National Science Challenges

PŪHORO
he waka eke noa

The next steps for sites with elevated *E. coli* concentrations above water quality guidelines.

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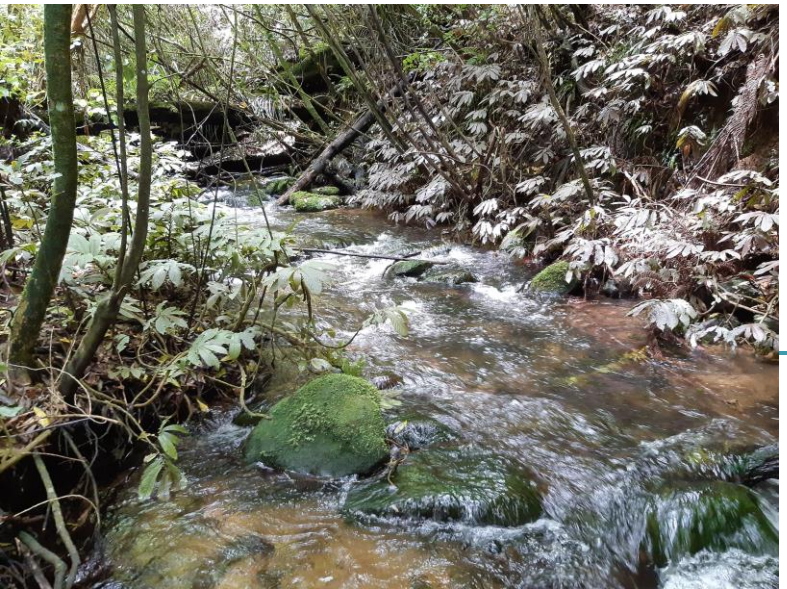
SWIM webinar presentation – 30 Sept 2021

- Background
- Adrian – OLW-NSC Project: Faecal source tracking and the identification of naturalised *Escherichia coli* to assist with establishing water quality and faecal contamination levels
- Meg – OLW-NSC Project: Framework assessment for water quality



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Water Research

journal homepage: www.elsevier.com/locate/watres

Review

Fecal indicator bacteria from environmental sources; strategies for identification to improve water quality monitoring

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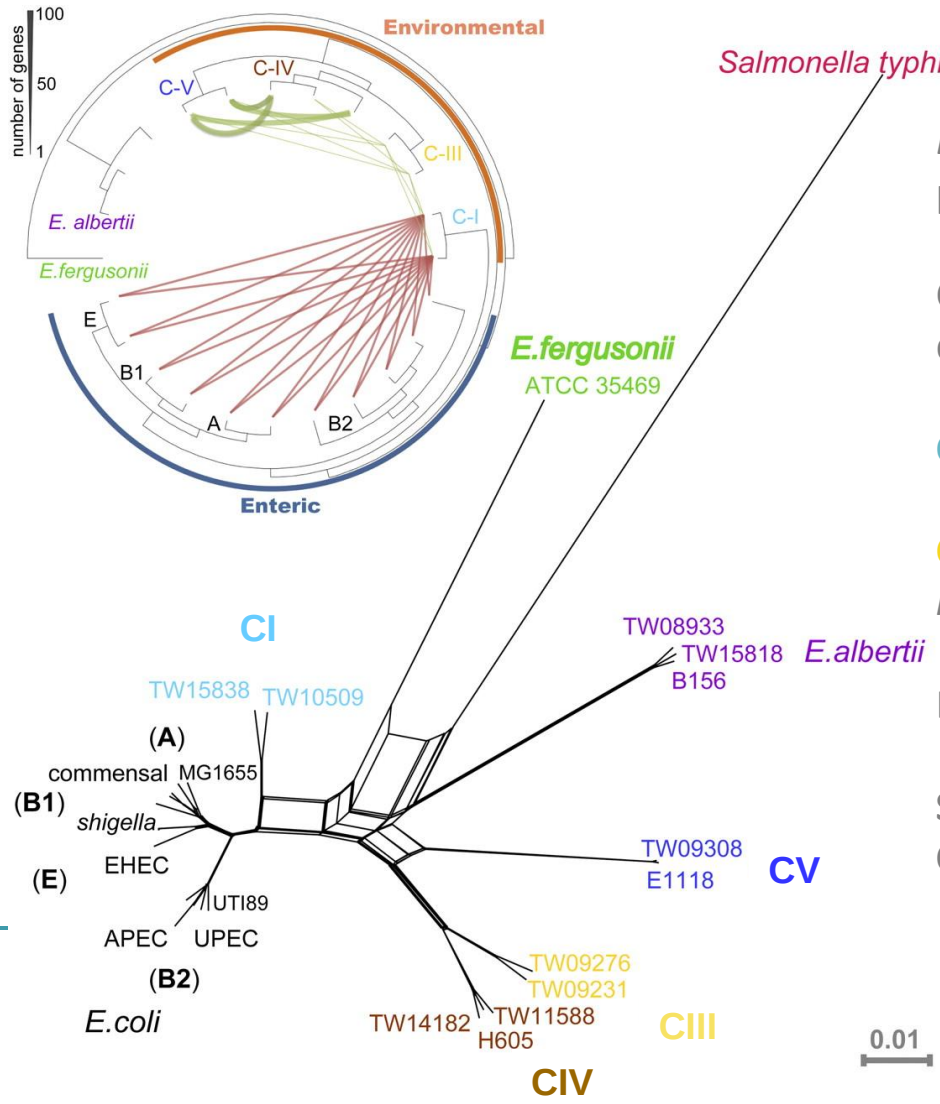
Background

