

Emergence of *Campylobacter jejuni* ST-6964 in poultry and humans in New Zealand: a new twist in the campy story

French NP¹, Williamson DA^{2,3}

Biggs R⁴, Biggs PJ¹, Bloomfield S¹, Dyet K², Gilpin BJ², Heffernan H²,
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One Health Aotearoa 2016, Wellington

Collaborating Centre



^mEpiLab

Tē Kūnenga
ki Pūrehuroa



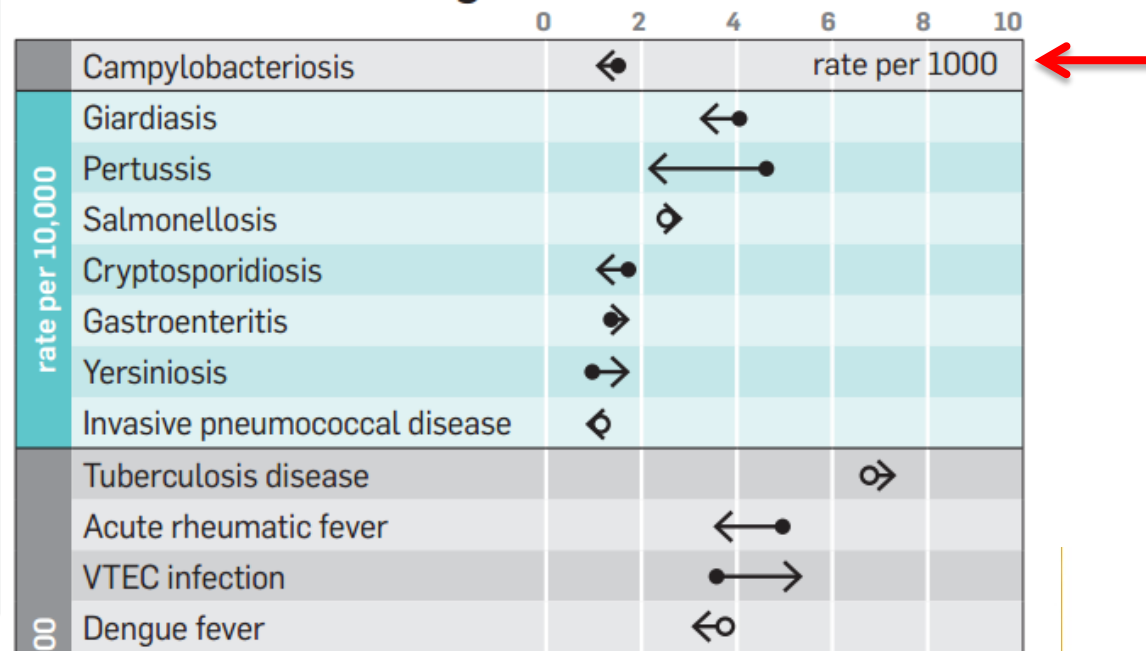
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Campylobacteriosis still high on the list of notifiable diseases in New Zealand, but declining

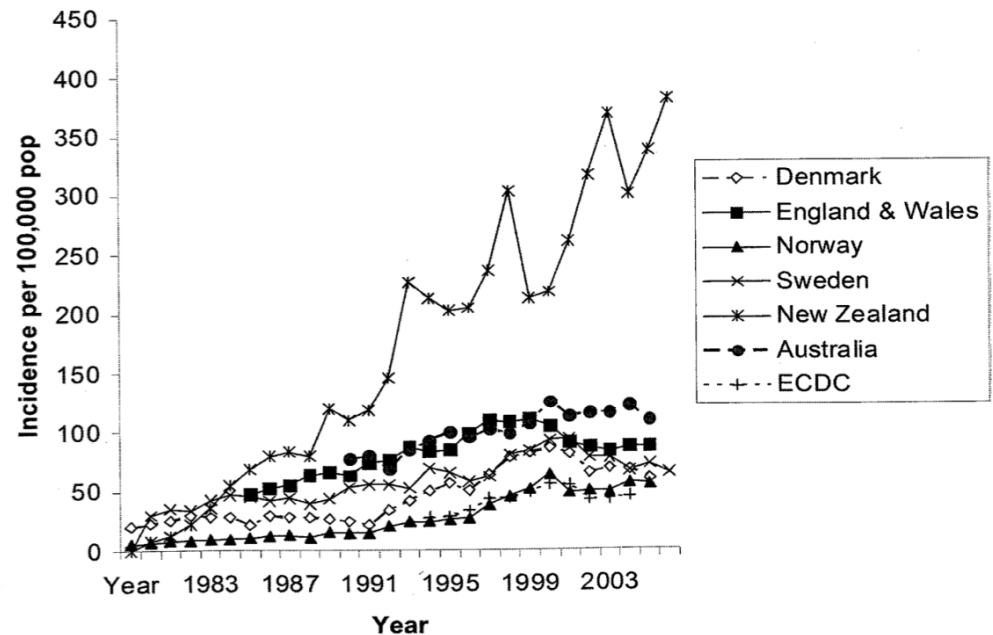
New Zealand Public Health Surveillance Report
September 2015: Covering April to June 2015

Source: ESR Ltd

National surveillance data 12-monthly notification rate changes¹



Campylobacter in NZ: 1980-2006



THE NEW ZEALAND MEDICAL JOURNAL

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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

Manawatu sentinel site 2005-2015

- 'One health' approach
- >7000 samples
- Identify MLST types common to particular sources
- Modelling (reservoir attribution)

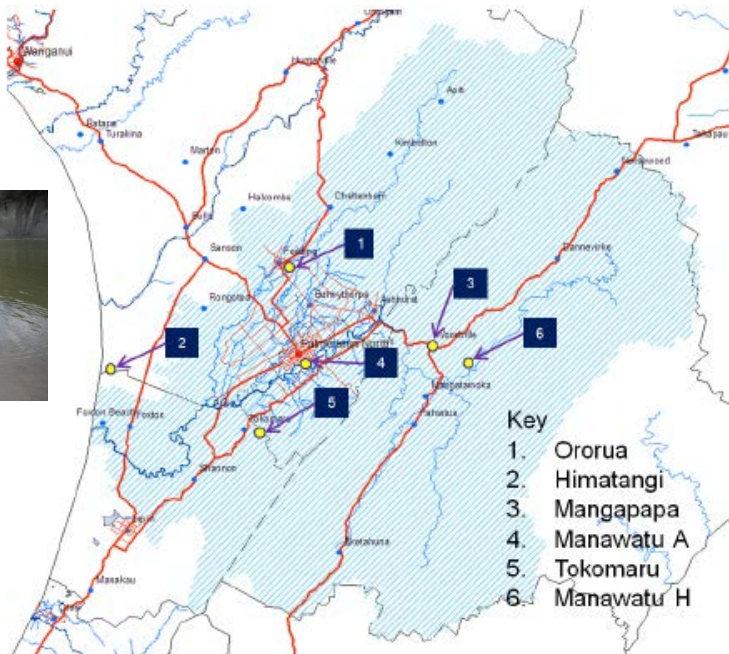
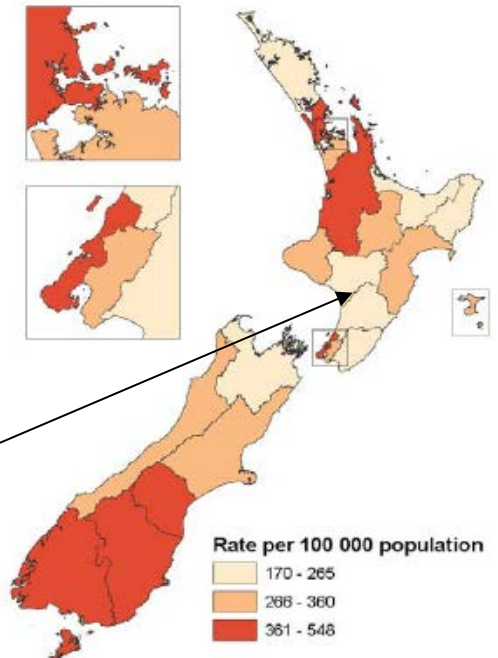


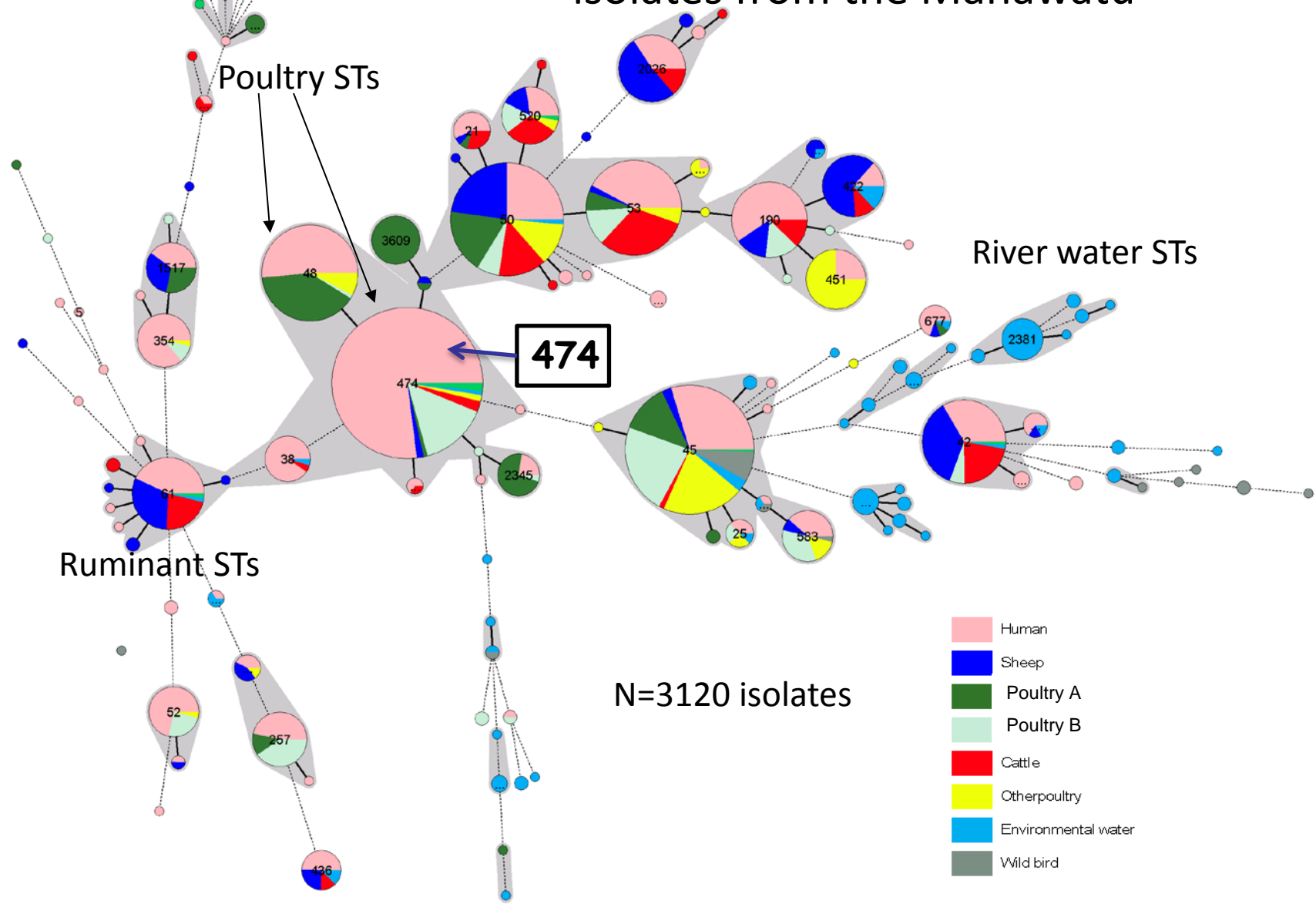
Figure 7. Campylobacteriosis notifications by DHB, 2004



