

Delineating the interaction of *Escherichia* species with freshwater pathogens and land-use

A project to add value to Ministry for the Environment's Freshwater Pathogen QMRA study that low numbers of *E. coli* phylotype B1 and a more diverse distribution of phylotypes were associated with recent faecal contaminations.

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Escherichia coli (*E. coli*) is a crucial indicator in water quality assessments for monitoring faecal contamination. This bacterium, commonly found in the intestines of warm-blooded animals, including humans, serves as a reliable marker for the presence of waterborne faecal pathogens including *Campylobacter*, *Salmonella*, *Cryptosporidium* and *Giardia*.

The use of *E. coli* as a faecal indicator bacterium offers several advantages in assessing water quality and ensuring public health safety. Since *E. coli* is predominantly associated with the gastrointestinal tracts of mammals and birds, its increasing prevalence in water samples is strongly associated with recent faecal contamination and allows for a more accurate assessment of the potential presence of harmful pathogens associated with human and animal waste.

E. coli is also easy to culture and quantify through various laboratory methods, making it a practical choice for routine water quality monitoring. Standardized techniques such as enzyme-substrate tests and membrane filtration, enzyme-substrate tests, and polymerase chain reaction (PCR) assays are an efficient and reliable means of assessing *E. coli* levels in water samples. The assessment of *E. coli* levels in water is also linked to regulatory standards and guidelines, aiding in the establishment of thresholds for safe recreational use of water.

Using PCR, *E. coli* can be delineated into several different phylogenetic groups or phylotypes (such as A, B1, B2, D etc), which are thought to have different host specificities and environmental survival characteristics. Recent studies utilising high-resolution genomics have demonstrated that several benign 'cryptic' *Escherichia* clades, indistinguishable from generic *E. coli* phenotypically, may also survive and persist in the environment, however, current culture-based methods cannot distinguish between environmental *Escherichia* clades and faecal strains. This lack of discrimination could lead to a potential overestimation of health risk using standard culture-based methods of *E. coli* determination.

In this collaboration between AgResearch, Massey University and ESR, detailed analysis of *E. coli* phylotypes, freshwater pathogens, land-use, and host-associated bacterial markers genes from human, ruminant and avian sources was undertaken from 199 freshwater samples collected between October 2022 and July 2023. These water samples were obtained from 41 sites where dairy, sheep and beef, urban, mixed farming (sheep, beef and dairy) was the dominant land-use, from low impact sites where native or exotic forests dominated, or sites designated as 'avian' using prior observational information provided by regional councils that noted large numbers of avian faecal contamination events caused by wildfowl flocks or bird roosts. Freshwater samples were taken as part of routine monitoring programs by council staff in support of the New Zealand Ministry for the Environment's (MfE) Freshwater Pathogen Quantitative Microbial Risk Assessment (QMRA) studies as an update to the 1998–2000 Freshwater Pathogen QMRA study.

In total, 20 separate *E. coli* isolates were taken from each of the 199 samples and all 3980 were examined using PCR to identify the *E. coli* phylotype or if whether 'cryptic' *Escherichia* clades.

- Eight different phylotypes were identified; B1 was the most abundant (48.0%), followed by B2 (14.9%) and A (14.8%), and the 'cryptic' *Escherichia* clades were rare (0.7%).
- Phylotypes A and B1 were overrepresented in dairy and urban sites which had the highest levels of overall *E. coli* contamination. In contrast, B2 were overrepresented in low impact sites which had the lowest *E. coli* contamination levels.
- *E. coli* contamination was also positively associated with increased the bacterial pathogens *Salmonella* and *Campylobacter*, but there was no interaction between *E. coli* and contamination by protozoa, *Cryptosporidium* or *Giardia*.
- Water samples from dairy land-use sites had a higher prevalence of *Campylobacter*, bacterial pathogens and protozoan pathogens.
- A higher prevalence of *Salmonella* was noted in water samples from avian and urban land-use sites.
- Pathogens were still detected in some water samples from low impact sites with a low *E. coli* concentration where human, ruminant and avian faecal sources were absent.

This study demonstrated that the prevalence of some phylotypes was clearly associated with land-use.

Exploratory analysis of the phylotype B1 abundance data from sites with diverse faecal sources suggest that it can be used as a convenient metric for assessing faecal age. For example, because phylotype B1 is thought to be able to survive and persist in the environment, high numbers of phylotype B1 were associated with aged or non-recent faecal contaminations, whereas low numbers of B1 and a more diverse distribution of phylotypes was associated with more recent faecal contaminations.

Further work is planned that will assist with understanding how many sampling events may be required to understand the dominant faecal sources likely to (intermittently) impact a site allowing an improved understanding of the human health risk associated with recreational water contact.