- '*E. coli*' is used as an FIB
- 2 groups of naturalised/environmental '*E. coli*'
  - Indicators of non-recent faecal inputs and able to persist (B1/B2)
  - Ancient relatives of *E. coli* lineages – inhabit environmental reservoirs/waterways (*E. marmotae*/*E. ruysiae*)
- Both groups phenotypically identical to *E. coli* – identified as FIB by conventional water testing methods
- Correlation with health risk…?

- MBIE-SI – genomics/prevalence of FIB in bush vs. agricultural/urban sites

Whole genome phylogeny of *Escherichia*



E. coli 'sensu stricto' separated into at least 8 phylogenetic groups (A, B1, B2, C, D, E, F, G)

Cryptic *Escherichia* clades CI to CV are genetically distinct but phenotypically 'identical'

CI sits with *Escherichia coli 'sensu stricto'*

CIII and **CIV** – *Escherichia ruysiae*, **CV** – *Escherichia marmotae*

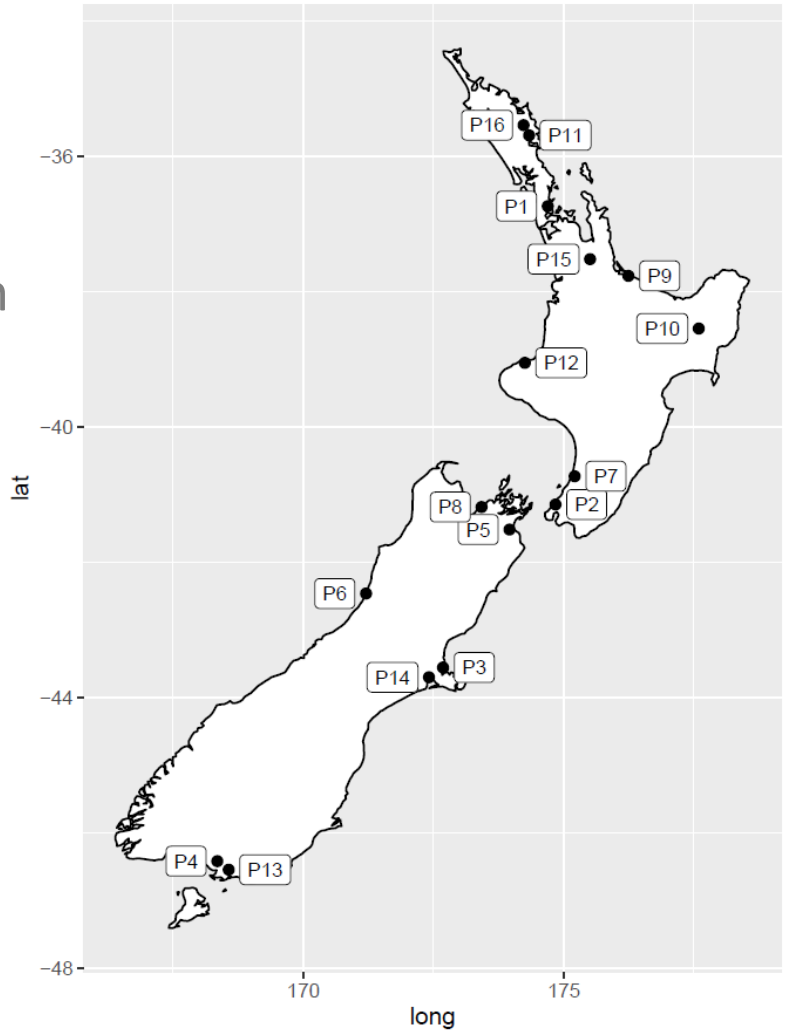
Limited genetic exchange between CIII, CIV and CV