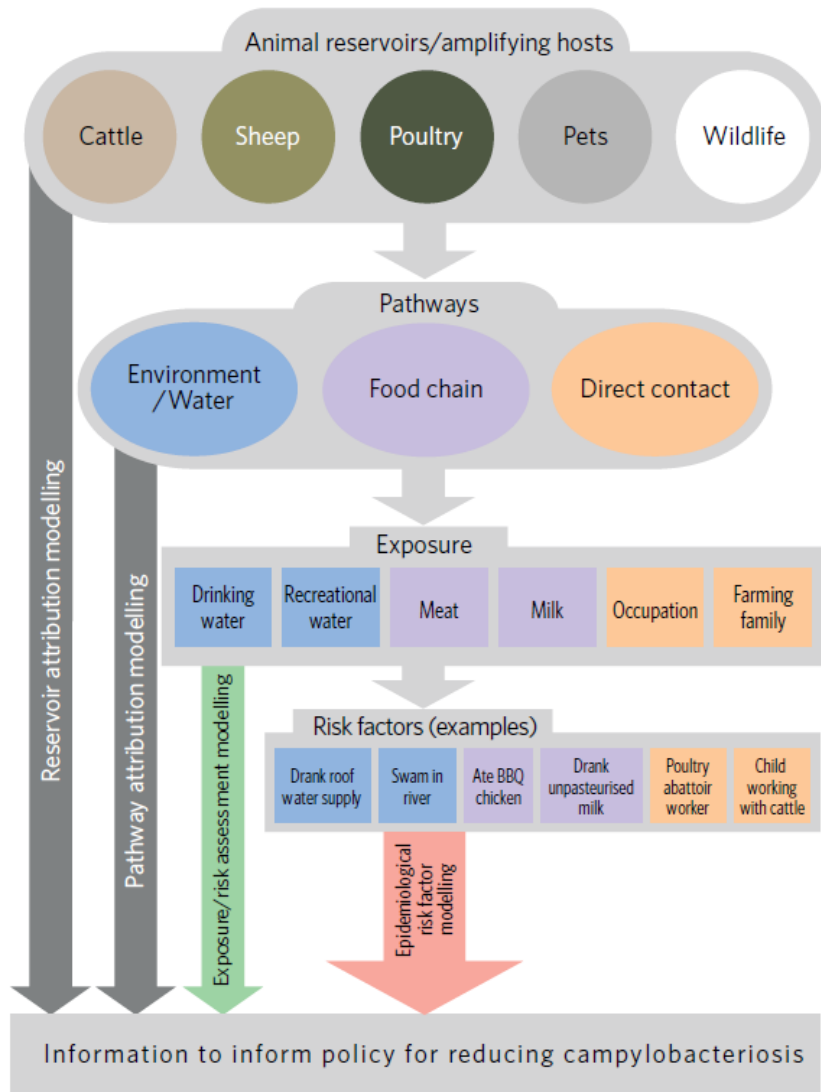
Multi-locus sequence typing

Unique pattern of genotypes

isolates from the Manawatu



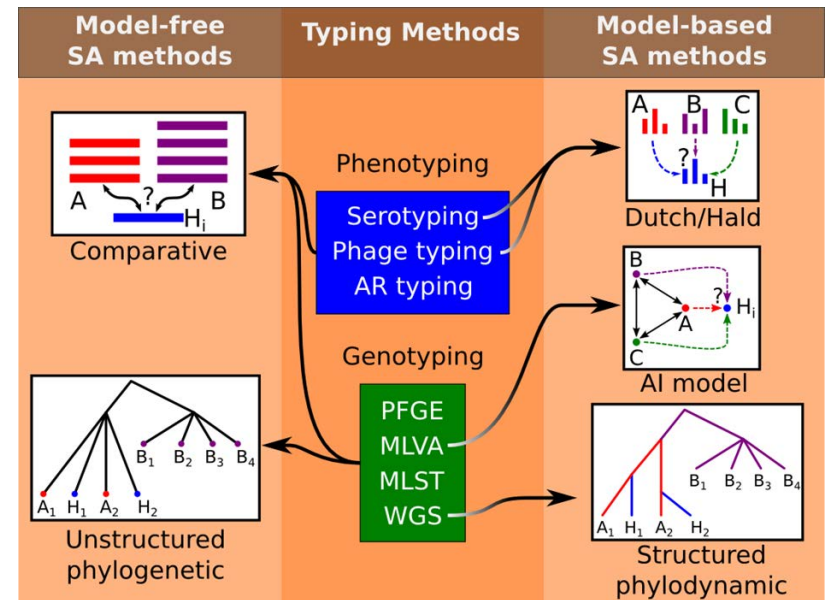
Source attribution and transmission frameworks



forever discovering

Wagenaar, French and Havelaar, 2013. *Clin Infect Dis*, 57, 1600-7

Preventing Campylobacter at the source: why is it so difficult?

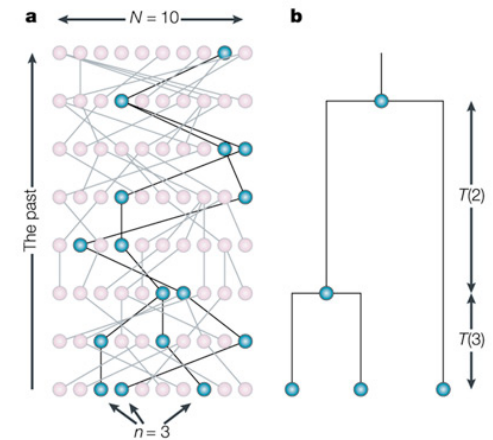


Mather, Vaughan and French, 2015. *Clin Infect Dis*. 61 S259-65

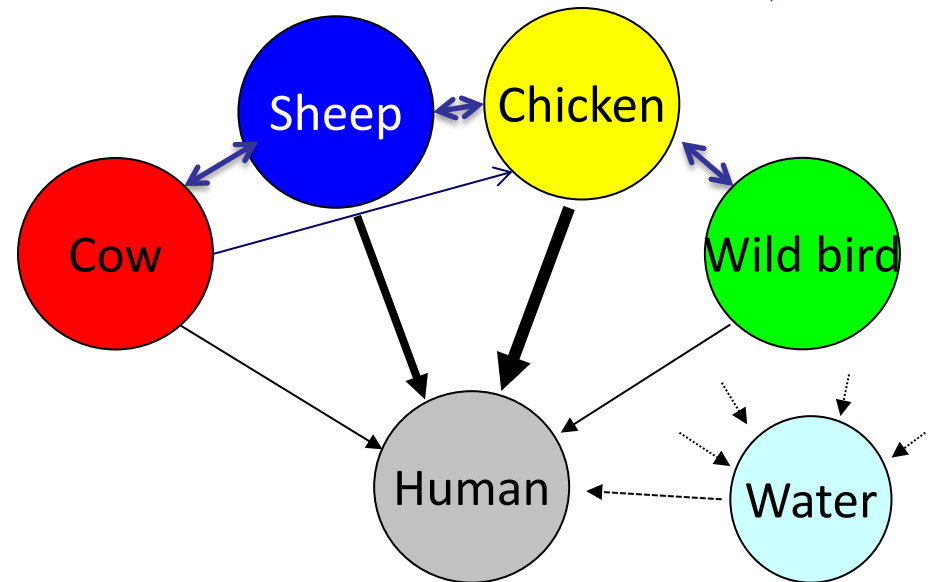
Molecular Approaches to Understanding Transmission and Source Attribution in Nontyphoidal Salmonella and Their Application in Africa.

Reservoir attribution 'Island model'

- Population genetics / evolution approach
- Reconstruct genealogy
- Used to find out source of human infections
- Flow into the human "island" from animal "islands"



Nature Reviews | Genetics



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PLoS GENETICS

Tracing the Source of Campylobacteriosis

Daniel J. Wilson^{1a*}, Edith Gabriel^{2ab}, Andrew J. H. Leatherbarrow³, John Cheesbrough⁴, Steven Gee⁴, Eric Bolton⁵, Andrew Fox^{4,5}, Paul Fearnhead¹, C. Anthony Hart^{6†}, Peter J. Diggle²

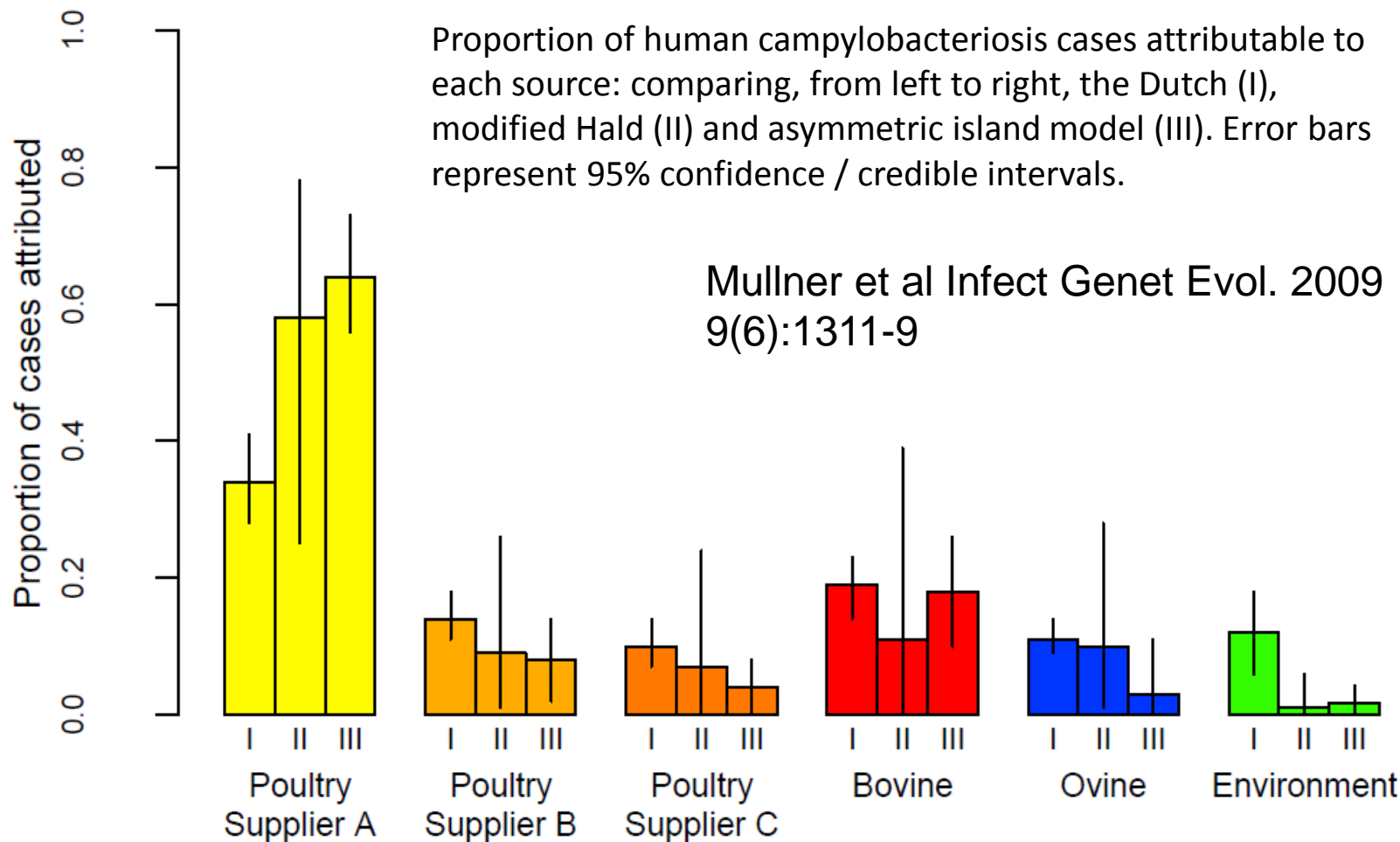
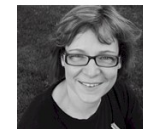
Forever discovering

