Science for Communities

Overview of the power, limitations and interpretation of microbial source tracking in recreational freshwater quality management

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EXECUTIVE SUMMARY

Freshwater recreational use is highly valued by New Zealanders. Contamination from point and non-point discharges of sewage and animal faeces presents a risk to human health. Water quality managers need to understand the risks to human health from faecal contamination to manage and improve recreational freshwater water quality and protect public health. Human faecal contamination may have human specific pathogens, such as viruses, but zoonotic pathogens from animal sources may also cause disease in humans. Monitoring recreational waters against the microbial criteria is routinely undertaken in the bathing season using the faecal indicator bacteria (FIB), *Escherichia coli* (*E. coli*). Where *E. coli* criteria are exceeded water quality managers need to identify the source of contamination to better understand the risk and target interventions that will improve water quality. Data from surveys of pathogens and faecal indicators in freshwater in New Zealand in 1998-2000, 2020 and 2021 showed that pathogen contamination comes from rural and urban activities.

As pathogens cannot be measured directly microbial indicators are used. This report presents the value of using FIB to alert water managers to faecal contamination events and the value of also using microbial source tracking (MST) techniques to identify the sources of faecal contamination. While host species specificity is an advantage of MST, there are still some disadvantages which are discussed. Key disadvantages are cross-reaction of MST markers to other non-host animals and geographical differences in prevalence and specificity. The common MST markers for human, ruminant and avian faecal contamination are discussed with information on cross reaction with non-target species from New Zealand.

Field data from the 2020, 2021 studies of human pathogens and FIB in New Zealand rivers is used to highlight the value of using FIB to detect faecal contamination and the presence of pathogens and using MST to identify sources. While more data is required for robust statistical analysis, MST results showed that observed land use can be a poor indicator of faecal source, with contamination from varied, and on occasions unexpected, sources. Repeated sampling events with multiple MST is a useful sampling strategy, not just for ensuring that faecal sources are not missed, but also to overcome cross-reaction with non-target species. Use of three markers for human MST provided more robust results. Risk cannot be determined by comparing the concentrations of MST as they target different micro-organisms or microbial interactions. Priority should be given to further investigation where there are significant concentrations of human MST as human viruses present the highest risk to human health.

A two-page summary is provided to inform Health Protection Officers about the application and interpretation of MST.

1. INTRODUCTION

Freshwater recreational use is highly valued by New Zealanders. Contamination from point and non-point discharges of sewage and animal faeces presents a risk to human health. The health risk for gastrointestinal illness (GI) is assessed using the criteria in microbial water quality guidelines. International microbial water quality criteria are based on epidemiological studies on the assessment of the risk of GI from swimming in water contaminated with untreated human wastewater (WHO 2021, EPA 2018, EU 2006, Health Canada 2012). Quantitative Microbial Risk Assessment (QMRA) shows that the greatest risk to swimmers' health is from human viruses (Boehm et al 2018) but direct deposition of bovine faecal material may carry a similar risk of GI from *Campylobacter,* a zoonotic pathogen carried by animals (Soller et al 2010). Data from surveys of pathogens and faecal indicators in freshwater in New Zealand showed that pathogen contamination comes from rural and urban activities (McBride et al 2002, Leonard et al 2020, Leonard et al 2021). New Zealand's freshwater guidelines are based on a relationship between *Campylobacter* and the faecal indicator bacteria (FIB), *Escherichia coli* (*E. coli*) (McBride et al 2002, MfE 2003).

Water quality managers need to understand the risks to human health from faecal contamination to manage and improve recreational freshwater water quality and protect public health. Human faecal contamination may have human specific pathogens, such as viruses, but zoonotic pathogens from animal sources may also cause disease in humans. Potential sources of faecal contamination are sewage discharges, leaking sewage pipes, cross connections of sewage and stormwater, combined sewer stormwater overflows, on-site wastewater treatment and disposal systems, direct defecation by animals and birds, runoff from pastures contaminated with animal faeces or discharges of animal waste. At certain locations, there may be more than one source of contamination and different sources of faecal contamination will have different risks.

The National Policy Statement Freshwater Management 2020 (New Zealand Government 2020) also requires improvement of water quality to increase the number of sites that comply with freshwater criteria. A Sanitary Inspection is traditionally undertaken to find the faecal source(s), using site inspections, observations of surrounding land use and information on consented discharges and activities (WHO 2021, MfE 2003). However, contamination sources are not always obvious, eg the discharge pipe may not be recorded, there may be multiple sources, or contamination events may be sporadic. FIB also have a disadvantage in that they are not specific to a particular faecal source. WHO guidelines recommend microbial source tracking (MST) techniques as a potential tool to identify sources of faecal contamination (WHO 2021).