Sampling and genetic analysis suggests CIII, CIV and CV environmentally adapted

Luo et al., 2011

MfE Pilot Study 2020

- Method development for 2021 study
- 16 freshwater sites with historically high counts of '*E. coli*'
- 3 observed land-uses: urban, dairy, sheep & beef
- Sampled by RC staff on 5 occasions in summer/autumn 2020
- Pathogen analysis:
  - bacteria (Salmonella, STEC, Campylobacter)
  - viruses (HAV, Noro, Entero)
  - Protozoa (Giardia, Cryptosporidium)



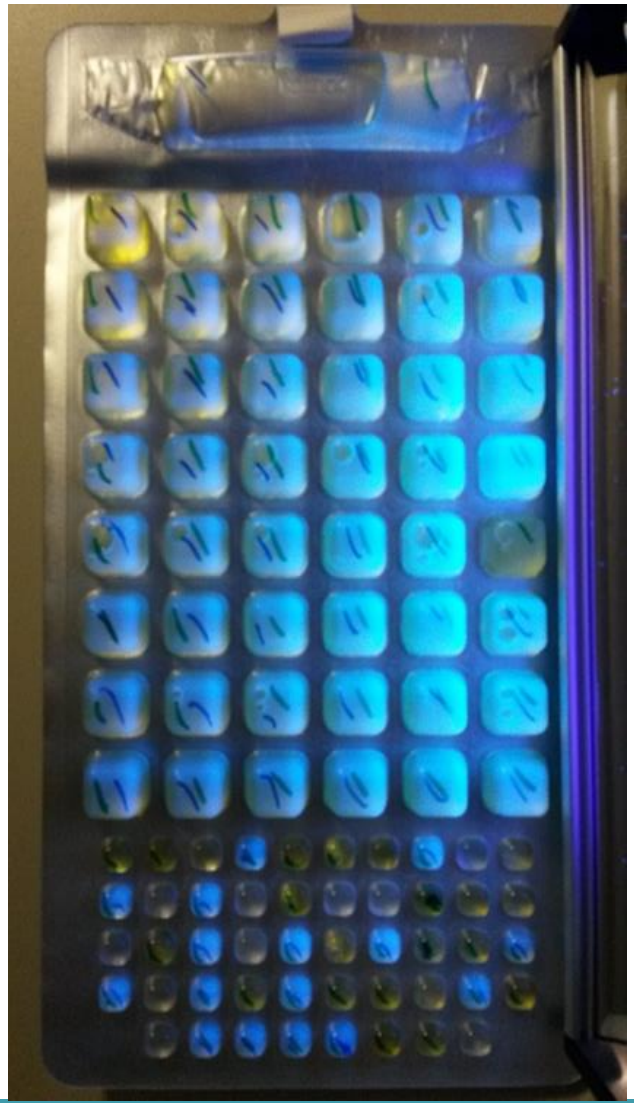
Motivation

Collaboration with ESR to 'add value' to MfE Pilot Study 2020 through:

- Detailed analysis and subtyping of '*E. coli*' from freshwater samples
- Link *E. coli* phylogenetic groups with pathogen presence/absence data
- Understand relative abundance of naturalised *E. coli*/*Escherichia* species in freshwater samples with historically high '*E. coli*' levels

