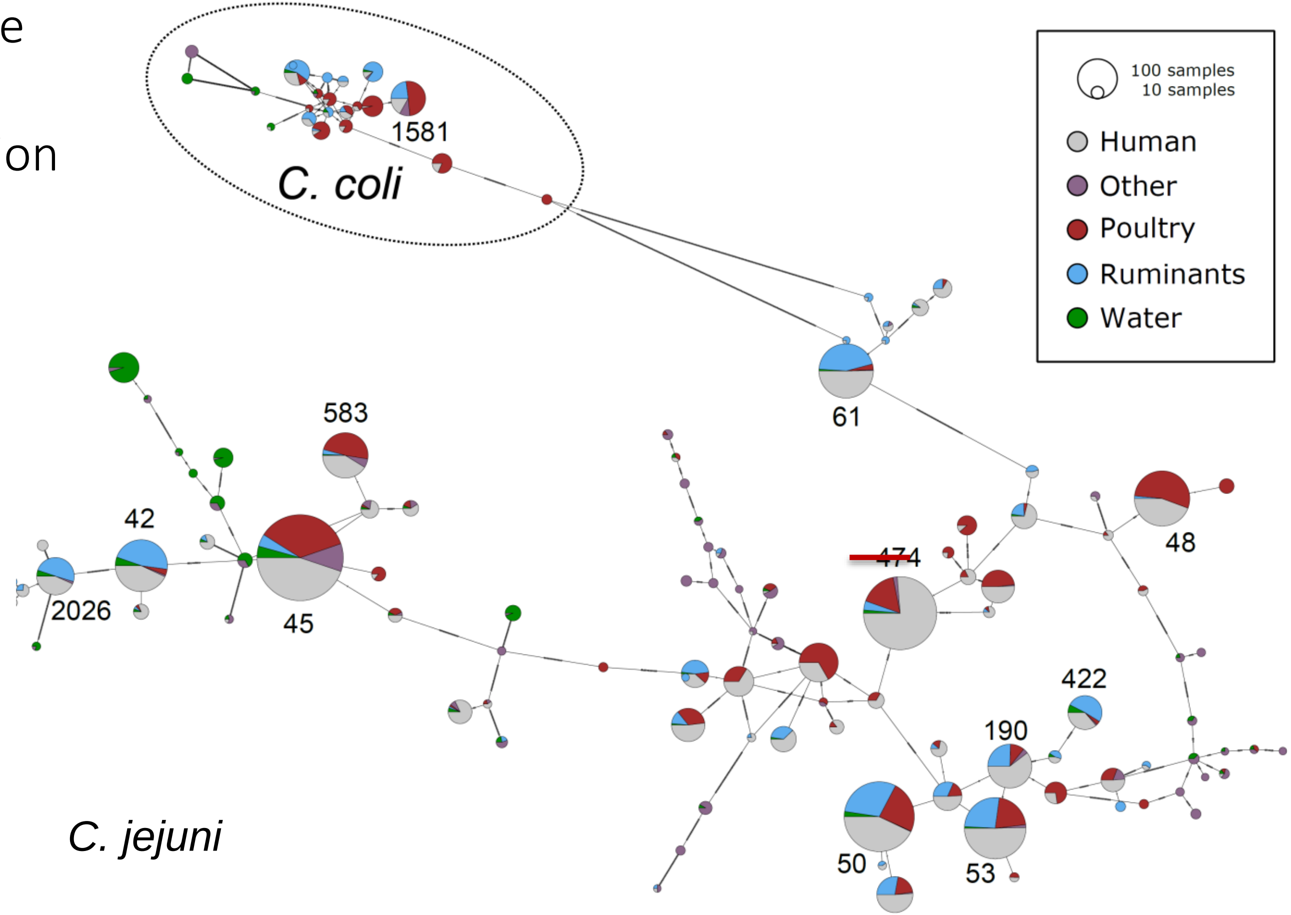


Figure 7. Campylobacteriosis notifications by DHB, 2004

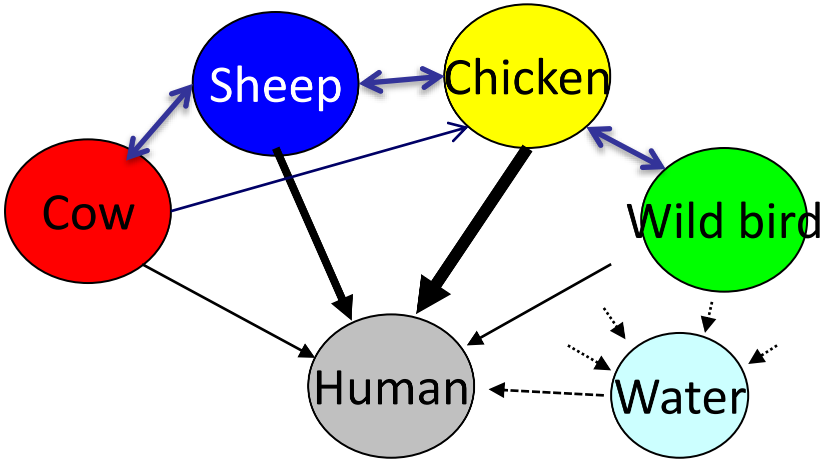


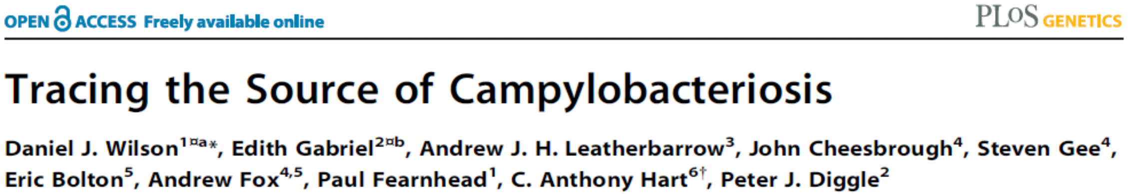
Manawatu sentinel site
2005-18
Campylobacter population structure:
according to 7-genes



Reservoir attribution
‘Asymmetric Island model’ (AIM)

- Population genetics / evolutionary modelling approach
- Used to find out source of human infections
- Flow into the human “island” from animal “islands”