Decisions on water quality improvements may involve health professionals. This report provides an overview of microbial tools that can help inform these decisions. This is an overview of the literature to assist in the interpretation of FIB and MST data from a public health perspective. It summarises the advantages and disadvantages of the most common FIB used for freshwater quality criteria. While MST tools are not recommended as indicators in the WHO guidelines, they are recognised as having significant value in water quality management (WHO 2021). This report presents data from the Ministry for the Environment's freshwater survey of pathogens and indicators to illustrate the benefits and limitations of MST tools and aid the interpretation of MST markers alongside FIB data.

2. INDICATORS

2.1 INDICATOR CHARACTERISTICS

The risk to public health arises from the presence of pathogens which may be ingested while swimming or through other recreational activities. However, direct measurement of pathogens is not feasible as they may only occur sporadically in populations of people and animals, are present in comparatively low concentrations, expensive to analyse and there is a lack of standardised indicators and methodologies. Despite recent research using viruses such as the mottled pepper virus or adenovirus, viruses have not been recommended for use for setting criteria for recreational water quality (WHO 2021). Instead, microbial indicators which are continuously prevalent in faeces and identified in high concentrations are used to alert water quality managers or health officials to the presence of faecal contamination and therefore to the potential presence of pathogens which may cause GI.

Indicator micro-organisms need to have key features such as:

1. continually excreted ie high prevalence in faeces
2. present in high concentrations
3. easily detected
4. quick and inexpensive to analyse
5. standardised methodology
6. survival characteristics are similar to pathogens
7. a relationship with health risk
8. don't replicate in the receiving environment.

These characteristics are discussed below for FIB (enterococci and *E.coli*) and MST.

2.2 USING *E. COLI* AND ENTEROCOCCI AS INDICATORS OF RECREATIONAL FRESHWATER QUALITY

Faecal indicator organisms, including *E. coli* and enterococci, have been used to assess the quality of recreational waters for over a century (Korajkic et al. 2018), and despite their limitations and research on alternative microbial indicators, they remain an important tool in monitoring the health risk in recreational water. A summary of candidates for alternative indicators (bacteriophage, enteric viruses and *Clostridium perfringens*, MST) were assessed in the WHO guidelines but none were proposed as suitable alternatives in the recent guidelines (WHO 2021). Either *E. coli* or enterococci are used exclusively for freshwater recreation in some guidelines and jointly in other guidelines.

- *E. coli*, used exclusively in the New Zealand guidelines (MfE 2003)
- *E. coli* and enterococci are used jointly in the guidelines in EU (EU 2006), Canada (Health Canada 2012) and the US (US EPA 2012),
- enterococci is used exclusively in the guidelines by WHO (WHO 2021) and in Australia (NHMRC 2008).

2.2.1 Advantages of FIB

The most common microbial indicators currently used are *E. coli,* a member of the faecal coliform group, and species of enterococci of the genus *Enterococcus.* They are a normal component of the gut of warm-blooded animals. Both are facultative anaerobic micro-organisms which means they can tolerate both the anaerobic environment of the gut and the aerobic receiving environment. Their presence in water is indicative of faecal contamination, and therefore, the potential presence of pathogens. Being intestinal microflora, they are continuously excreted and occur in high concentrations. In the human colon *E. coli* concentrations are 10^8/mL, representing 1% of all the biomass, and enterococci are just below 10^8/mL (Garcia-Aljaro et al 2020).

Standardisation of test methods for *E. coli* and enterococci means the data is robust allowing comparison from different laboratories and in different geographical areas. Compared with analysis of pathogens the tests are inexpensive, although they require a 24-hour incubation period to culture. Standardised qPCR methodology (US EPA 2015) has enabled enterococci to be analysed within hours, although it is more expensive than culture methods. Rapid results, however, are an advantage at highly populated swimming beaches, such as in the US.

2.2.2 Association with health risk

The recent revision of the WHO guidelines has confirmed enterococci as the indicator for freshwater using epidemiological studies with randomised cohort methodology. The epidemiological study is proposed as a superior method to other studies. As discussed in Leonard and Eaton (2021) there are other approaches, such as the norovirus outbreak investigation reported by Joosten et al (2017), which support *E. coli* as a better indicator of the risk of infection for freshwater. The Korajkic et al. (2018) literature review examined 23 studies for indicator-pathogen relationship in freshwaters. Only 13 reported a statistically significant relationship between at least one indicator and at least one pathogen and *E. coli* was the indicator that had the greatest number of significant pathogen relationships.

Leonard and Eaton (2021) also highlighted literature that show that there is a relationship between enterococci and GI only when the faecal contamination is human sewage (Arnold 2016, Yau et al 2014, Colford et al 2012). This has important implications for New Zealand where animal faecal sources are significant sources in the environment.