Te Kunenga
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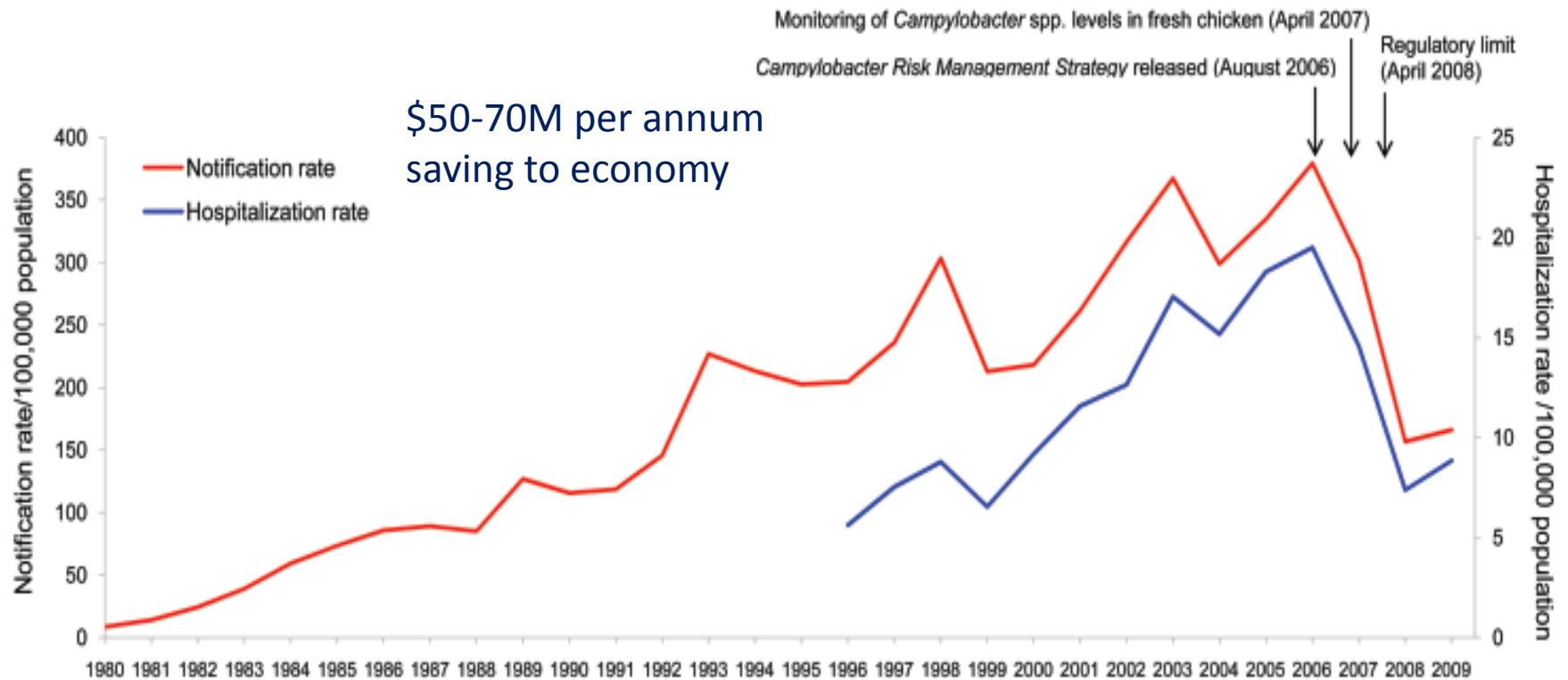
Reservoir attribution (Mullner et al)





Campylobacter in Poultry – Risk Management Strategy 2007 - 2010

Relationship between campylobacteriosis notifications and hospitalisations (A. Sears)

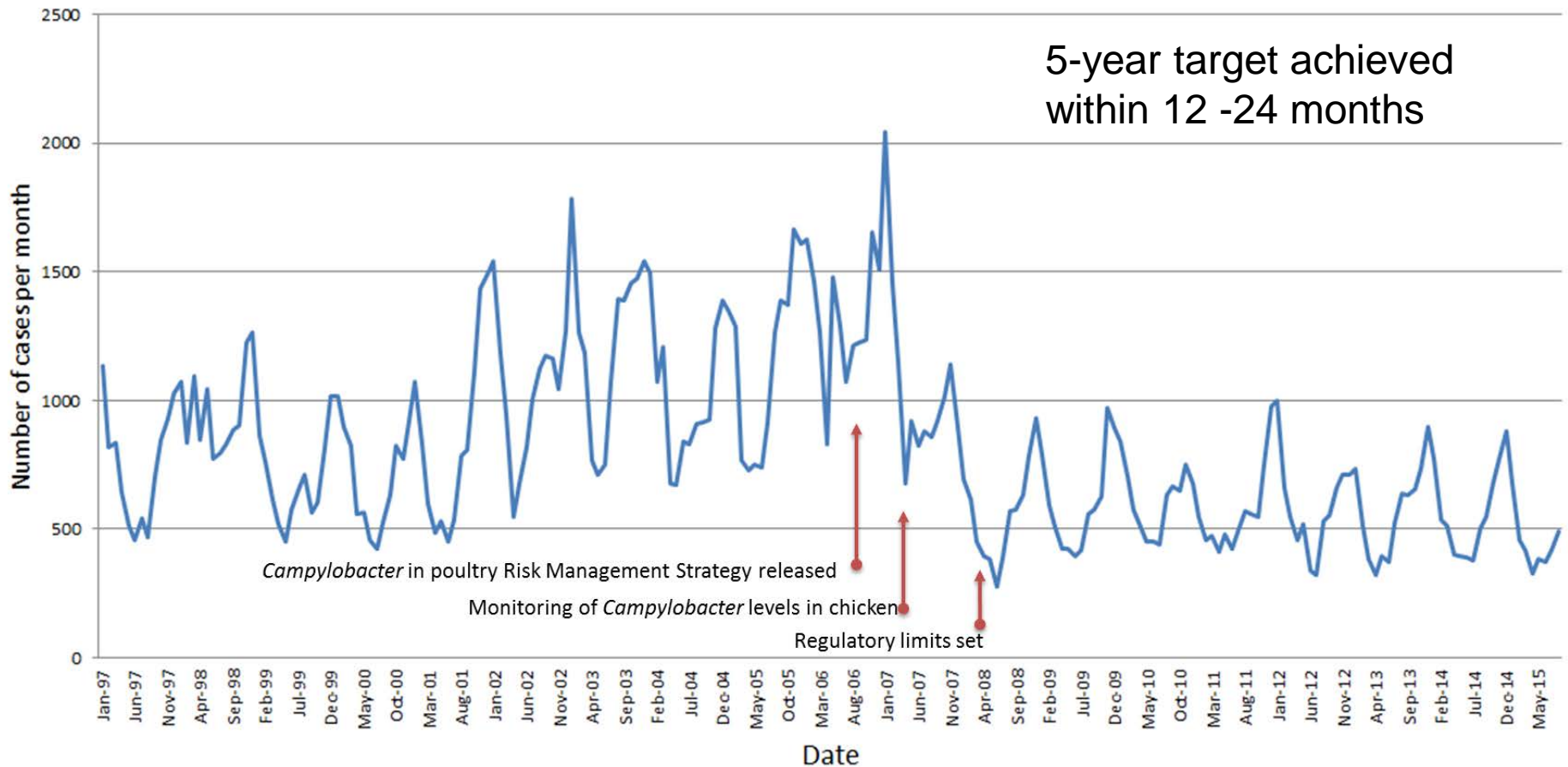


Data Sources: ESR Ltd notification data; NZHIS hospitalisation data (filtered)