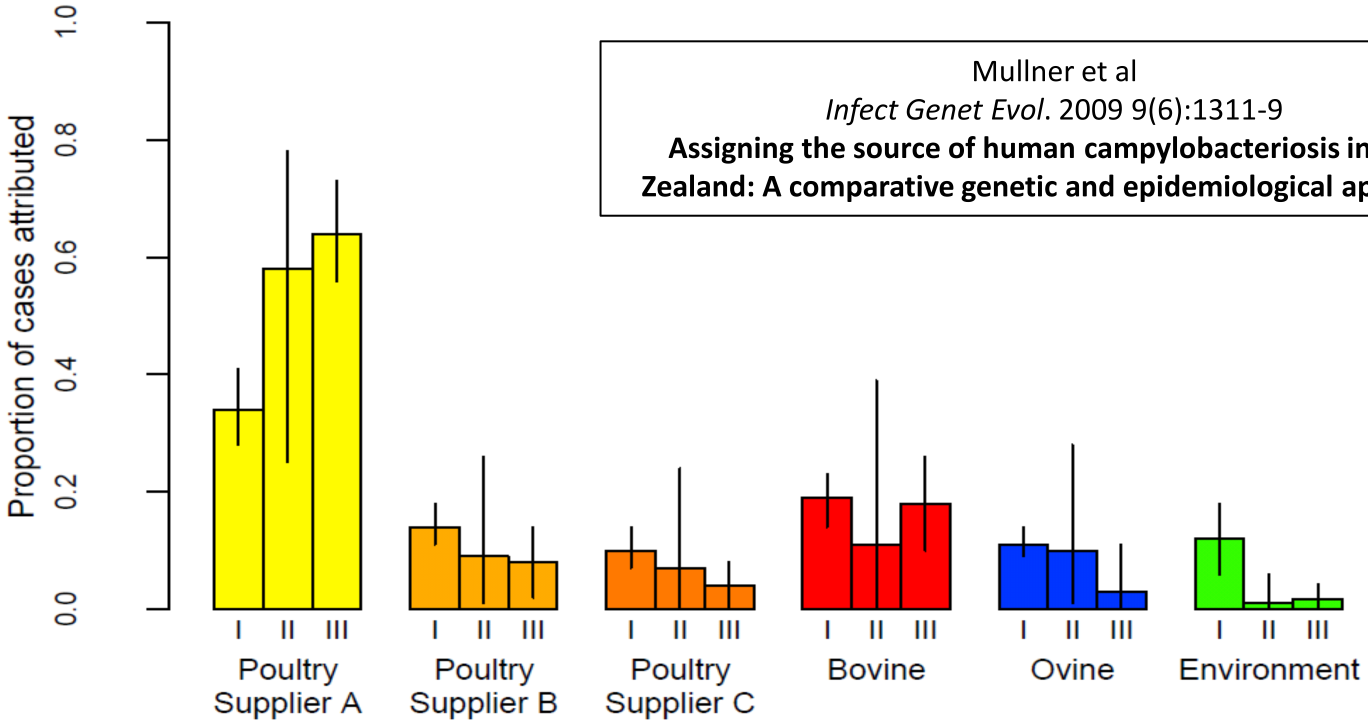

OPEN ACCESS Freely available online

PLoS GENETICS

Tracing the Source of Campylobacteriosis

Daniel J. Wilson[1¤a*], Edith Gabriel[2¤b], Andrew J. H. Leatherbarrow[3], John Cheesbrough[4], Steven Gee[4], Eric Bolton[5], Andrew Fox[4,5], Paul Fearnhead[1], C. Anthony Hart[6†], Peter J. Diggle[2]

Reservoir attribution



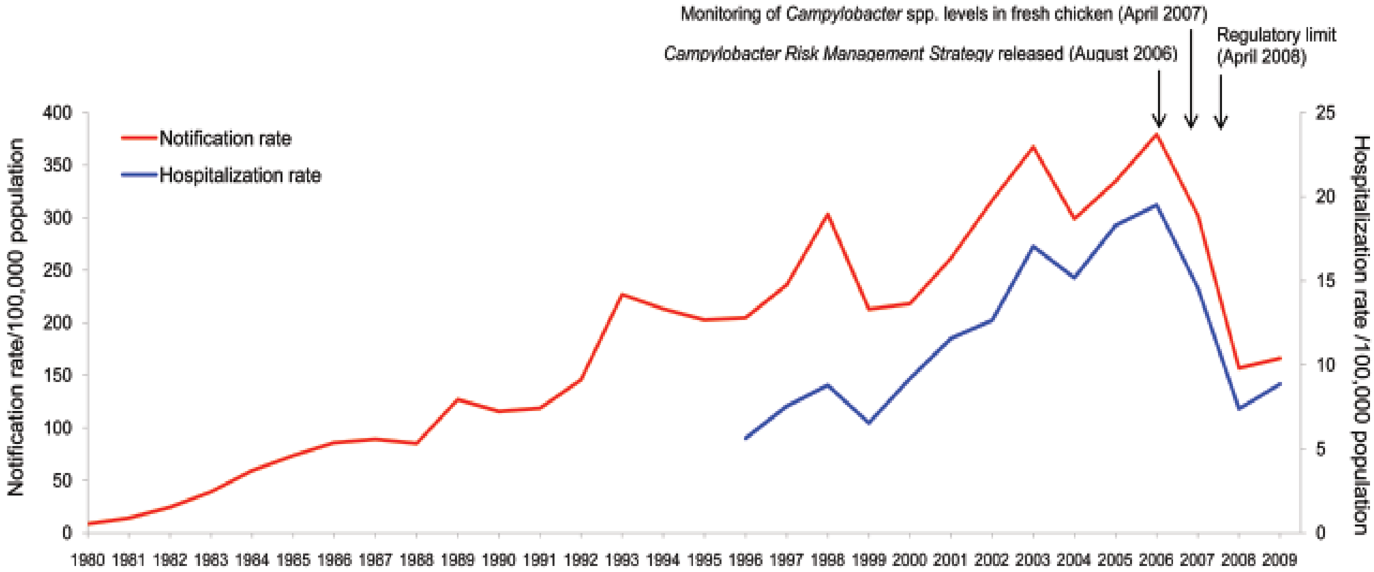
Mullner et al
Infect Genet Evol. 2009 9(6):1311-9
Assigning the source of human campylobacteriosis in New Zealand: A comparative genetic and epidemiological approach

NEW ZEALAND FOOD SAFETY AUTHORITY

Campylobacter in Poultry – Risk Management Strategy

2007 - 2010

Relationship between campylobacteriosis notifications and hospitalisations

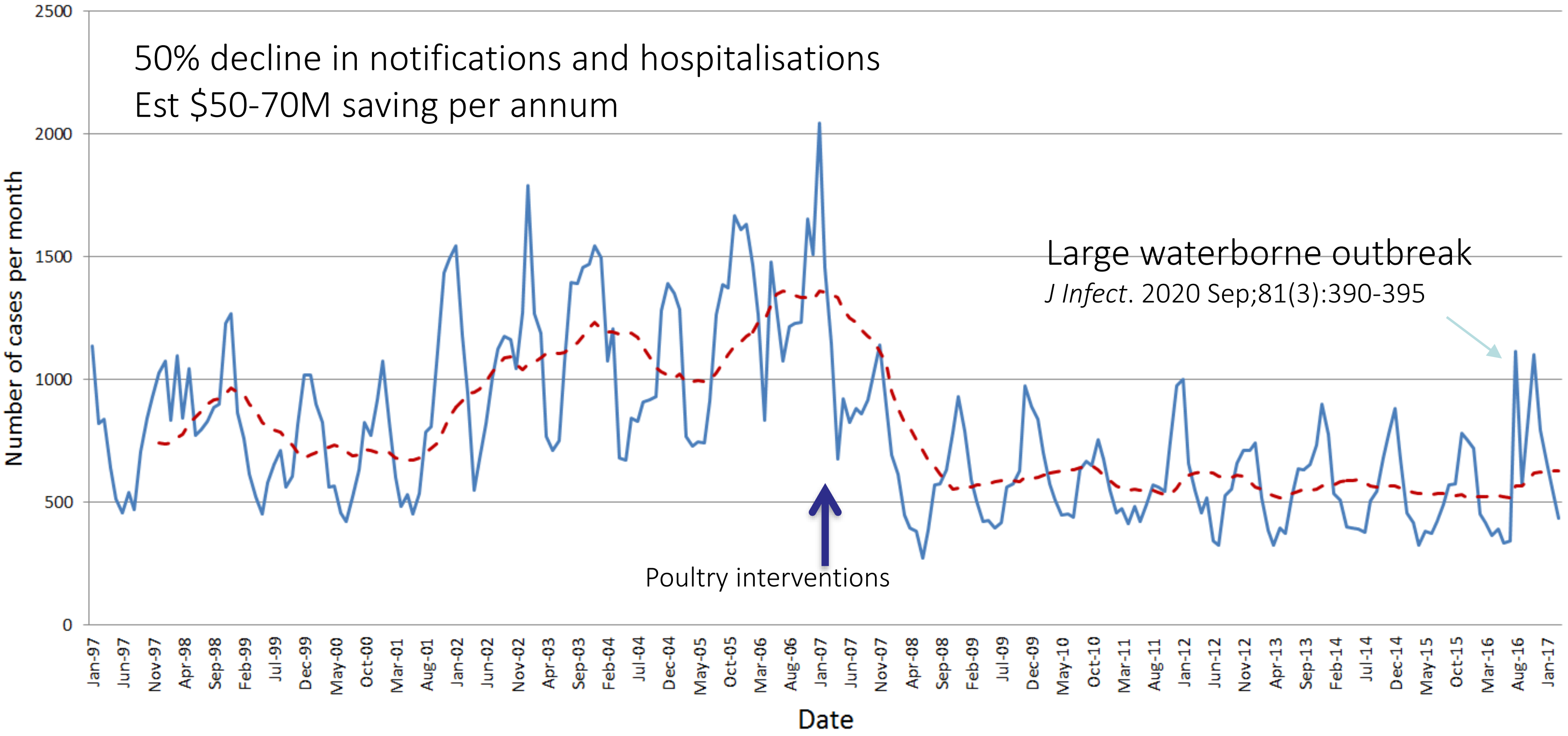


Data Sources: ESR Ltd notification data; NZHIS hospitalisation data (filtered)