2.3 IMPLICATIONS AND INTERPRETATION

As previously described, an assumption in using indicator organisms as a proxy for health risk is that the presence and concentration of microbial indicators varies consistently with that of pathogens (Harwood et al 2014). Differences in survival characteristics due to wastewater treatment, or exposure to the environment, have implications in terms of understanding the risk to human health and water quality management.

The epidemiological studies used to determine the microbial water quality criteria for recreational water were mostly undertaken where there were untreated sewage discharges to water bodies (Carbelli et al 1982, Kay et al 1994, Fleisher et al 1996, Wade et al 2010). This does not address:

- the different survival characteristics between pathogens and FIB
- other sources of faecal contamination.

The underlying assumption is that significant risk occurs above an FIB criterion, with an acceptable risk below the criterion. However, sewage treatment and disinfection will reduce the bacterial concentrations but the effect on the concentrations of protozoa and viruses will be different and the ratio of indicator:pathogen will have changed (Table 1). As viruses are considered the primary risk to health from water contaminated with sewage, other indicators that are specific to human faecal contamination would be of great value in understanding the overall risk from recreation in a water body. Identification of human faecal sources, therefore, is a key benefit that MST can inform.

3. MST

MST markers are based on genetic material from gut micro-organisms or host-bacterial interactions. They are more species specific than FIB, allowing more effective mitigation measures to be implemented. MST are recognised in the WHO and Canadian guidelines (WHO 2021; Health Canada 2012) as valuable tools to help identify source(s) of faecal contamination. They can inform the initial sanitary inspection which is used for classification of the recreational area where there is a discrepancy between the sanitary inspection and the FIB. For example, there may be no obvious point source discharges but FIB concentrations are consistently high. MST marker analysis is also useful where there is a contamination event and the source of contamination needs to be identified and mitigations put in place. In New Zealand, the information can also be used by councils for water quality improvement to meet the government's NPS FM targets to increase the number of freshwater bodies that are within the microbiological guidelines for recreational water use. (New Zealand Government 2020).

However, MST have not been included as indicators of risk in the WHO guidelines, partly because method development and standardisation are still developing and also because WHO requires epidemiological studies that show an association between indicator and illness. There are other limitations in terms of specificity of markers which cross-react with non-target animals (false positives), and the relationship of MST markers with pathogens. An understanding of these limitations is important in the interpretation of MST data.

3.1 MST

Microbial source tracking (MST) was developed in the early 2000s using polymerase chain reaction (PCR) techniques to target the DNA of gut micro-organisms unique to a particular animal species (host) or to target actual host DNA (e.g., mitochondrial DNA of an animal). More than 40 microbial source tracking (MST) markers have been developed (WHO 2021). Application of this technique allows water quality managers to be able to distinguish between avian, human, ruminant, domestic animals such as cats or dogs, or feral animals such as possum faecal contamination. MST markers can also be used to provide greater host species detail such as the species of ruminant: cow, sheep, goat or deer. This allows interventions to improve water quality to be better targeted.

3.1.1 Species specificity of the host animal targets

MST marker specificity is much improved compared to the non-specific *E. coli* and enterococci. Specificity of MST markers to a particular animal host, however, is not absolute. Before using MST markers they need to be assessed for:

- Sensitivity - How many of the target host faecal specimens test positive?
- Specificity - How many non-target animal specimens test positive?

Both the frequency of detection within an animal species and in non-target species may differ across geographical locations (Green et al 2012, Balleste et al 2021, Devane et al 2013). This relates to different interactions between animals and humans, including co-habitation, different feeding and farming practices. Cross-reaction of MST markers may occur between domestic pets and humans, across groups of farmed animals, or across animals with similar digestive physiology eg humans and pigs.

It is important to test both aspects in animals that are likely be present in a catchment. Possums for example are found across rural and urban New Zealand. A MST marker for possum faeces cross reacted with two widely used human MST markers, HF183 and HumM3, yet in the field, the possum marker was not found in human sewage and only found infrequently in concentrations which were orders of magnitude lower in human faeces (Devane et al 2013). Specificity may also be consistent in different countries but the prevalence differs between regions. Seagulls, geese, chickens and ducks had 100% specificity for the avian marker GFD across New Zealand, the United States and Canada (Green et al 2012) ie no non-avian species tested were found to cross react with GFD. However, the prevalence of GFD was lower in chickens in New Zealand compared to the West Coast of the USA and Canada.

It is therefore important that MST markers are tested on faecal material from species in New Zealand.