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

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

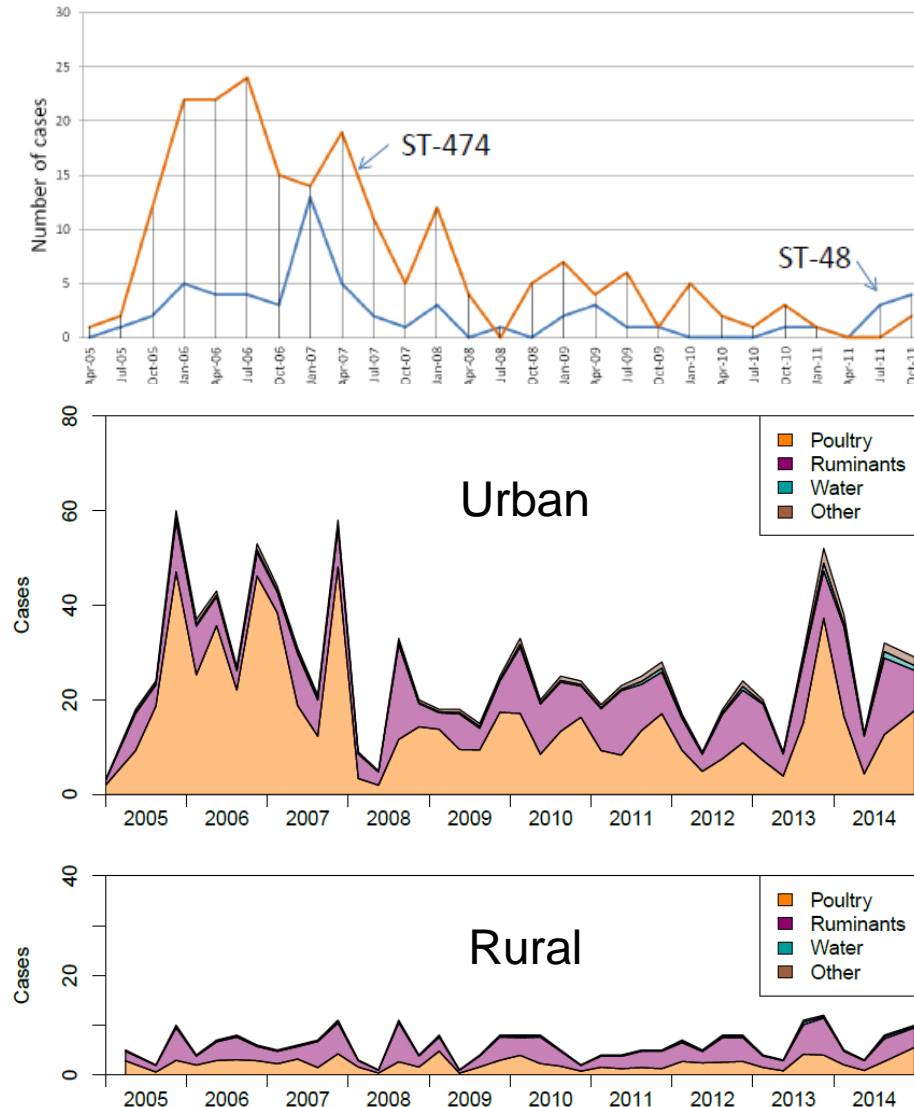
Ann Sears, Michael G. Baker, Nick Wilson, Jonathan Marshall, Petra Muellner, Donald M. Campbell, Robin J. Lake, and Nigel P. French



Surveillance and monitoring: Rise and fall of *C. jejuni* ST 474

- ST-474 found in multiple sources later in epidemic
- Genome sequencing revealed rapid evolution*
- Recombination > mutation
-Waiting for next 'epidemic' strain?

*Biggs et al 2011, PLoS One, 6 (11) e27121
French et al, 2014. Campylobacter Ecology and Evolution, 221-240.



Arrival of ST-6964 and AMR.....

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

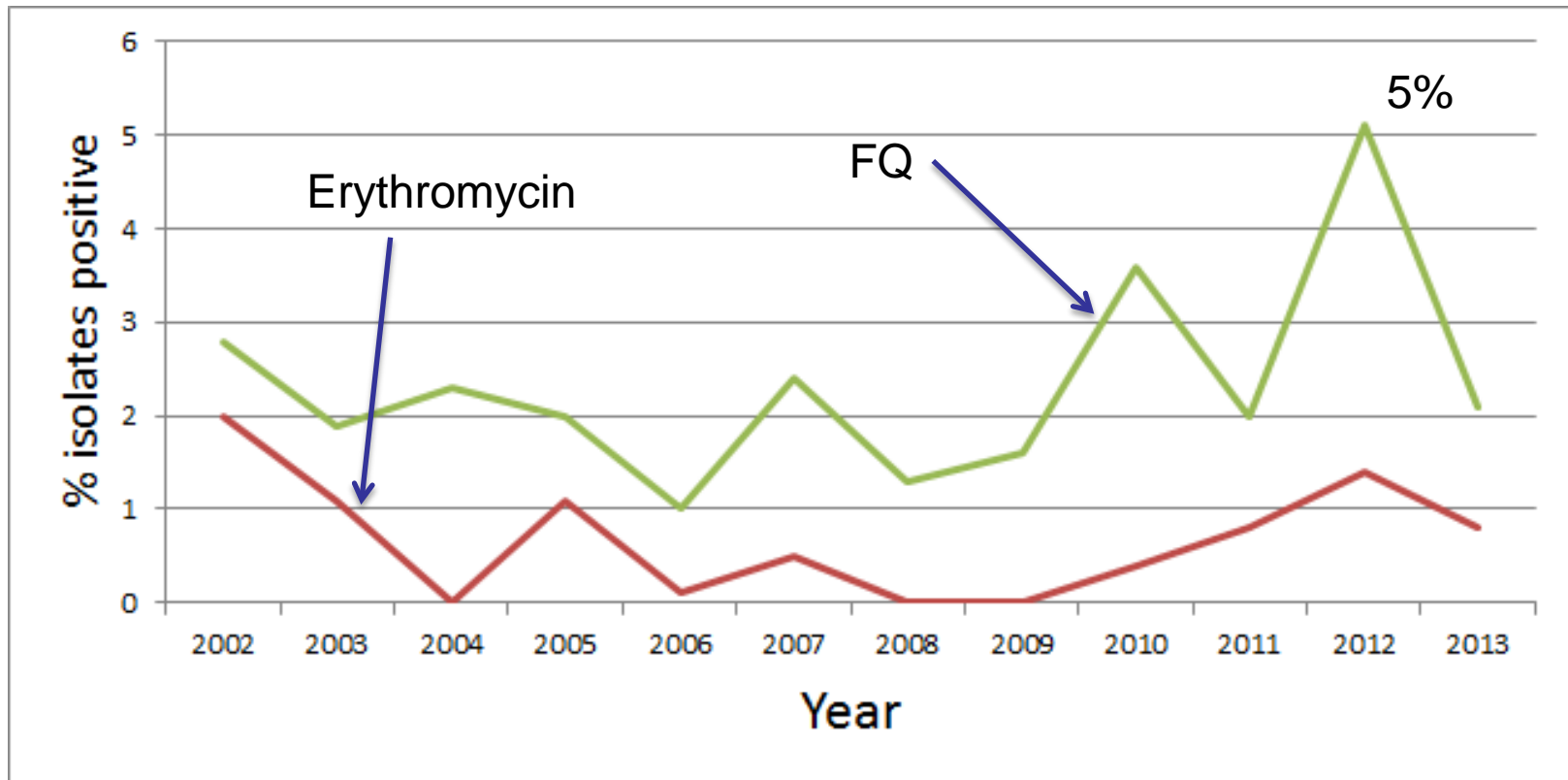
Emergence of ST-6964

(in collaboration with Dr Debbie Williamson, MDU)

- August 2014: first two human cases of new *C. jejuni* ST-6964 detected in Manawatu sentinel surveillance site
- Found in all 3 largest poultry companies
- Resistant to tetracycline and fluoroquinolones
- ST-6964 member of Clonal Complex 354, only other isolates in PubMLST database from China

AMR in *Campylobacter* in NZ:

Human cases to 2013 (data from ESR Ltd)



Low levels of resistance by international standards (UK >25%)

AMR in Campylobacter in NZ: Poultry

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 | Zone size (mm) | | | | | | | | | | | | | |
|-----------------|------------------------|----------------------|----------------|------|----|----|----|----|----|----|----|----|----|----|----|-----|
| | | | ≤6 | 7-15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | ≥28 |
| Erythromycin | 15 | 0.5 | 1 | | | | 1 | | | 2 | 3 | 4 | 8 | 19 | 12 | 21 |
| Ciprofloxacin | 5 | 0 | | | | | | | | | | | 1 | 1 | 1 | 2 |
| Enrofloxacin | 5 | 0 | | | | | | | | | | | 1 | | 3 | 4 |
| Nalidixic acid | 30 | 0 | | | 2 | 2 | 3 | 8 | 12 | 13 | 19 | 24 | 22 | 33 | 17 | 12 |
| Chloramphenicol | 30 | 0 | | | | | | | | 1 | 1 | | | | 9 | 10 |
| Tetracycline | 30 | 0 | | | | | | | | | | | | | 1 | |

^a Concentration of drug within the disc

^b Percentage of isolates with zone sizes within the resistant category for that drug

Very low by international standards

Poultry survey 2015

- Samples:
 - 193 pooled caecal samples
 - 1 week of slaughter
 - 62 farms, 158 sheds
- 37% +ve for ST-6964 tet/cip resistant strain
- 0% +ve on South Island
- All 3 main NI companies +ve (27-62% of samples)
- Also isolated from 'parent' stock