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

Sears et al 2011,
Emerging Infectious Diseases 17, 1007-15



50% decline in notifications and hospitalisations

Est $50-70M saving per annum

Large waterborne outbreak

J Infect. 2020 Sep;81(3):390-395

Poultry interventions

Arrival of ST-6964 in 2014 and antibiotic resistance

SUNDAY STAR★TIMES
November 22, 2015
NEWS A3

Superbug found in chicken

Scientists are alarmed at the unprecedented discovery of a strain that resists drugs and has crossed into humans around NZ, writes Susan Edmunds.

A new superbug has been found in chicken from three of New Zealand's four major poultry suppliers. Groundbreaking research reveals the new antibiotic-resistant strain of campylobacter spreads to humans, which could make it hard to treat serious cases of infections.

Campylobacter occurs naturally in the gut of chickens but is the leading cause of food poisoning, with about 7000 cases reported in New Zealand each year.

The antibiotic-resistant strain was first found in 2014 and has now been identified in human cases in Manawatu, Auckland and Wellington.

The study, by Nigel French of Massey University and ESR microbiologist Debbie Williamson, found three of the major poultry suppliers in the North Island tested positive for the strain. A fourth was still waiting for test results. The pair would not name the companies.

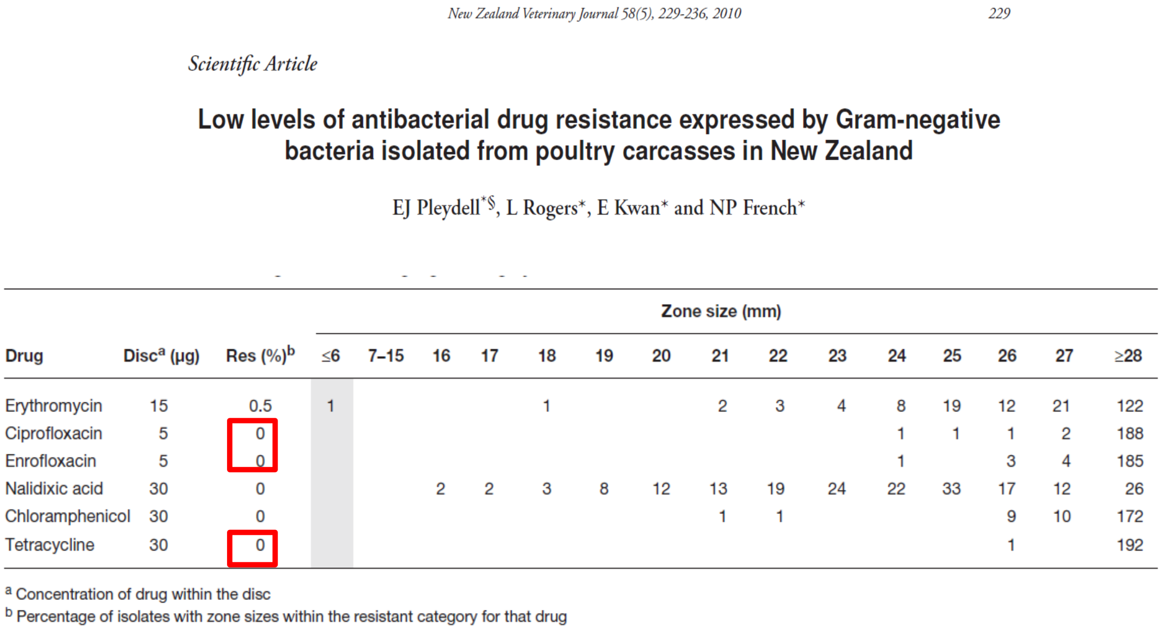
The resistance means two antibiotics – fluoroquinolones and tetracyclines – would fail in treating the infection. But erythromycin, which is most

Which comes first?

The chicken or the campylobacter

Photo: CHRIS McKEEN / FAIRFAX NZ
Graphic: NEIL BOND

Antibiotic resistant Campylobacter in NZ



New Zealand Veterinary Journal 58(5), 229-236, 2010 229

Scientific Article

Low levels of antibacterial drug resistance expressed by Gram-negative bacteria isolated from poultry carcasses in New Zealand

EJ Pleydell*§, L Rogers*, E Kwan* and NP French*

Drug	Disc[a] (µg)	Res (%)[b]	≤6	7–15	16	17	18	19	20	21	22	23	24	25	26	27	≥28
			Zone size (mm)														
Erythromycin	15	0.5	1				1			2	3	4	8	19	12	21	122
Ciprofloxacin	5	0											1	1	1	2	188
Enrofloxacin	5	0											1		3	4	185
Nalidixic acid	30	0			2	2	3	8	12	13	19	24	22	33	17	12	26
Chloramphenicol	30	0								1	1				9	10	172
Tetracycline	30	0													1		192

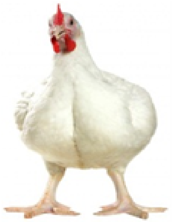
[a] Concentration of drug within the disc
[b] Percentage of isolates with zone sizes within the resistant category for that drug

Resistance in humans similarly low ~1-3% FQ

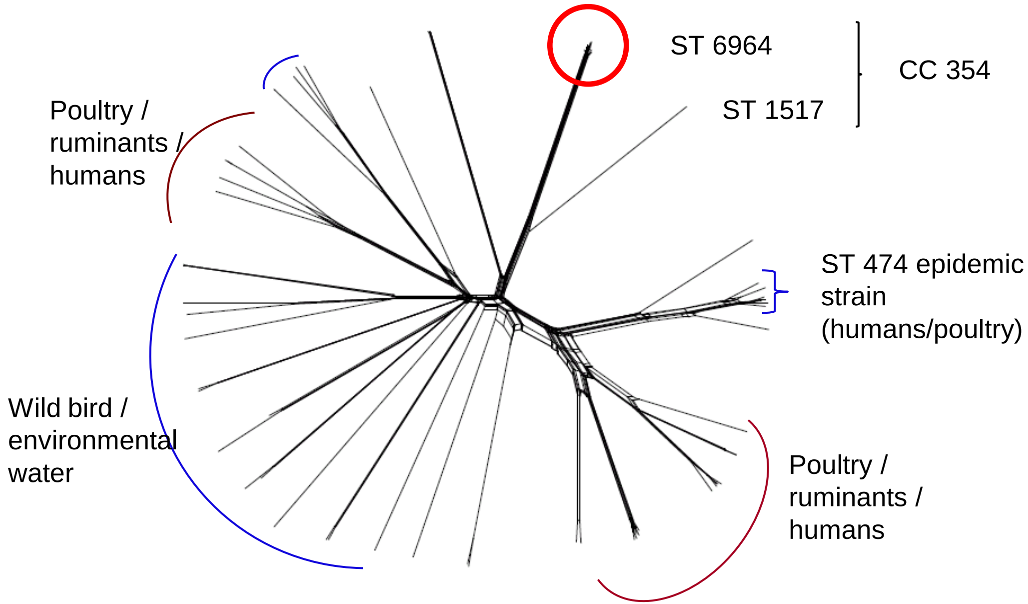
Very low by international standards
Low use of Abs in New Zealand poultry industry

Whole genome sequencing and Antimicrobial Resistance: Emergence of new strain: *Campylobacter jejuni* ST-6964

- August 2014: first two human cases of new *C. jejuni* ST-6964 detected in sentinel site
- Found in 4 poultry companies and 'breeder' (parent) flocks
- Resistant to tetracycline and fluoroquinolones
  - **0->37% in poultry**
  - Sharp increase in AMR and ST 6964 in human cases across NZ (ESR study)
  - Chicken liver outbreak, Wellington



Whole genome sequencing showed AMR strain was new to New Zealand



Key questions?