3.1.2 Commonly used MST in New Zealand recreational freshwater

A key group of MST are the anaerobic bacterial genetic markers *Bacteroides* and *Bifidobacterium* genus. *Bacteroides* and *Bifidobacterium* genus are present in higher concentrations in the human gut than FIB (Ahmed et al 2011), and, as obligate anaerobes, are less likely to grow in the environment than traditional FIB. GenBac3 is a MST marker that targets the taxon Order *Bacteroidales* and is identified in concentrations, in the human colon of 10^{11}/mL, around three orders of magnitude higher, than *E. coli* and enterococci (Garcia-Aljaro et al 2020). GenBac3 is a marker of general faecal contamination because it has low specificity being detected at high concentrations in human faeces and the faeces of animals such as possum, cows, sheep, pig duck and black swans in New Zealand (Devane et al 2013).

HF183, a genetic marker from *Bacteroides dorei,* has been used extensively to characterise the human faecal input from raw sewage and in QMRA that model the risk to human health from mixed sources of faecal contamination (Boehm et al 2015, Boehm et al 2018, Boehm and Soller 2020, Schoen et al 2020). It has high sensitivity, being detected in low concentrations of sewage, and has high host specificity, although it has been detected in quantifiable concentrations in Australian cat and chicken faeces but not in cow, deer, goat, horse, pig or sheep (Ahmed et al 2019). *Bifidobacterium adolescentis* (BiAdo) is also used to detect human faecal contamination (Matsuki et al 2004) and is present in high concentrations in human faeces, but may also be present in seagulls, possums, dog, duck and swan (ESR MST specificity sheet).

MST markers of *Bacteroides* spp. and *Bifidobacterium* can be used to differentiate between faecal sources eg humans and ruminant (Bernhard and Field 2000). Specific MST have been developed that target faecal sources from pig and horse (Dick et al 2005), human (Reischer et al 2007) and ruminant faecal contamination (Reischer et al 2006). BacR is a *Bacteroidetes* ruminant marker found to be present in cattle, deer, chamois, sheep and goat and absent in human sewage, horse, pig, cat dog, chicken, turkey, swan and duck (Reischer et al 2006).

Further MST markers have been developed to discriminate between faecal contamination from cows or sheep. For example, CowM2 targets specific bovine faeces genetic markers derived from host-bacterium interactions that are very specific for cow, occurring in high concentrations in cattle herds compared to other sources (Shanks et al 2008). However, it is not present in the same high concentrations as the *Bacteroidetes* ruminant marker, and

therefore, there needs to be a high level of faecal contamination such as fresh faeces for it to be detected. For example, the BacR marker concentration needs to be approximately 1000 copies/100 mL to ensure detection of CowM2 (Devane et al. 2020, 2021). There are MST markers for other ruminants, such as sheep and deer, but if the markers are not present in high concentrations in the faeces, very fresh inputs are required before analysis is worthwhile. These MST markers are, therefore, used subsequent to detection of BacR at ≥ 1000 copies/100 mL.

Bacteriophage, viruses that infect bacteria, have also been identified as useful MST markers. In human faeces, some bacteriophage are present in concentrations 10-100 times higher than gut bacteria (Garcia-Aljaro et al 2019). The crAssphage is a bacteriophage which has high specificity for human faecal contamination and has been identified in high concentrations in New Zealand sewage plant influent and effluent (Gyawali et al 2021). . Testing on New Zealand animal and avian faeces show that the crAssphage marker has a low level of cross-reactivity to livestock, and feral animals (possums and rabbits) compared with HF183. The crAssphage marker was, however, identified infrequently in cat and seagull faeces in quantifiable concentrations (Gyawali et al. 2021). It was not detected in black swan, Canada geese, chicken, cow, dog duck, horse, sheep, goats or rabbits.

For avian contamination GFD, a genetic marker present in *Helicobacter* spp, is used (Green et al 2012, Ahmed et al 2016). It has been tested on New Zealand animals and sewage and found to have moderate sensitivity and is absent from human, cow, sheep, deer, horse, goat, pig, rabbit, possum, cat and dog faeces (Green et al 2012).

3.2 SURVIVAL OF FIB, MST AND PATHOGENS IN THE ENVIRONMENT

Ideally, a pathogen and an indicator decay at a similar rate: the lower the concentration of FIB the lower the concentration of pathogen. However, Table 1 illustrates that viruses and protozoa have much longer die-off times in the environment than *E. coli* and enterococci. Micro-organisms are also affected differently by the receiving environment. *E. coli* and enterococci die-off rates are similar in freshwater, but *E. coli* dies-off much quicker than enterococci in marine water (Pachepsky et al 2014).

Under different discharge scenarios the age of the faecal contamination will be different and may be a mixture of recent and aged contamination. This will affect the overall concentrations of MST, FIB and pathogens.