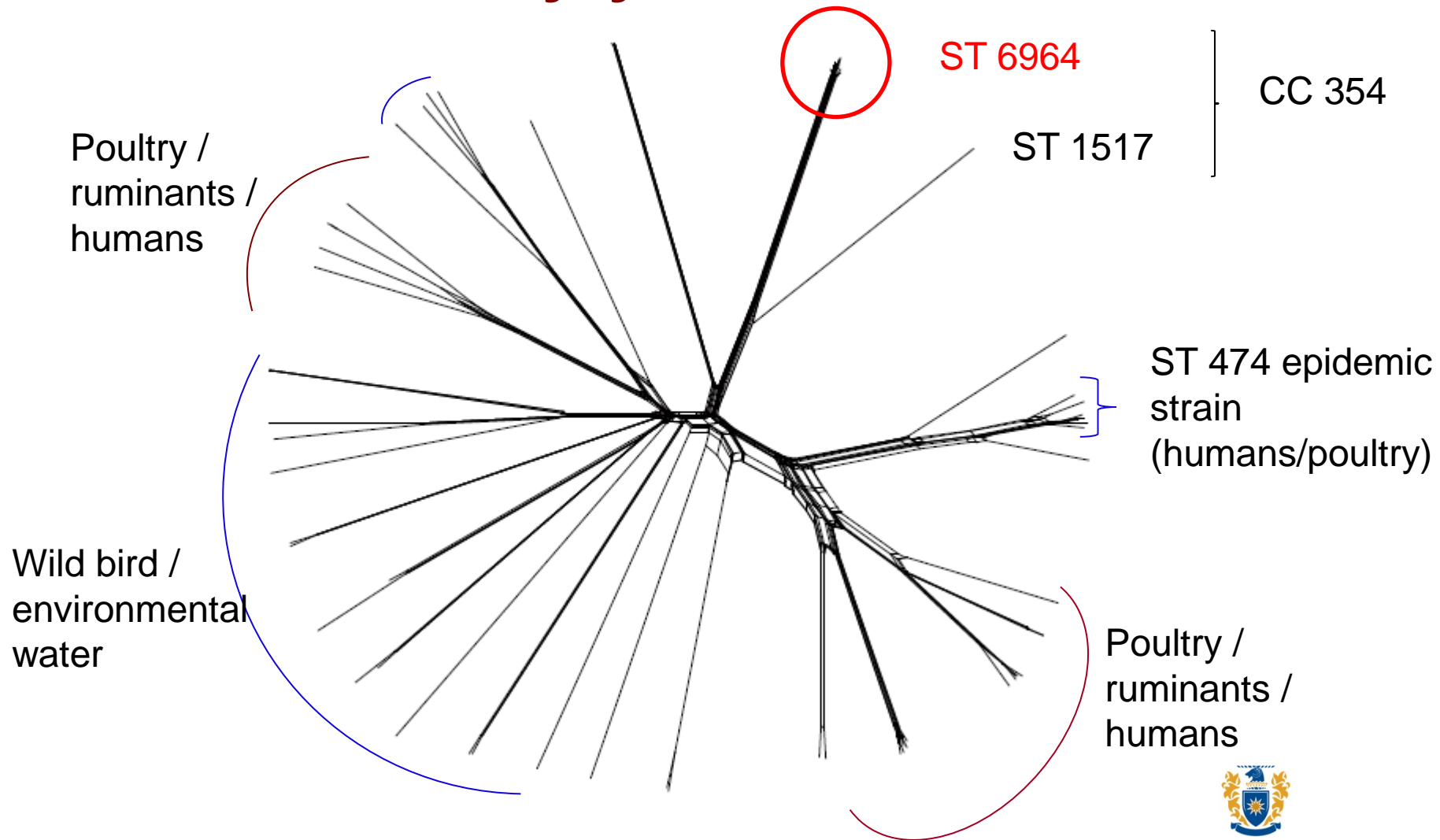


Human survey 2015

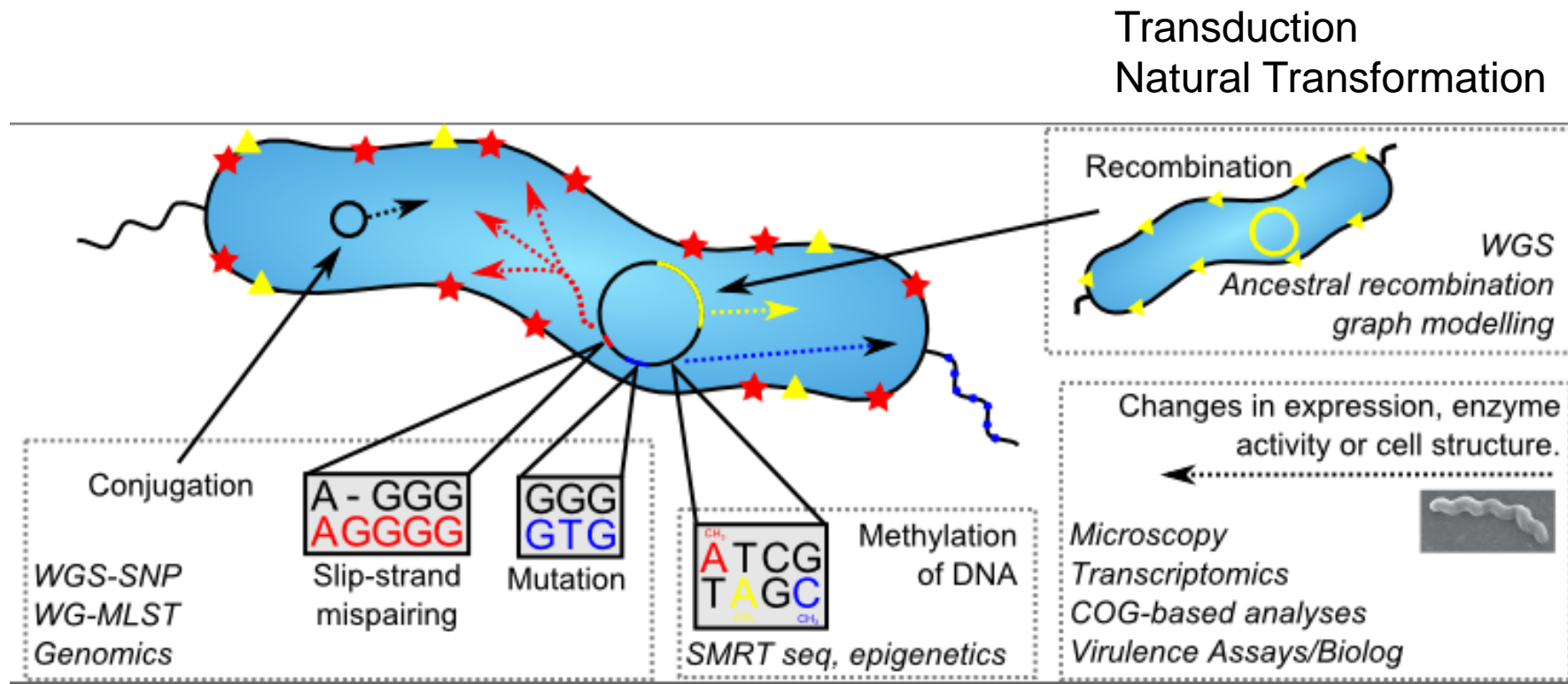
- ESR survey (Debbie Williamson and Kirsten Dyet, presented at Antimicrobials 2016, PP2.3)
- May-October 2015, ~300 isolates
- Increased prevalence of FQ and tet resistance
- Most (73%) ST-6964
- Wellington outbreak (chicken liver)