- How long has it been in NZ?
- How has it been transmitted between poultry companies?
- What has driven the emergence?
- What is the main source of human infection?
- How is it evolving?
- Genetic basis for resistance?

These could only be addressed by Whole Genome Sequencing and a One Health approach

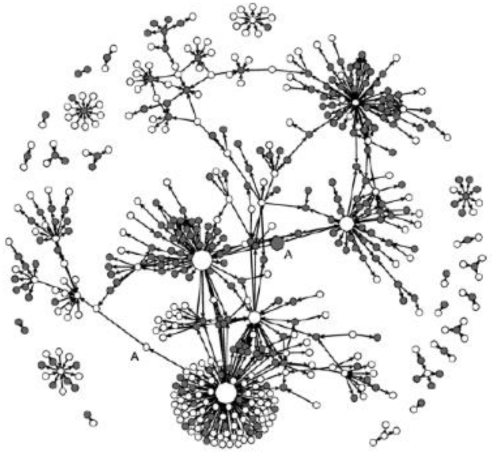
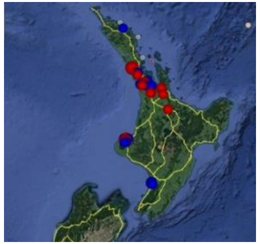
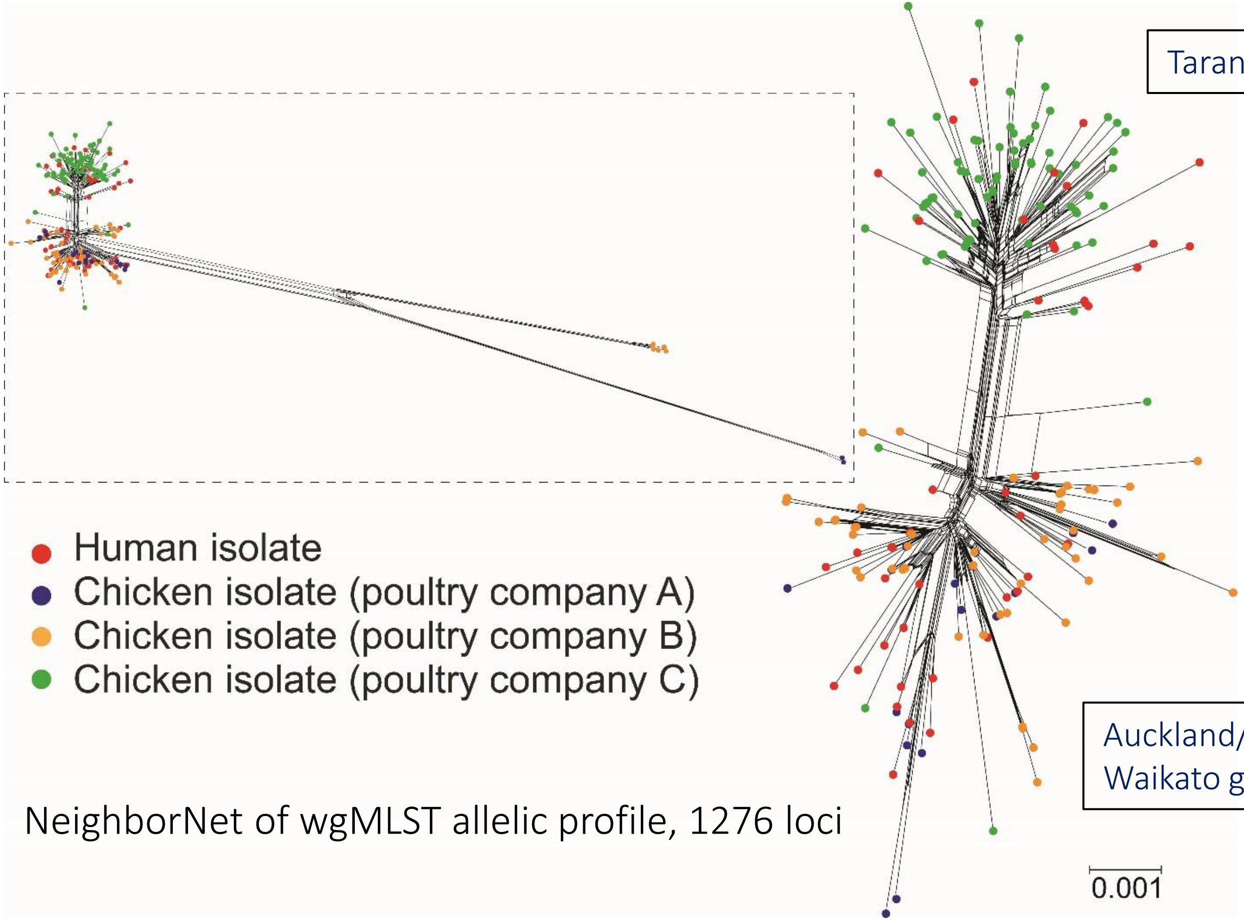


Fig. 2. Social network analysis of feed-related contacts in the New Zealand

Pacbio and Illumina sequencing of 230 *C. jejuni* ST-6964 isolates from poultry farms and human cases