A review of decay rate data in the literature by Boehm et al 2018, shows that the mean daily $\log_{10}$ decay rate constant for HF183 (0.063 $\log_{10}$ /day) is similar to enterococci (Table 1), slower than *Campylobacter* and quicker than *Salmonella* or *E. coli* O157. Dick et al (2010) compared decay rates for HF183 and *E. coli* in a river microcosm at 25°C and 15°C and determined no significant difference. The mean daily $\log_{10}$ decay rate of crAssphage was reported as -0.19 $\log_{10}$ /day (Schoen et al 2020) which is slower than the human bacterial MST, but still faster than pathogens such as viruses or protozoa (Table 1). This slower decay rate means crAssphage may be more useful than HF183 for assessing the health risk from viruses, *Giardia*, *Cryptosporidium* and STEC. *Helicobacter* spp. is not a strict anaerobe like *Bacteroides* and *Bifidobacterium* and is therefore also expected to have a slower decay rate in the environment than HF183 (Ahmed et al 2016).

Agricultural runoff of faecal material is a key pathway for contamination of freshwater bodies. Cowpats dry out quickly forming an outer protective crust which protects pathogens and FIB. The ratio of BacR:GenBac3 eluted from cowpats can provide information about the source of

contamination (Devane et al 2020). Water samples with a BacR:GenBac3 ratio of 17%, or higher, indicate that the majority of contamination detected is attributed to ruminant (in this case, bovine) sources. There are significant changes to the microbial communities as cowpats age, and CowM2 is absent from cowpats after 42 days, despite being present in high concentrations in fresh faeces (Devane et al 2020). It is recommended that CowM2 is only tested if concentrations of BacR are ≥ 1000 gene copies /100 mL. Devane et al (2020) also identified that *E. coli* is persistent in decomposing cowpats and may be mobilised by flood events from a cowpat months after deposition, creating a source of FIB contamination, when MST markers are no longer detected or present in low concentrations in the waterbody.

3.3 MST AS A MICROBIAL INDICATOR OF HEALTH RISK

To be a recreational water quality microbial indicator, WHO (2021) requires evidence of a relationship between the indicator and pathogens. This has not been established for MST. In the review by Korajkic et al (2018) only one of eight freshwater studies showed a direct significant relationship between MST and pathogens. In another study, *Campylobacter* was shown to be related to a human MST marker, HF183, despite the waterbody being observed to be more likely to be impacted by agriculture rather than human sewage and cattle MST markers related to *E. coli* O157 and *Salmonella* (Walters et al 2007).

Human MST markers such as HF183 have been used in QMRA modelling studies as a surrogate for the presence of sewage. Boehm et al (2015) derived concentrations of the human MST markers, HF183 and HumM2, that are likely to align with the US acceptable risk of 30 GI per 1000 swimmers. The model assumed the sewage contamination was constant and fresh, raw sewage, as they noted that treatment reduces the concentrations of MST markers. Another QMRA model was run using different ages of raw and treated sewage to determine risk-based water quality thresholds for HF183 (Boehm and Soller 2020). For unaged, treated effluent the Risk Based Threshold (RBT) would be 10 times lower, meaning lower concentrations of the MST marker could equate to risk from persistent pathogens because of the faster decay rate of the marker compared with pathogen die-off during treatment. Using a gull MST marker and modelling the risk to human health from *Campylobacter* and *Salmonella*, it was predicted that *Campylobacter* died off quickly and after 2.5 days *Salmonella* presented the higher risk.

Use of RBT is still in developmental stages. Boehm and Soller (2020) highlighted information gaps in the die-off rates of relevant MST markers and norovirus. At this time the value of MST lies in the ability to better understand faecal sources and mitigate them.

3.4 IMPLICATIONS AND INTERPRETATION

3.4.1 Disadvantages of FIB

It is important to understand how the survival characteristics of indicator microorganisms reflect the survival characteristics of pathogens. There are many factors which affect the survival of micro-organisms in the receiving environment such as temperature, salinity, sunlight. Therefore, it can be difficult to compare studies where the same environmental conditions are not used. Using within study comparisons, Pachepsky et al (2014) showed that *E. coli* survives longer than *Salmonella* in lake water but that *E. coli* dies off faster than enterococci and *Salmonella* in marine water. Studies of the survival of viruses and *E. coli* and enterococci also highlight variations. The daily rate constants for die-off have been derived for common pathogens from a review of decay rate data in the literature and are compared in Table 1 with indicators.