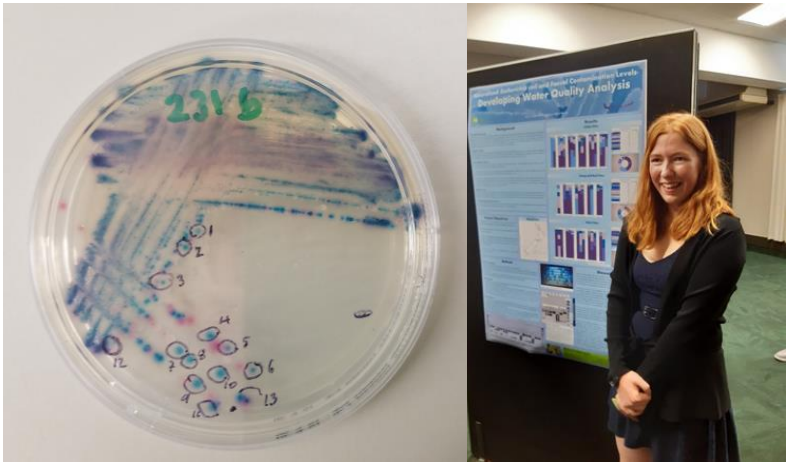
Methodology 1

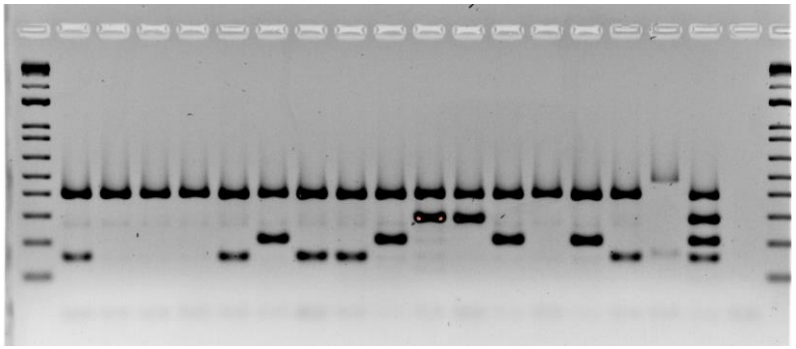
- ***Day1*** – water samples taken
- ***Day2*** – water samples received at ESR Christchurch - Colilert and '*E. coli*' MPN
- ***Day3*** – Post-incubation Colilert trays received at Hopkirk Research Inst., Palmerston North
- For each Colilert, growth from each '*E.* coli'-positive well pooled & stored in glycerol at -80°C
- Data from 42 Colilert samples included in this study



Methodology 2

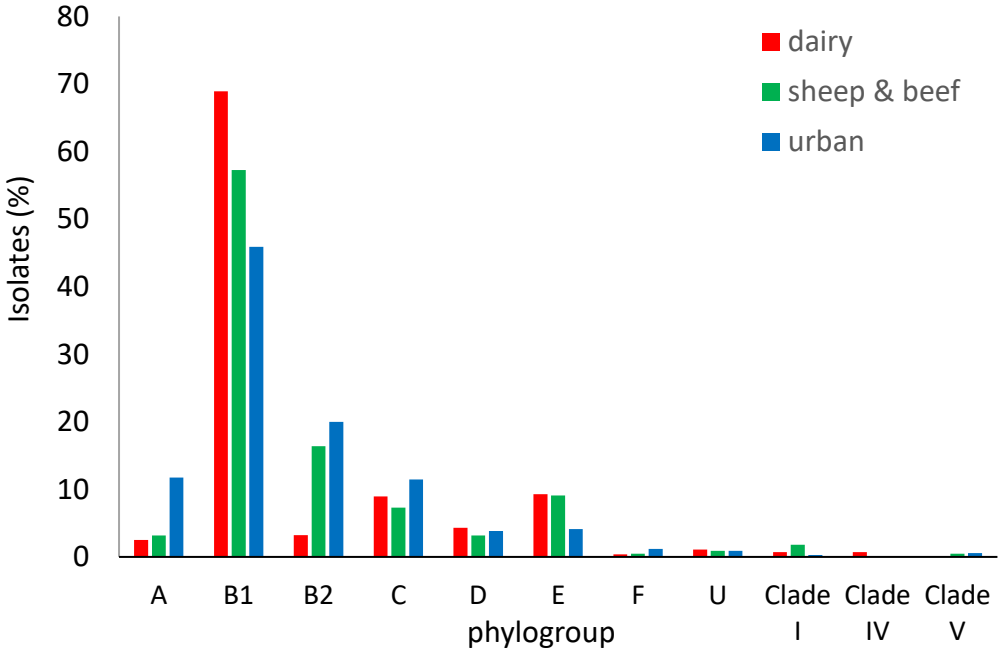
- 20 colonies recovered from each stored sample (n=42): 840 total
- All underwent phylogenetic PCR typing to:
  - Identify the 'true' *E. coli* phylogroups (A to G)
  - New 'environmental' *Escherichia* species (*E. marmotae* and *E. ruysiae*)





Results

- Phylogroups B1 and B2 can persist in the aquatic environment
  i.e., *non-recent faecal pollution*
- Such faecal *E. coli* linked with persistent pathogens? Health risk?
- Pathogens detected in 95.2% (40 of 42) samples incl. in this study
- Average (mean) '*E. coli*' 1,427 MPN per 100ml
- Phylotype B1: mean 10.9 per 20 isolates, B2: 2.6 per 20 isolates