Whole Genome MLST: NZ *C. jejuni* isolates

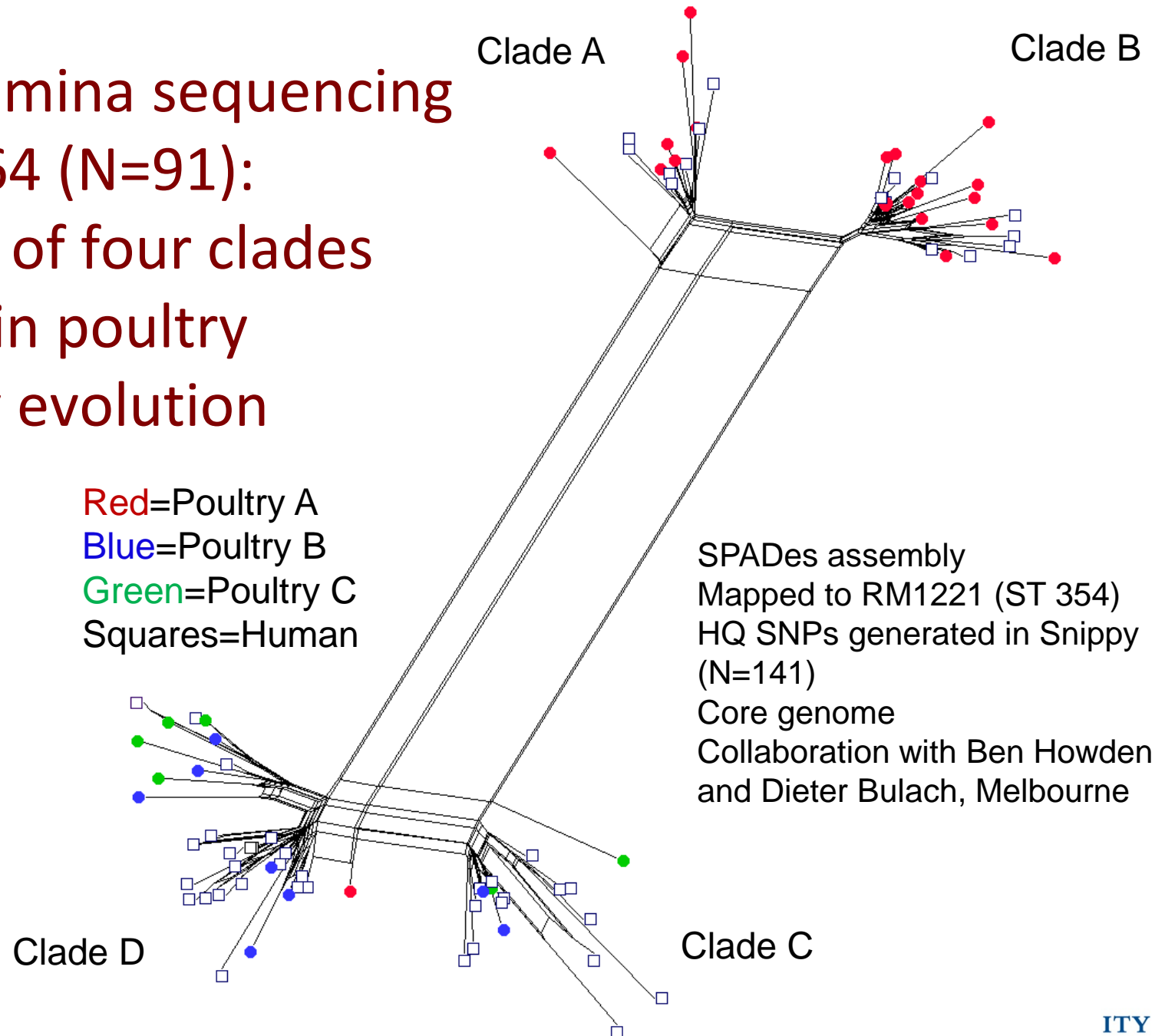


Adaptive mechanisms deployed by *Campylobacter*

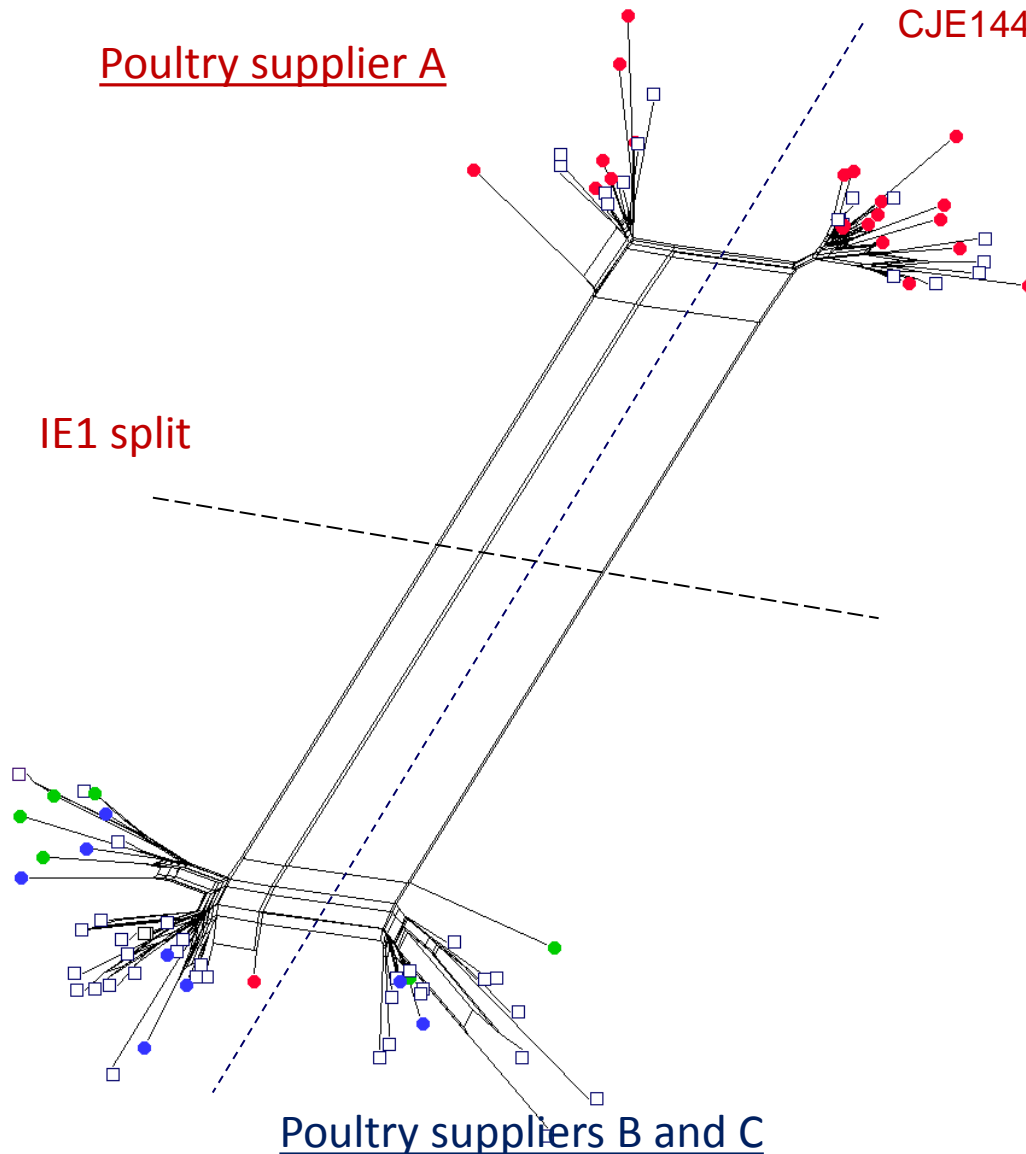


Creates a vast array of possible phenotypes

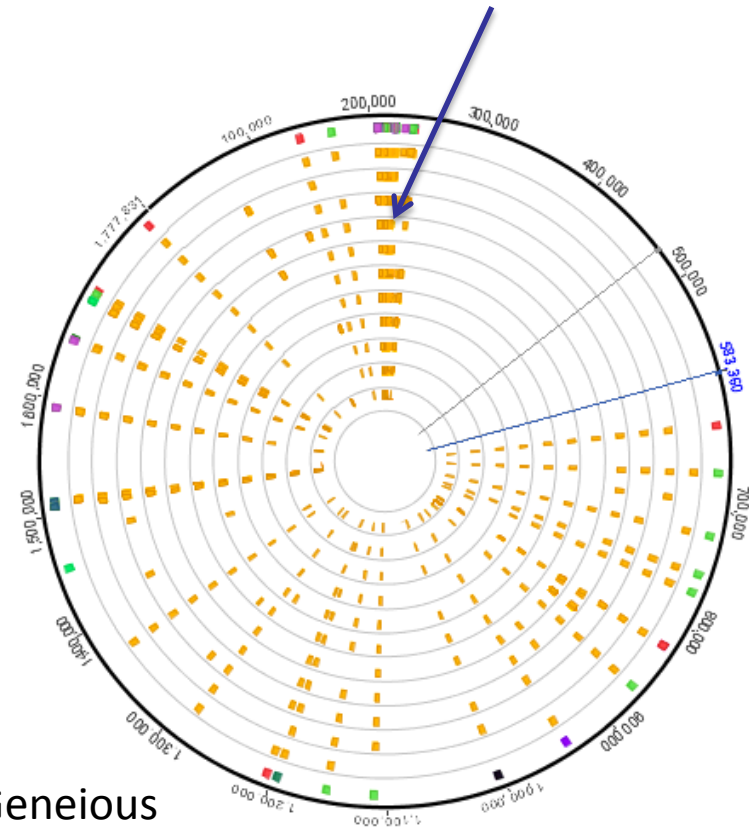
Initial Illumina sequencing of ST-6964 (N=91): evidence of four clades and within poultry company evolution



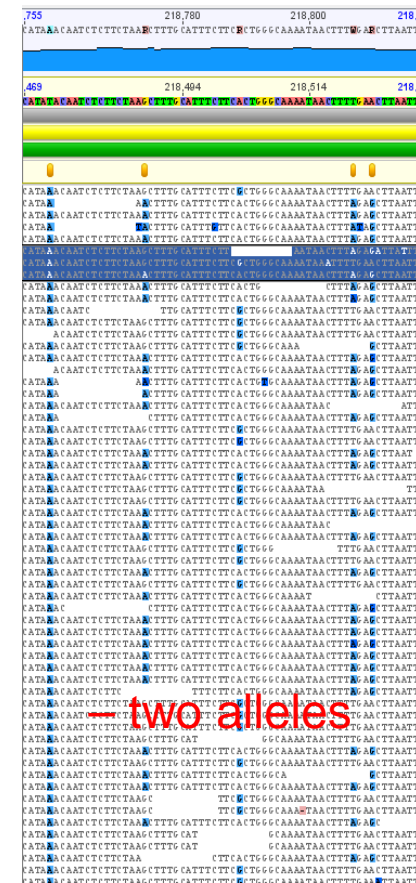
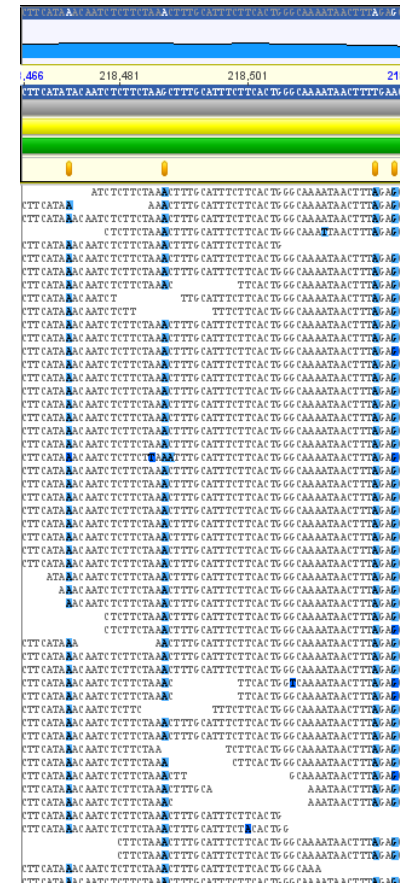
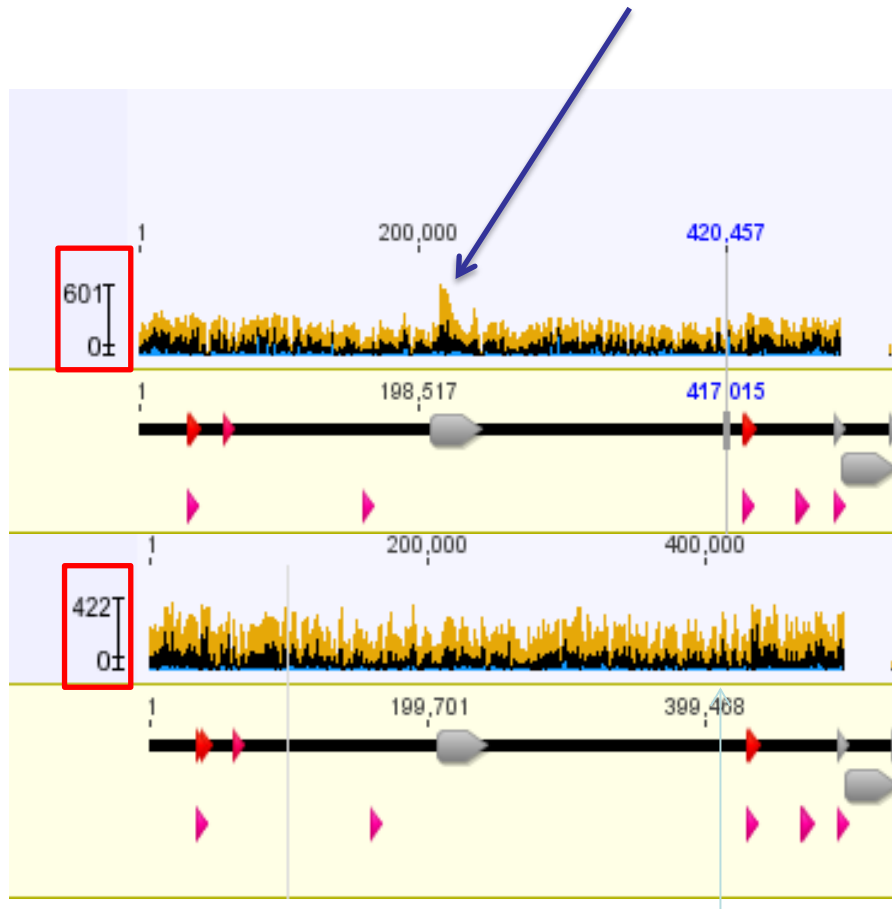
SNPs associated with mobile elements (prophage integrated elements)



High density of SNPs in Integrated element 1 (mu-like prophage)



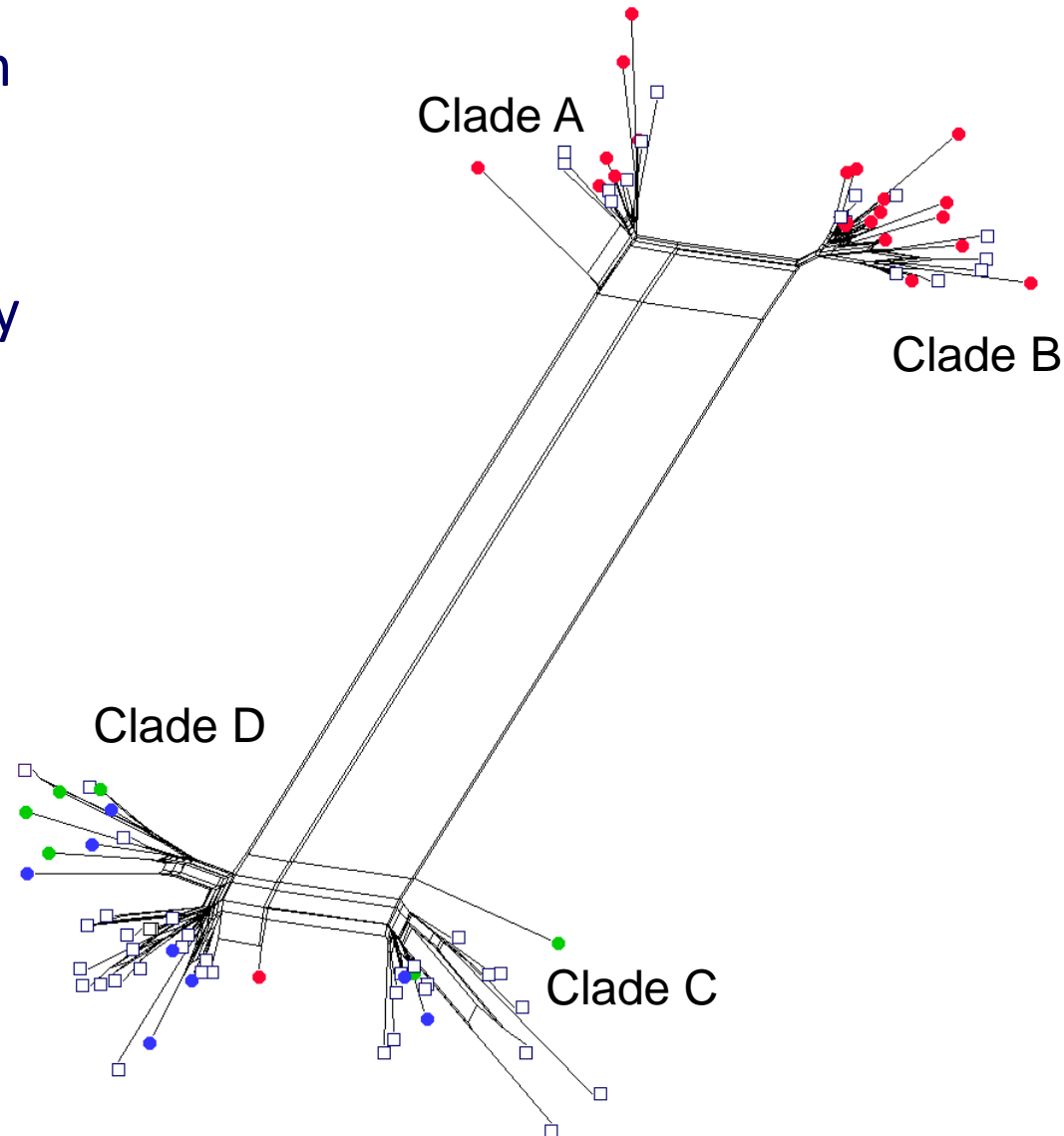
Ectopic mapping – evidence of extra IE1-like prophage in Supplier A?