Table 1 Comparison of rate constants for die-off of indicator and pathogenic micro-organisms

Micro-organism	Daily mean log_{10} decay constant	Reference
Enterococci	0.068	Boehm and Soller 2020
Campylobacter	0.45	Boehm and Soller 2020
Salmonella	-0.13	Boehm and Soller 2020
viruses	-0.81	Bohem et al 2018
E. coli O157	-0.43	Bohem et al 2018
Giardia	-1.36	Schoen et al 2020
Cryptosporidium	-1.39	Schoen et al 2020

These mean daily rate constants show bacteria have faster die-off rates than viruses or protozoa, with *Campylobacter* having the fastest die-off rate. In fresh faecal inputs, therefore, FIB are good indicators of faecal contamination events, and suggestive of pathogen presence. However, there is an increasing disconnect between FIB and pathogens when the contamination is from an aged/non-recent sources. Non-detection of FIB in such circumstances may not alert authorities to a potential health risk from the more persistent pathogens.

In tropical and subtropical climates, *E. coli* can replicate in the environment and naturalised *E. coli* have been identified which persist after the faecal contamination event, as reviewed by Devane et al (2020). The routine microbial water quality tests may also pick up other environmental micro-organisms which belong to the genus of *Escherichia* but are not associated with faecal contamination from humans or livestock.

While it is an advantage that FIB are intestinal micro-organisms that are continuously shed, they are not specific to a particular animal species as they are present in the gut of all warm-blooded animals. However, different hosts have different FIB prevalence, with 90% prevalence in the human gut, 23% in birds and 56% in wild mammals (Tenaillon et al 2010). Concentrations in faeces also differs from 10^7-10^9 /g faeces in humans to 10^4-10^6 /g faeces in domestic animals (Tenaillon et al 2010).

3.4.2 Multiple MST markers

Holcomb and Stewart (2020) highlight the use of a toolbox approach to identify faecal contamination, using MST for multiple sources to provide a more robust interpretation than reliance on a single MST. Detection of human MST markers is critical due to the high risk associated with viruses in human sewage. Investigation for the presence of human MST markers is, therefore, key in any situation where *E. coli* criteria are exceeded, even if a sanitary inspection does not indicate human contamination.

All MST markers have a degree of cross-reaction to the microbes present in the faeces of non-host animals. The concentration of the MST marker, however, is usually 3-4 orders of magnitude lower than the concentration identified in the host faeces (Devane et al 2013, Gyawali et al 2021). This means that dilution of the non-target faecal input into a water body will decrease the likelihood of incorrectly identifying the faecal source. Cross reactivity is the reason why routine monitoring for human contamination utilises three markers that target different microbes in the human intestinal environment. A sample is considered to have human faecal input when two of the three are present in significant concentrations (Devane et al 2019). A combined concentration of human markers greater than 1,000 copies/100 mL would be considered significant (Leonard et al 2020, 2021). While HF183 is the most widely used MST marker for identifying human faecal contamination, cross reactions with other animal species likely to be present in an urban, farming or site of low impact (ie native bush or forest) means that on its own it may not be reliable. The longer survival time of crAssphage makes it a useful choice as it is present in very high concentrations in the intestine and in sewage in New Zealand (Gyawali et al 2021) and has fewer cross reactions

compared with HF183. Table 1 indicates that it will better reflect the survival of viruses than bacteria MST markers.

3.4.3 Comparison of MST for different types of animals

Initially non-quantitative PCR was used to give presence/absence data, but quantification by qPCR has become more common. However, an important consideration when interpreting the data is that concentrations of MST markers that target different host animals are not directly comparable. Each MST marker is measuring a different organism or host-bacterium interaction. Different microbial targets have varying survival rates.

Even with the same MST marker, concentrations need to be interpreted on a site-specific basis. For example, comparisons between sites when using the same MST markers may not be informative if there are mixtures of fresh and aged faecal contamination at one of the sites, or sporadic contamination events from rainfall. Longitudinal sampling events that account for seasonal differences and land use practices at a site may be required to provide certainty of the dominant faecal sources and to assess effectiveness of mitigation measures. Longitudinal sampling is occurring as part of the Ministry for the Environment survey of FIB and pathogens in New Zealand freshwater bodies.

3.4.4 Sampling strategies

There are many different scenarios which may lead to faecal contamination of a waterbody such as:

- A continuous direct sewage discharge or broken sewer pipe.
- An intermittent discharge in response to environmental conditions like rainfall such as combined stormwater sewage overflows, overloaded on-site wastewater drainage field, or runoff from pasture or tile drainage systems after rainfall.
- Intermittent discharge events
- Intermittent direct faecal deposition events such as birds or cattle in the stream

All these scenarios may be further complicated if there are multiple sources. The freshwater survey of pathogens and faecal indicators in 2020 and 2021 showed avian contamination in 90% of samples, which is not unusual as birds are ubiquitous. Care needs to be taken to ensure that other faecal sources have not been missed using multiple sampling events, particularly to include rainfall and seasonal variation. Multiple sampling is important to ensure consistent interpretation of the MST data eg if a number of samples show human MST markers, it can indicate a continuous source such as a single broken sewer pipe. A systematic approach to sampling and data interpretation is given in Devane et al (2021).