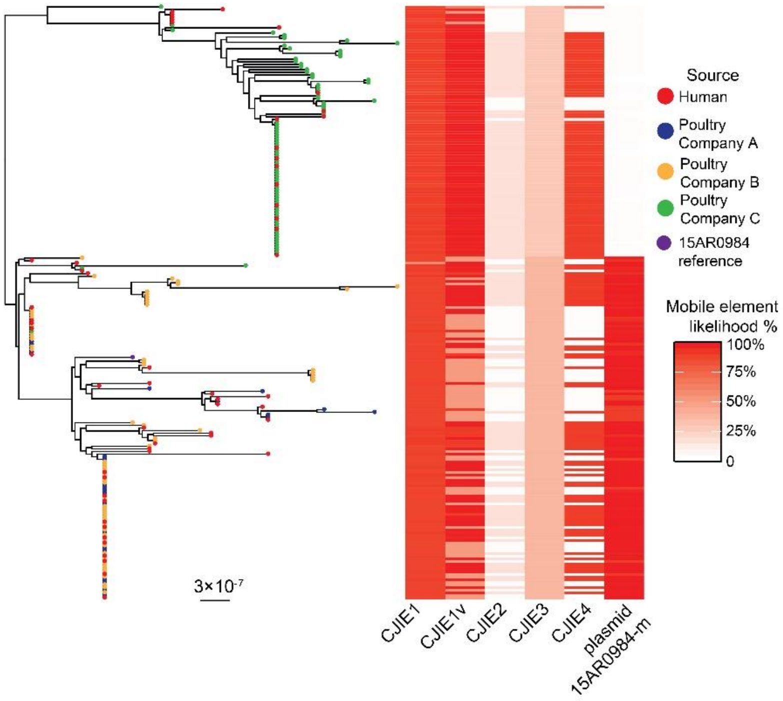


Taranaki group

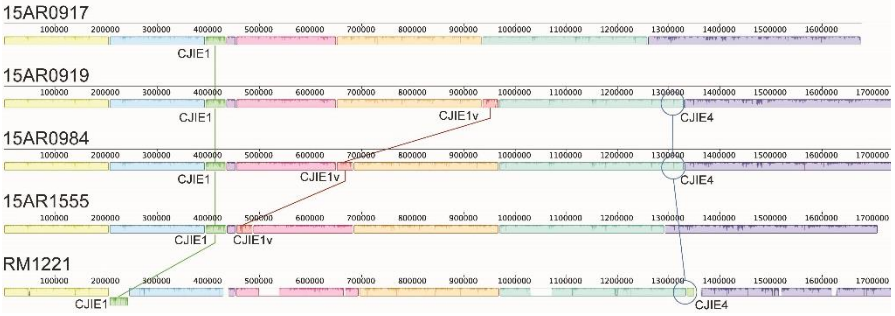
Auckland/
Waikato group

NeighborNet of wgMLST allelic profile, 1276 loci

SNP-based phylogeny, plasmid and prophage integrated elements



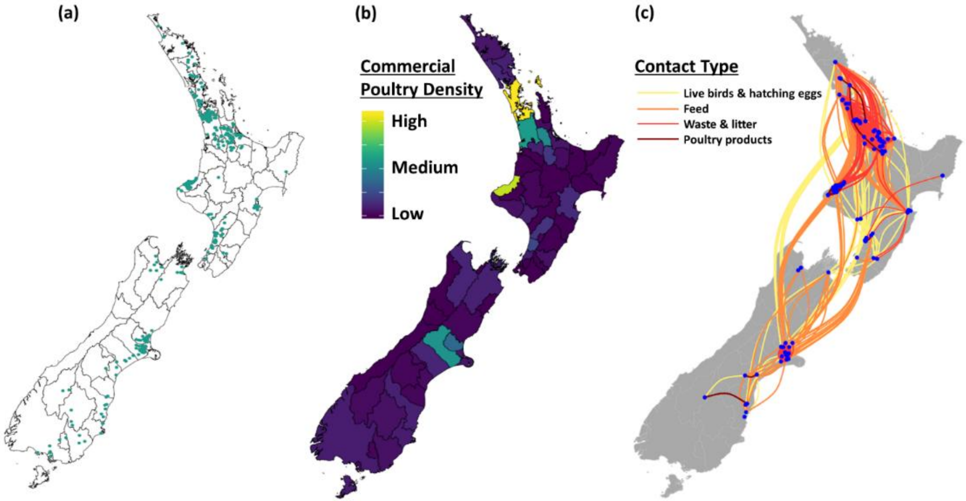
- Plasmid absent from company C
- Evidence of 3 prophage integrated elements
- CJIE1v identified in 3 locations in PacBio reference strains



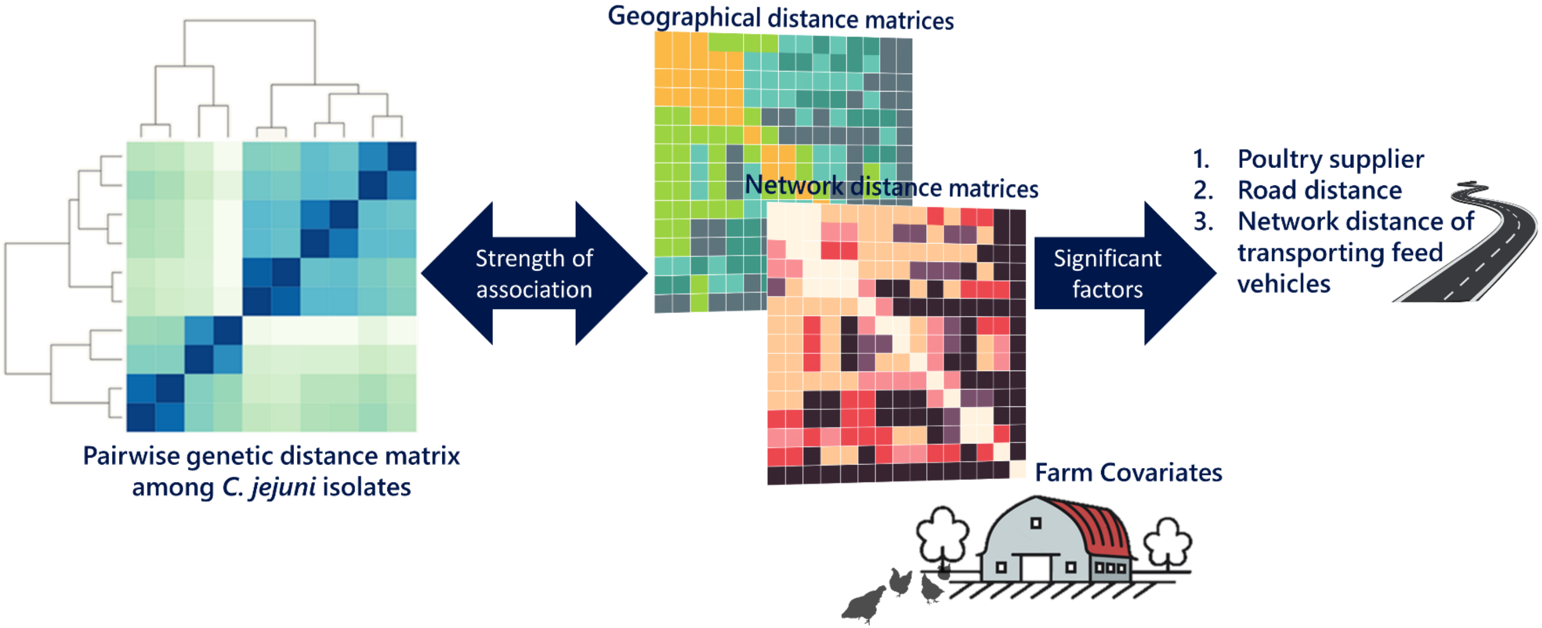
French et al *Emerg Infect Dis*. 2019 Dec;25(12):2226-2234

Transmission of *Campylobacter* in the poultry industry

- Within company?
- Local spread?
- Spread through contact networks?
  - Feed
  - Chicks
  - Personnel