IE1

SMRT (Pacbio) sequencing (MDU)

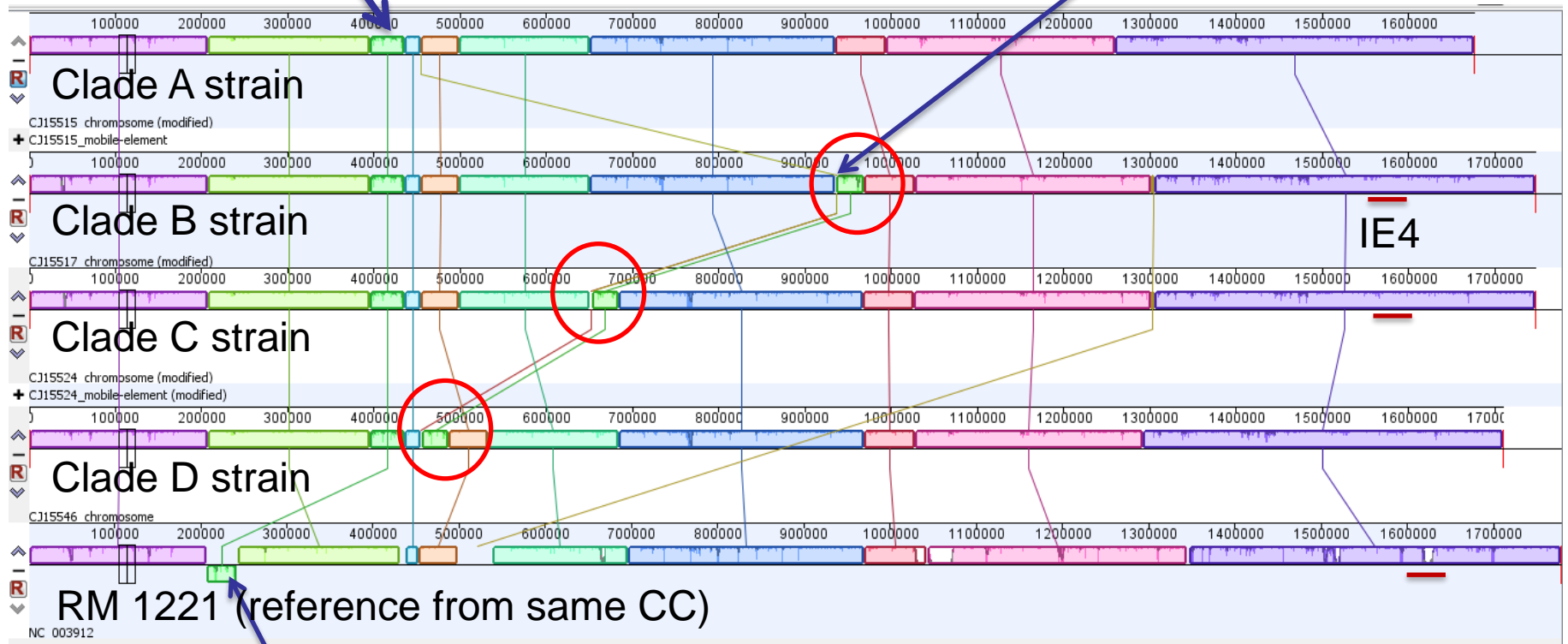
- Single isolates selected from 4 clades
- Sequenced at MDU, Melbourne (hybrid assembly using Illumina short reads)
- Complete annotated genomes (single contigs)
- Revealed different genome sizes
- Mobile (phage) elements
- Confirmed remnant pTet plasmid in C and D



Multiple phage insertions?

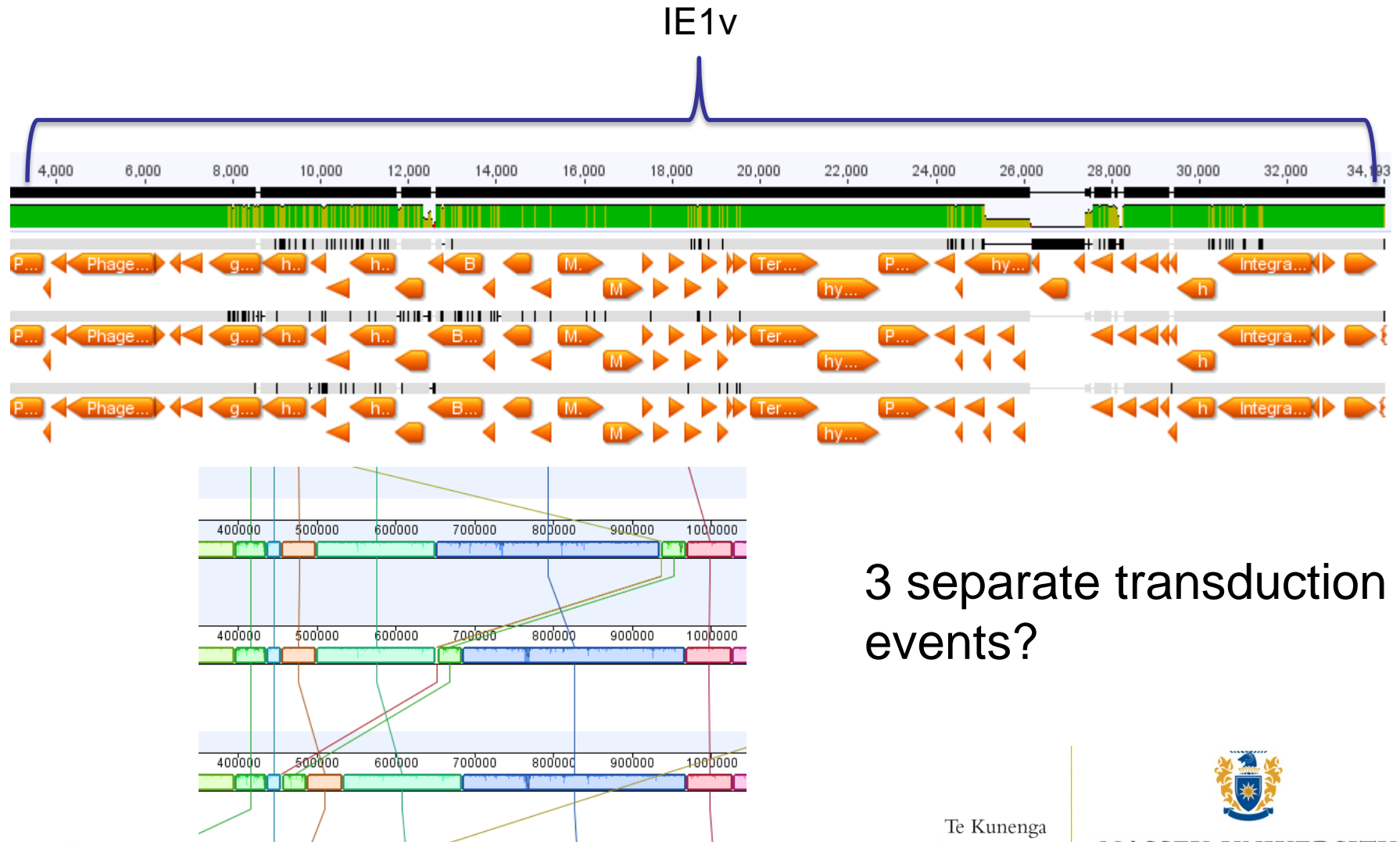
IE1

IE1variant (++ seq. variation and 3 insertion sites)

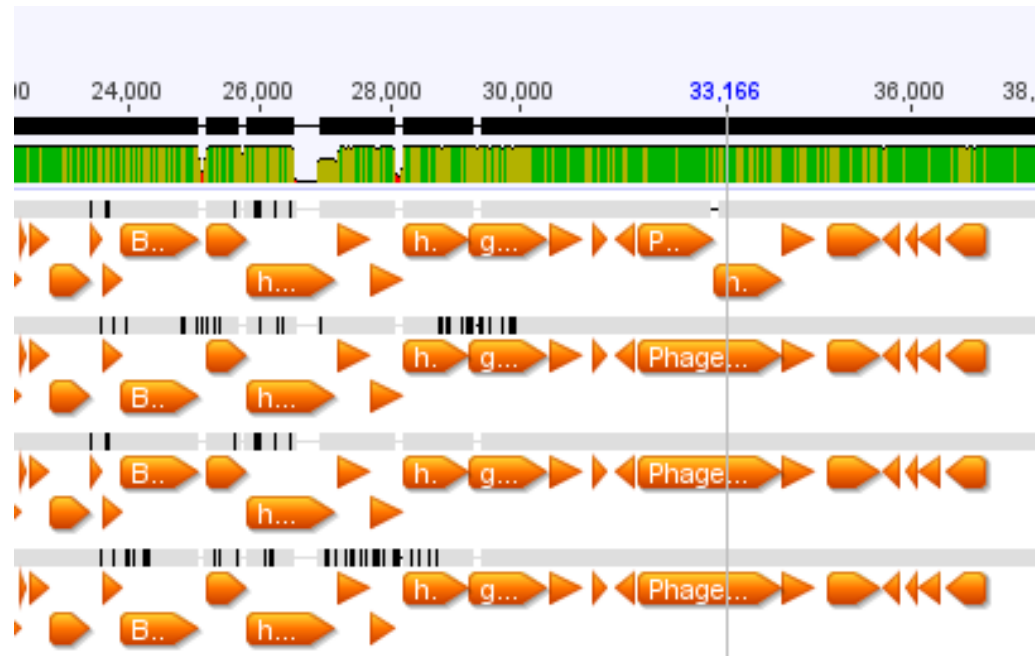


Inverted

IE1variant: highly polymorphic phage insertion



Number of genes with point mutations in coding regions of core genes low (~10)