3.4.5 Using MST to change classification of recreational water

WHO guidelines are based on human faecal contamination where the highest risk is from human viruses (WHO 2021). Using MST to identify other sources for a QMRA model of risk to human health has predicted that the risk is an order of magnitude less when faecal contamination is non-human, when the US EPA water quality criteria (35 enterococci/100 mL) is met (Soller et al 2015). WHO guidelines acknowledge that a better grading may be an option if only non-human sources are detected using MST but cautions non-human faecal pollution may still be an important source of pathogens, as shown in the QMRA on New Zealand freshwater which found the main risk to human health was *Campylobacter*, a zoonotic pathogen (McBride et al 2002). A QMRA model by Soller et al (2010) indicated that direct deposition of *Campylobacter* presented a similar risk as viruses to human health using FIB criteria and an acceptable GI of 3/1000 people.

4. FIELD DATA

The Ministry for the Environment is currently undertaking a survey of pathogens (*Campylobacter*, *Salmonella*, STEC, *Giardia*, *Cryptosporidium*) and indicators (FIB and MST) to build a field database to revise the QMRA used as the basis of the New Zealand microbial freshwater recreational water quality guidelines. The sites have been chosen because they have poor water quality and therefore likely to have faecal contamination and pathogens. They are not a reflection of recreational water quality across New Zealand. The data highlights the benefits and limitations of MST. Key interpretations of the FIB and MST data are presented to better understand health risk, as well as the limitations.

4.1 OBSERVED LAND USE AND FAECAL CONTAMINATION

Observation of land use has in the past been the key mechanism of determining faecal contamination sources and informing mitigations. For rural land with sheep and cows it would be expected that ruminant contamination would dominate and in urban areas, human contamination would be the main faecal source. However, Figure 1 illustrates the discrepancy between a static observed land use and variable faecal sources. The sites are classified according to land use, but MST markers show that other faecal sources may be dominant at the time of sample collection. Samples with significant concentrations of ruminant or human MST are shown against the land use in Figure 1. Some sites had significant concentrations of both human and ruminant and are classified as “Human & Ruminant” faecal sources. As 90% of samples had avian MST markers in the 2020-2021 samples, avian is only shown as the dominant faecal source in the absence of significant concentrations of ruminant or human MST. Samples classified as “unidentified” had none of the MST markers tested ie no ruminant, avian or human MST.