- B1, n= 475, 56.5%;
- B2, n=113, 13.5%)
- *E. ruysiae*/Clade IV, n=2, 0.24%
- *E. marmotae*/Clade V, n=3, 0.36%

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Human health risks

- Where a variety of different *E. coli* phylotypes (A to G) – water body may contain recent faecal inputs
- Where ≥ 15 of 20 B1 and/or B2 (naturalised *E. coli*) per sample – water body may contain non-recent faecal inputs
- Where B1 and/or B2 ≥ 10 isolates per water sample

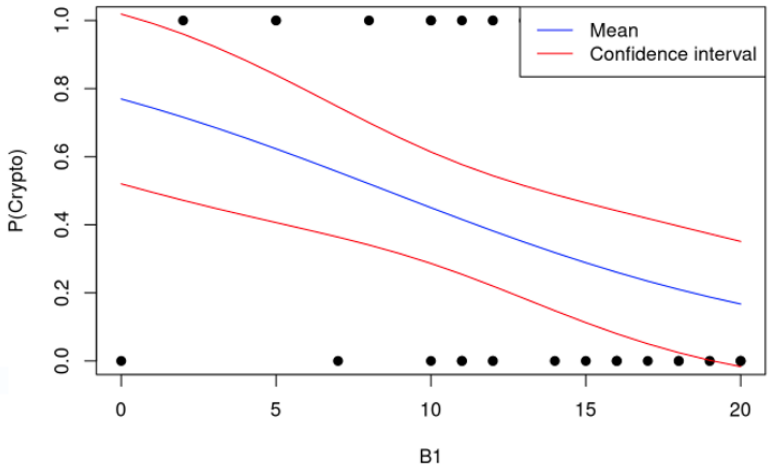
  ≥ 1 pathogen detected in 93.1% (27 of 29 samples)
- Where B1 and/or B2 ≥ 15 isolates per water sample

  ≥ 1 pathogen detected in 88.2% (15 of 17 samples)
- Samples with high B1 and/or B2 suggest non-recent faecal inputs, but presence and identification of pathogens = health risks

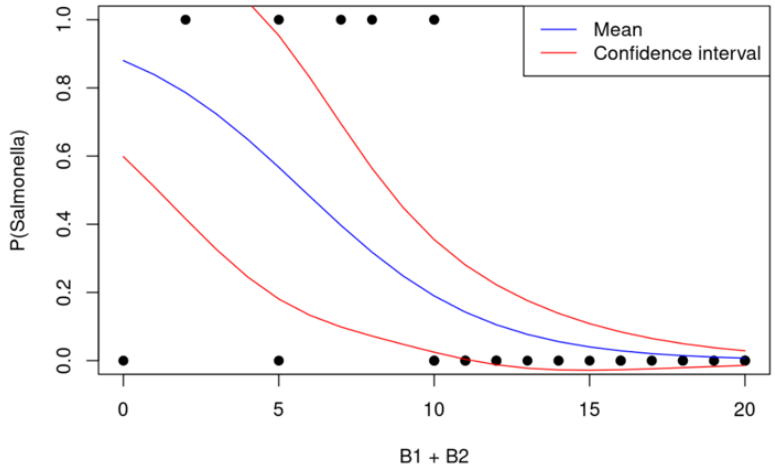
Data modelling – logistic regression

- ***From 42 samples*** – *'E. coli'* MPN per 100ml water data appeared to be predictive for Salmonella, Norovirus GI, Norovirus GII, and 'viruses' ($p<0.05$)

- High B1 as an indicator of aged faecal material – lower likelihood of sample containing cryptosporidium ($p=0.021$)



- High B1 and B2 as an indicator of aged faecal material – lower likelihood of sample containing Salmonella ($p=0.009$)



- Risk from other pathogens (*Campylobacter*, viruses, Giardia etc.) remains the same
- *E. coli* useful FIB: faecal contamination - ↑ likelihood of pathogens

Recommendations

- Where FIB exceedances occur with :
- ***Identification of faecal source markers*** – unnecessary to investigate naturalised FIB sources as *E. coli* from faecal inputs – PATHOGENS
- ***No identification of faecal source markers*** – investigate contribution of naturalised sources of *'E. coli'* (*E. marmotae* and *E. ruysiae*) in faecal contamination
- ***Identification of E. marmotae* and *E. ruysiae*** as dominant/sole source of increased '*E. coli*' – may represent a lower likelihood of health risk to recreational users – site-specific threshold may be required and ↑ testing

Acknowledgements

- Regional Council staff who provided 2020 samples
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