Integrated Element 1 (IE1)

Most are in Integrated elements (recombination hotspots)

One recombinant core gene (maf3) motility accessory factor in flagellar (FLAG) region

Consistent with recent common ancestor

SSRs and slip-strand mispairing

e.g. Polysaccharide deacetylase – Capsular polysaccharide

...aaaaaggccttggaatgtccatagaattcattatcttctgggtaaattgggggggggggatta
...aaaaaggccttggaatgtccatagaattcattatcttctgggtaaatt_ggggggggggatta



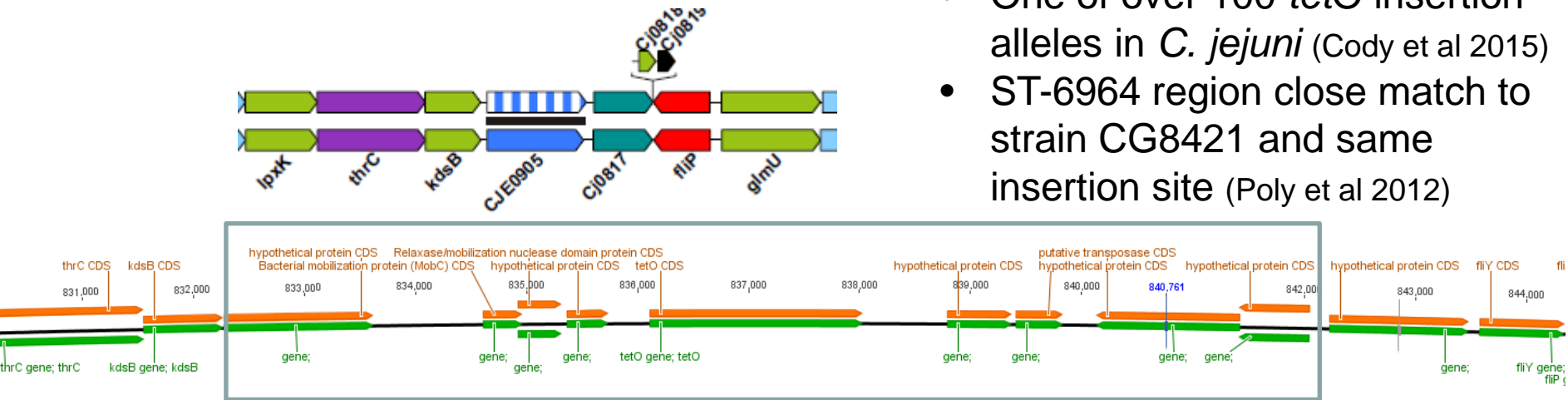
High frequency slip strand mispairing

On/off switching of genes involved in FLAG, CPS, LOS, OMP, phage resistance

15 SSR g/c and 39 SSR a/t variants identified,

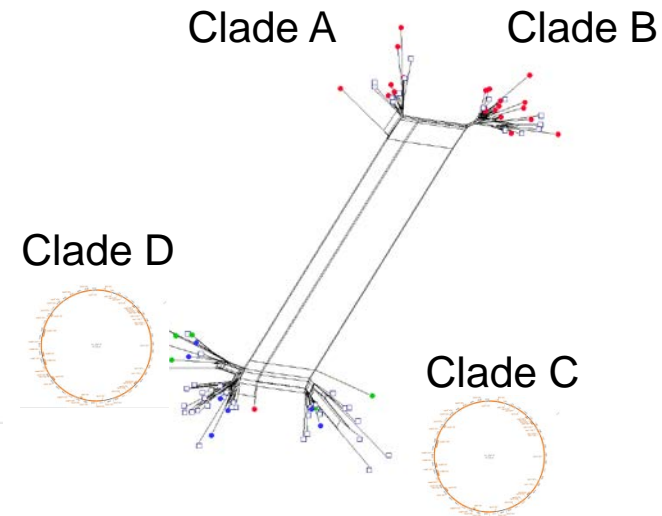
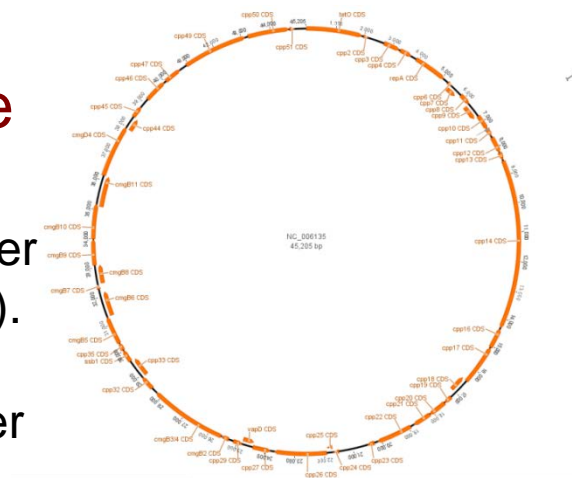
Confirmed *tetO* inserted in all between *kdsB* and CJE0905

- One of over 100 *tetO* insertion alleles in *C. jejuni* (Cody et al 2015)
- ST-6964 region close match to strain CG8421 and same insertion site (Poly et al 2012)



Remnant conjugative plasmid?

- Absent from poultry supplier A isolates (clades A and B). Present in clades C and D
- Strong homology with other *tetO* carrying plasmids



Key questions?

- Genetic basis for resistance?
 - *tetO* plasmid and C257T mutation in *gyrA*
- How long has it been in NZ?
 - Not long??—analysis complicated by SSRs and mobile elements.
- How has it been transmitted between poultry companies?
 - Shared parent and grandparent stock?
 - Feed
 - Complex interrelationships between suppliers (A and C shared parent stock, B and C shared feed)

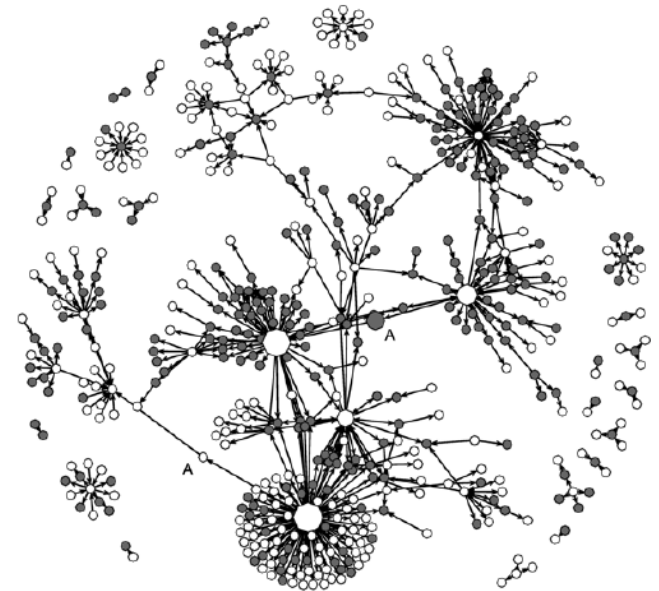
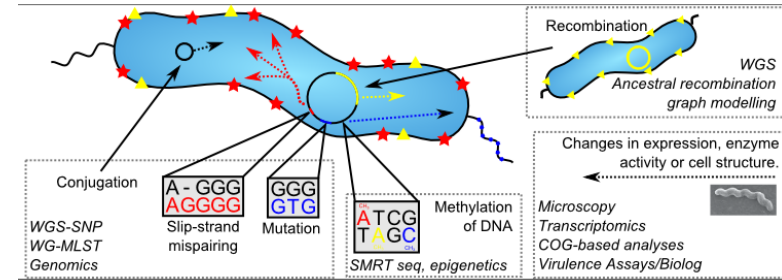


Fig. 2. Social network analysis of feed-related contacts in the New Zealand

Poultry farm feed network
(from Lockhart et al 2010)

Key questions?

- What has driven the emergence?
 - FQ use negligible
 - Limited tetracycline use in parent stock
- What is driving evolution?
 - Point **mutations?** – number v. small
 - **Natural transformation?** Only one core gene (*maf3*) with evidence of homologous recombination
 - **Singe Sequence Repeats** s – +++++ and in previously recognised and other locations
 - **Conjugation** – pTet plasmid, *tetO* chromosomal insertion
 - **Transduction** – multiple phage insertions
- Important point: WGS enabled higher resolution attribution to individual poultry supplier



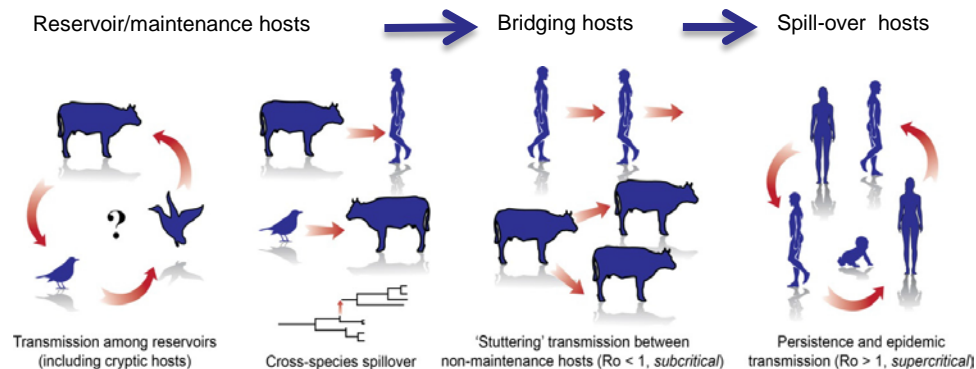
Conclusions

- Improved understanding of epidemiology of enteric zoonoses in New Zealand
 - One Health approach essential (working with Govt and Industry)
 - Application of molecular and genomic epidemiology
 - MLST and Whole genome sequencing – models of evolution and transmission
- Source attribution modelling
 - Tools advanced in recent years
 - Applied to *Campylobacter* in NZ and identified food, particularly poultry, most important source, informed policy
 - Major decline in human cases 2007-9
 - New antimicrobial resistant strain emerged in 2014
 - Recent introduction? But high diversity driven by mobile elements



Conclusions

- Important to use 'toolbox' approach
 - Space, time, risk factors
 - SA modelling
 - Population genetics/genomics/ecology
- Ongoing and future work
 - Source-assigned case control study (1400 isolates for WGS)
 - Work with poultry industry to reduce prevalence
 - Model development (attribution, ARG models)



Acknowledgements

- mEpiLab team: Patrick Biggs, Jonathan Marshall, Anne Midwinter, Julie Collins-Emerson, Rukhshana Akhter, Charlotte Bolwell, Jackie Benshop, David Hayman, Lynn Rogers, Sam Bloomfield
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- Poultry Industry Association of New Zealand – Dr Kerry Mulqueen, Dr Roy Biggs
- University of Melbourne, MDU Prof Ben Howden, Dr Dieter Bulach
- University of Otago: Prof Michael Baker,
- University of Auckland (Dr Tim Vaughan, Prof Alexei Drummond)
- Prof Paul Fearnhead (Lancaster)
- Prof Martin Maiden, Dr Danny Wilson, Dr Sam Sheppard
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