Transmission in the poultry industry



Pairwise genetic distance matrix among *C. jejuni* isolates

Strength of association

Geographical distance matrices

Network distance matrices

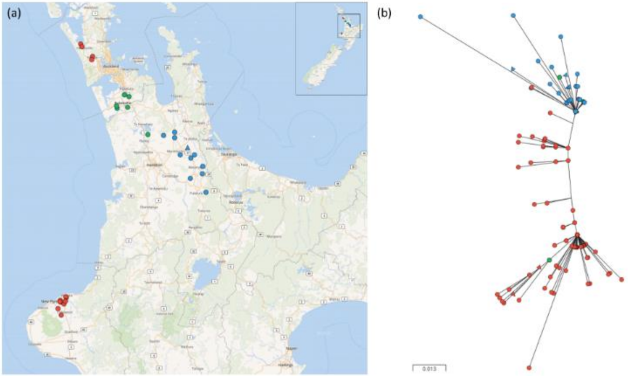
Farm Covariates

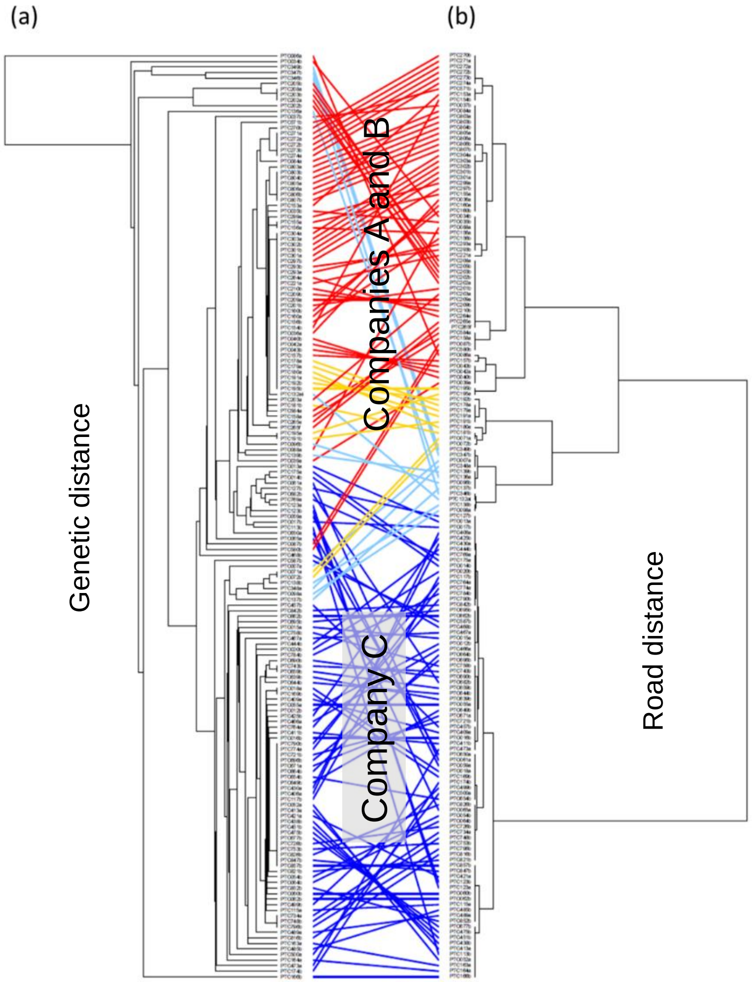
Significant factors

1. Poultry supplier
2. Road distance
3. Network distance of transporting feed vehicles

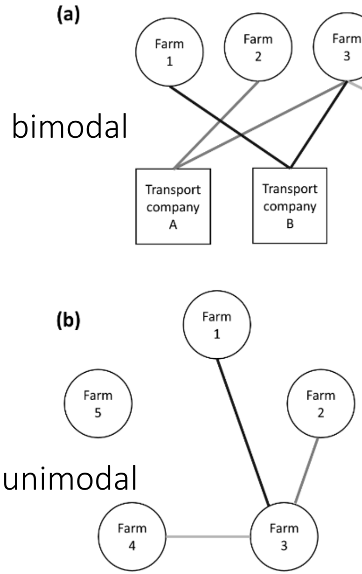
Greening et al 2020 under review

Comparing genetic relatedness of isolates revealed association with parent company and geographical location (evidence of local spread)



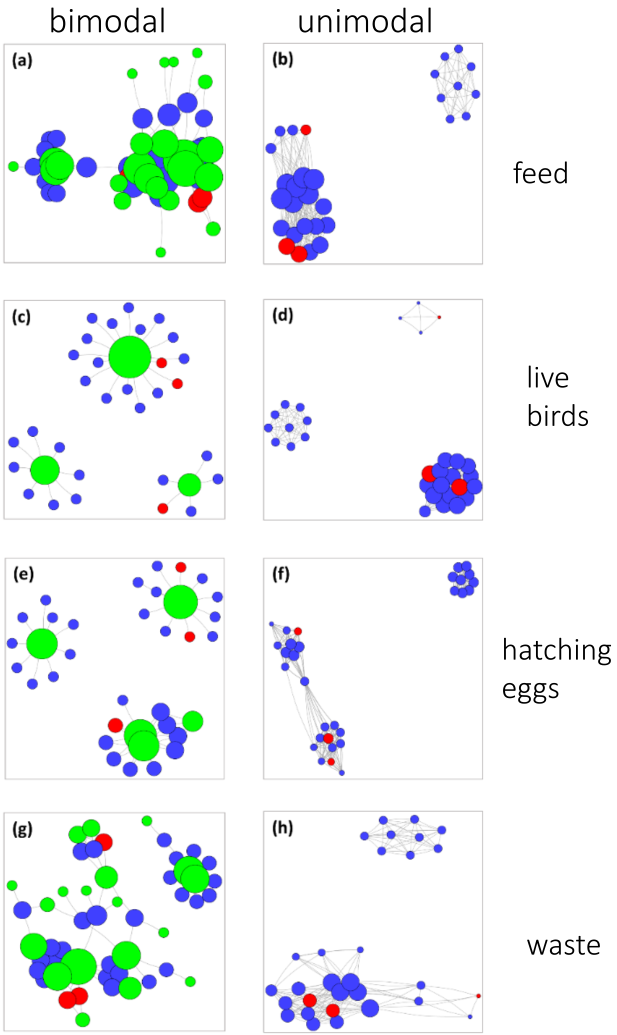


Modelling genetic distance and potential transmission networks



(c)

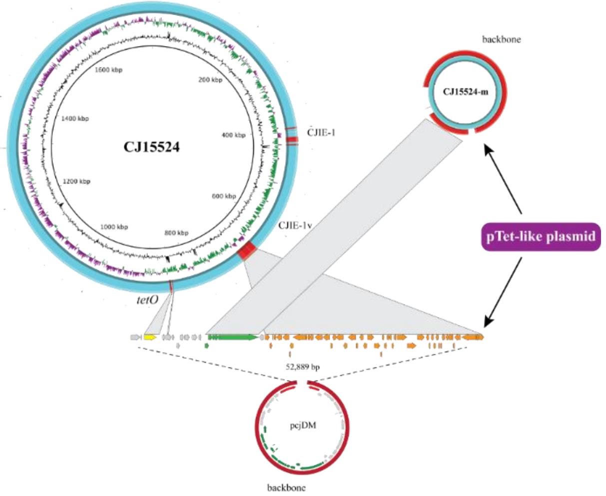
	1	**2**	**3**	**4**	**5**
1	0				
2	2	0			
3	1	1	0		
4	2	2	1	0	
5	99	99	99	99	0



Multivariable model (Distance-based redundancy analysis):
Parent company, farm and road and feed networks associated with genetic distance

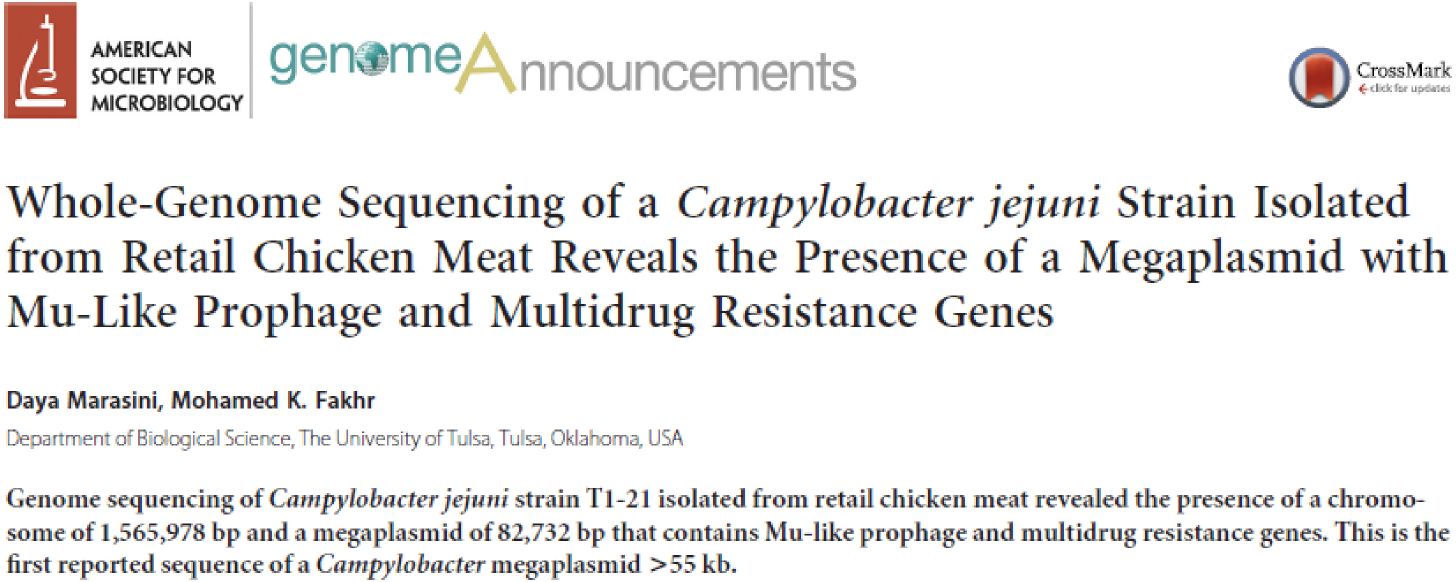
Key questions addressed by whole genome sequencing

- How long has it been in NZ?
  - ~mid-late 2013.
- How has it been transmitted between poultry companies?
  - Within company
  - Local spread – road/feed network
- What is the main source of infection?
  - All companies contributing to human infection
- Genetic basis for resistance?
  - megaplasmid and mutation



Genomic Analysis of Fluoroquinolone- and Tetracycline-Resistant *Campylobacter jejuni* Sequence Type 6964 in Humans and Poultry, New Zealand, 2014–2016

Nigel P. French, Ji Zhang, Glen P. Carter, Anne C. Midwinter, Patrick J. Biggs, Kristin Dyet, Brent J. Gilpin, Danielle J. Ingle, Kerry Mulqueen, Lynn E. Rogers, David A. Wilkinson, Sabrina S. Greening, Petra Muellner, Ahmed Fayaz, Deborah A. Williamson



AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements

Whole-Genome Sequencing of a *Campylobacter jejuni* Strain Isolated from Retail Chicken Meat Reveals the Presence of a Megaplasmid with Mu-Like Prophage and Multidrug Resistance Genes

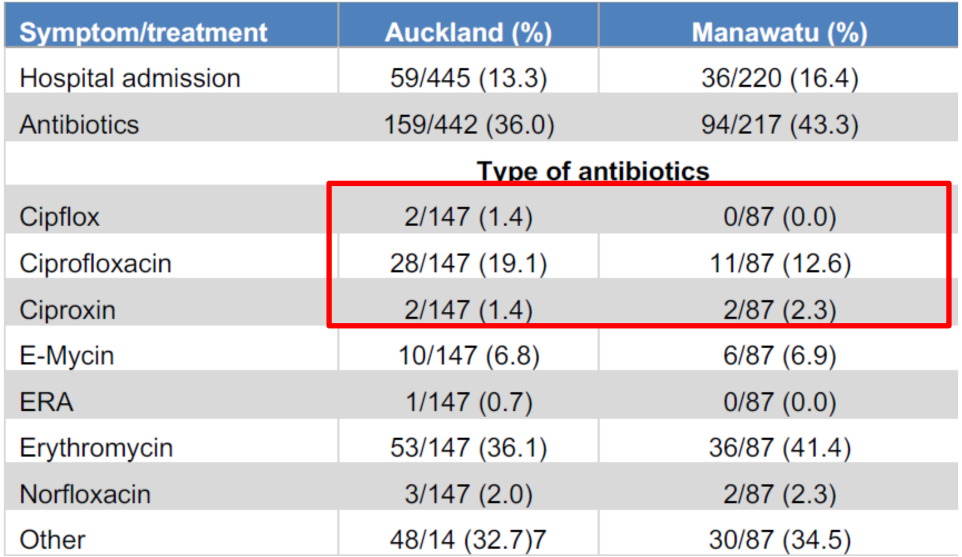
Daya Marasini, Mohamed K. Fakhr

Department of Biological Science, The University of Tulsa, Tulsa, Oklahoma, USA

Genome sequencing of *Campylobacter jejuni* strain T1-21 isolated from retail chicken meat revealed the presence of a chromosome of 1,565,978 bp and a megaplasmid of 82,732 bp that contains Mu-like prophage and multidrug resistance genes. This is the first reported sequence of a *Campylobacter* megaplasmid >55 kb.

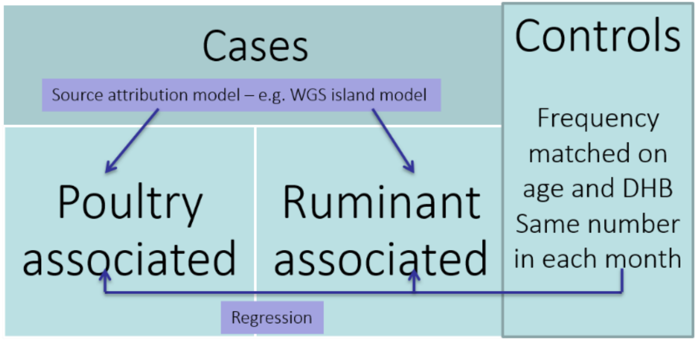
Public health significance?

- Resistant to two classes
  - Unless invasive, antibiotics not recommended
  - Sensitive to GP drug of choice, erythromycin
  - …but others still given by some GPs
- New strain hasn't caused increase in notifications
- But potential for spread of other, more virulent pathogens



Symptom/treatment	Auckland (%)	Manawatu (%)
Hospital admission	59/445 (13.3)	36/220 (16.4)
Antibiotics	159/442 (36.0)	94/217 (43.3)
	Type of antibiotics	
Cipflox	2/147 (1.4)	0/87 (0.0)
Ciprofloxacin	28/147 (19.1)	11/87 (12.6)
Ciproxin	2/147 (1.4)	2/87 (2.3)
E-Mycin	10/147 (6.8)	6/87 (6.9)
ERA	1/147 (0.7)	0/87 (0.0)
Erythromycin	53/147 (36.1)	36/87 (41.4)
Norfloxacin	3/147 (2.0)	2/87 (2.3)
Other	48/14 (32.7)7	30/87 (34.5)

Source Assigned – case control study design using WGS



Cases

Source attribution model – e.g. WGS island model

Poultry associated

Ruminant associated

Regression

Controls

Frequency matched on age and DHB
Same number in each month

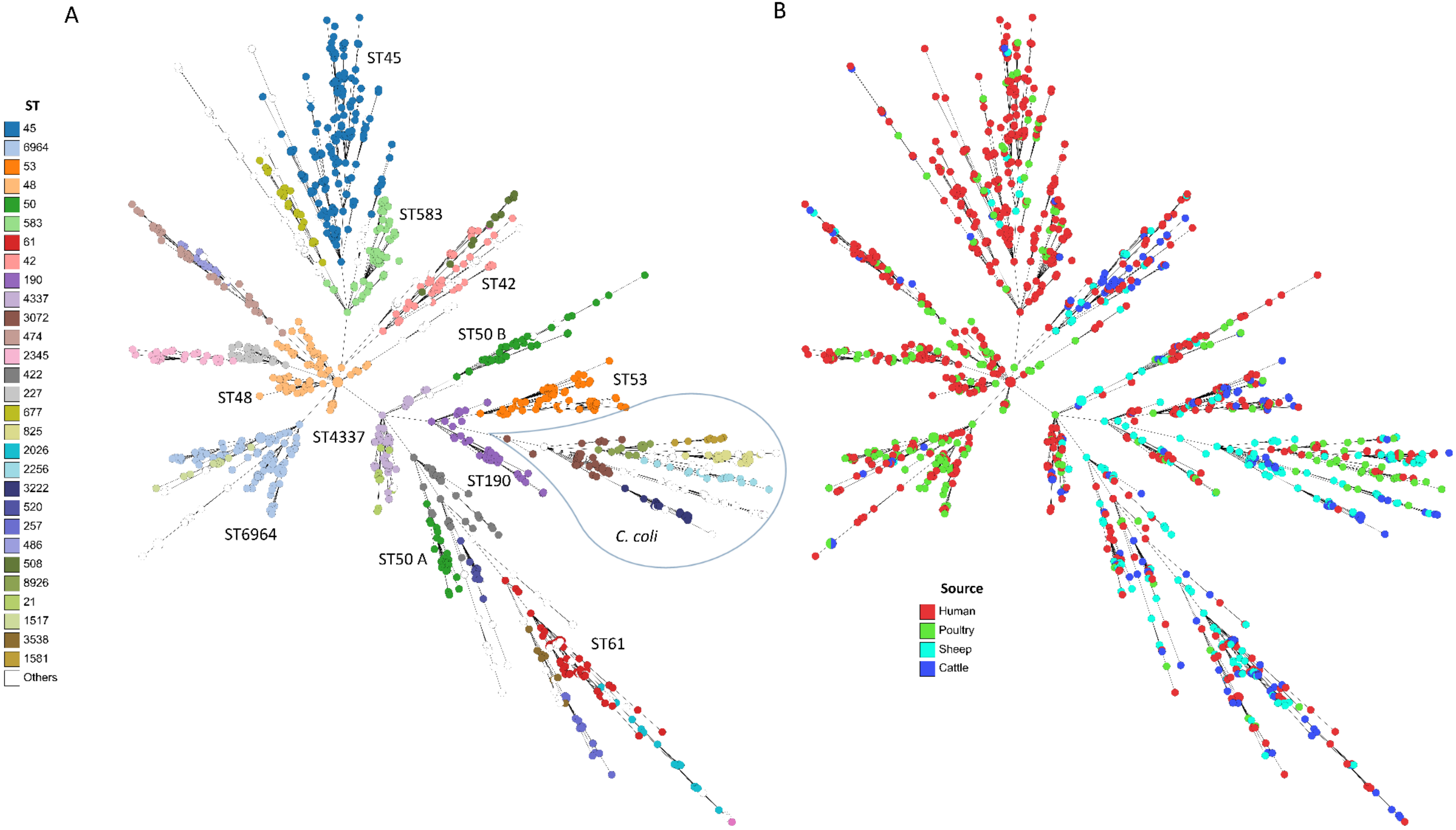
> Int J Infect Dis. 2020 Nov 19;S1201-9712(20)32479-6. doi: 10.1016/j.ijid.2020.11.167. Online ahead of print.

Source attributed case-control study of campylobacteriosis in New Zealand

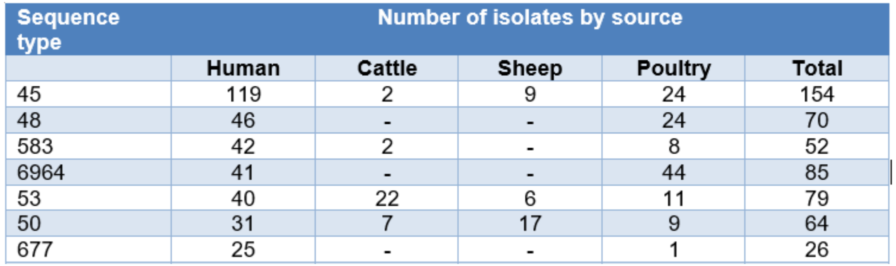
R J Lake [1], D M Campbell [2], S C Hathaway [3], E Ashmore [4], P J Cressey [5], B J Horn [5], S Pirikahu [6], J M Sherwood [7], M G Baker [8], P Shoemack [9], J Benschop [10], J C Marshall [11], A C Midwinter [10], D A Wilkinson [10], N P French [12]

- Conducted 2018-19
- >600 cases, 600 controls
- Over 150 isolates each from poultry, cattle, sheep
- >1200 isolates sequenced

- Risk factors associated with poultry and cattle


A
ST
45
6964
53
48
50
583
61
42
190
4337
3072
474
2345
422
227
677
825
2026
2256
3222
520
257
486
508
8926
21
1517
3538
1581
Others
ST45
ST583
ST42
ST50 B
ST53
ST48
ST4337
ST190
ST6964
ST50 A
ST61
C. coli
B
Source
Human
Poultry
Sheep
Cattle

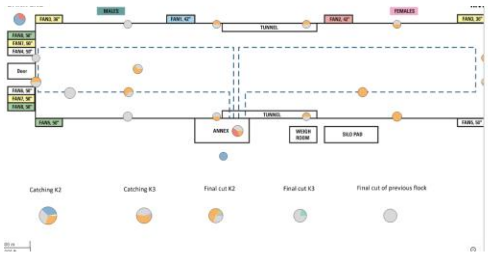
Current situation

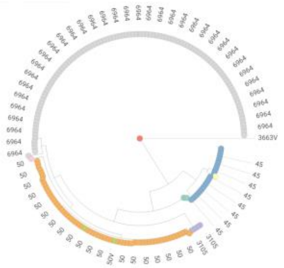


Sequence type	Number of isolates by source				
	Human	Cattle	Sheep	Poultry	Total
45	119	2	9	24	154
48	46	-	-	24	70
583	42	2	-	8	52
6964	41	-	-	44	85
53	40	22	6	11	79
50	31	7	17	9	64
677	25	-	-	1	26

Source SACNZ study

- ST-6964 was 4th most prevalent in human cases in 2018/19
- Most prevalent in poultry
- Recent work with industry indicates carry-over between flocks important for ST6964 transmission





https://microreact.org/project/ds4r5sfegYQaHg4mzttZCo

https://www.mpi.govt.nz/dmsdocument/39896-source-assigned-campylobacteriosis-in-new-zealand-study-sacnzs-report

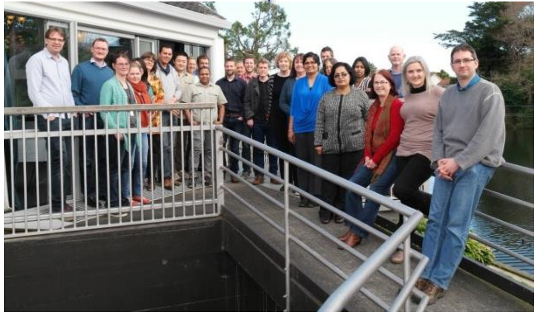
Conclusions

- Campylobacteriosis still concerning for New Zealanders despite significant gains in 2006/7
  - Sporadic food-associated cases
  - Large waterborne outbreak in 2016
  - Emergence of AMR linked to poultry consumption
- Poultry industry, government and research providers working together to achieve targets and further drive down contamination and notification rates
- AMR situation can change very rapidly
- MPI new strategy
- Advances in genome sequencing and modelling playing increasingly important roles in determining origin, evolution, transmission and prevention

Requires a One Health approach

Acknowledgements

- ^{m}EpiLab team: particularly David Wilkinson, Ji Zhang, Jonathan Marshall, Patrick Biggs, Lynn Rogers and Anne Midwinter
- Carolyn Gates, Sabrina Greening, Jing Liao, Marti Anderson, Petra Mullner and Martin Hazelton
- ESR: Rob Lake, Phil Carter, Brent Gilpin, Kristen Dyet
- University of Melbourne: Debbie Williamson, Ben Howden, Dieter Bulach, Glen Carter, Sarah Baines, Anders Gonçalves da Silva, Danielle Ingle
- University of Otago: Michael Baker
- MidCentral Public Health, MedLab Central
- Ministry for Primary Industries team: Donald Campbell, Steve Hathaway
- Poultry Industry Association



Funding

Poultry Industry
Association of
New Zealand (Inc)

Ministry for Primary Industries
Manatū Ahu Matua
NEW ZEALAND

MINISTRY OF BUSINESS,
INNOVATION & EMPLOYMENT
HĪKINA WHAKATUTUKI

NEW ZEALAND'S
BIOLOGICAL
HERITAGE
Ngā Koiora
Tuku Iho
National
Science
Challenges

ALLAN
WILSON
CENTRE

New Zealand
FOOD SAFETY SCIENCE
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