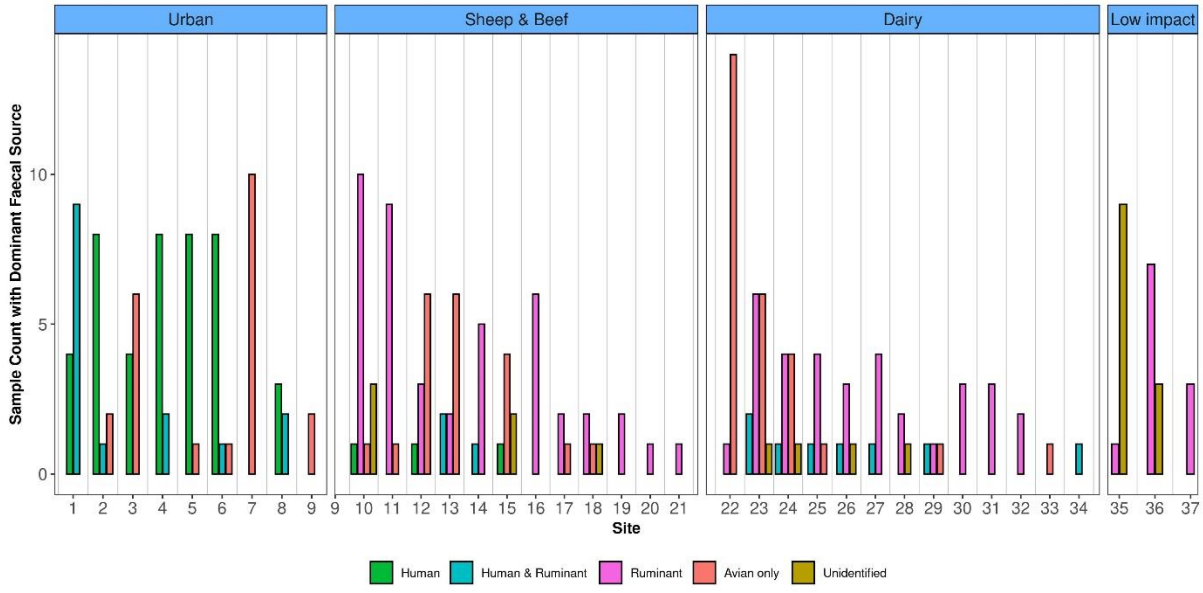

Figure 1 Summary of the MST markers for each site by observed land use in 2021

Figure 1 highlights the benefits of MST as six of the nine urban sites had a mixture of Human & Ruminant MST. The presence of ruminant MST markers in urban areas suggests significant upstream catchment contamination. One site accounted for 53% of the Human & Ruminant mixtures in the urban sites, suggesting some catchment specificity. Over half Only avian MST were measured in at least one sample in 6/9 sites (ie no significant concentrations of ruminant or human MST). The presence of Human MST markers at Sheep & Beef sites was unexpected. While significant concentrations of Ruminant MST was measured most frequently in samples from the Sheep & Beef sites, significant concentrations of Human and Ruminant MST markers (Human & Ruminant) were also measured at Site 13 in two samples and one sample at Site 14. High concentrations of Human MST were measured in one sample at sites 10, 12 and 15. Dairy sites most frequently had significant concentrations of Ruminant MST markers, but site 23 has two samples with Human & Ruminant and sites 25, 26 and 27 have one sample with the Human & Ruminant mixture. Of the 57 samples from dairy sites, 39 did not have significant concentrations of ruminant markers. Site 35 which is low impact and therefore would be expected to be avian only, had four samples with *E. coli* >959 MPN/100 mL. The MST marker concentrations of these samples indicated that one sample had significant concentrations of Ruminant MST and three samples were unidentified ie not avian, ruminant or human. Despite being observed as a low impact site, site 36 had significant concentrations of Ruminant MST markers in 7/10 samples.

In conclusion, Figure 1 highlights that observed land use is not always consistent with the sources of faecal contamination. While land use is static, MST highlights temporal variation.

4.2 PATHOGENS AND INDICATORS

4.2.1 Pathogens and *E. coli*

E. coli concentrations >550 MPN/100 mL is the water quality criterion in the Guidelines for recreational water quality that alerts water managers to the potential for risk to human health.[1] The data from 2020 and 2021 (309 samples) is cut into two categories >550 MPN/100 mL (96 samples) and ≤500 MPN/100 mL (212 samples) and the presence of pathogens assessed (Table 2). The data shows that where *E. coli* concentrations exceeded the water quality criterion there are more samples with *Campylobacter* and giving a higher median concentration, although the maximum concentration was the same. Data was not available for *Salmonella* and STEC as they were not quantified in 2021 due to the low prevalence and concentrations identified in the 2020 pilot study. The same pattern is observed for the protozoa, *Cryptosporidium* and *Giardia.* More data is required for a robust statistical analysis to determine the association of *E. coli* and the presence of pathogens and to calculate the risk to health with a QMRA.

[1] McBride and Soller (2017) note that this was changed to 540 MPN/100 mL in the NPS FM (2020) for technical reasons

Table 2 Frequency of pathogen detection and concentrations above and below recreational water quality criterion

	Campylobacter	*Cryptosporidium* oocyst/10 L	*Giardia* cyst/10 L
***E. coli* >550 MPN/100 mL**			
Median	4.1	0.27	3
Range	BDL - 1100	0.1-14	BDL-25
Pathogen detected in sample (%)	84	53	69
***E. coli* >550 MPN/100 mL**			
Median	0.34	BDL	BDL
Range	BDL - 1100	BDL - 6	BDL - 21
Pathogen detected in sample (%)	43	35	17

BDL below detection limit

4.2.2 MST and pathogens

As *Campylobacter* is more frequently present and in higher concentrations when water contains >550 MPN/100 mL *E. coli*, it would be important to identify the faecal source(s) to mitigate the potential health risk. The observed land uses where *Campylobacter* and *Salmonella* were detected where *E. coli* exceeded 550 MPN/100 mL, are presented in Table 3 and Table 4 with the MST markers for Human, Human & Ruminant, Avian and Unidentified. There were too few detections of STEC to assess.

In samples containing >550 *E. coli* MPN/100 mL, *Campylobacter* was detected most frequently in Urban land uses (Table 3). However, the measurement of significant concentrations of Ruminant and Human & Ruminant MST in these samples indicates potential contamination from rural activities in the surrounding catchment. Significant concentrations of Ruminant MST were measured in more than half of the samples (18/33 samples). If only observed land use was used to inform mitigation, it is clear from Table 3 that the important contributions of Human and Human & Ruminant would also be missed at Dairy and Sheep & Beef sites. Human or Human & Ruminant account for 15/23 and 17/27 results in Dairy and Sheep & Beef categories, respectively, with 6/23 and 6/27 results with Human MST and no Ruminant markers, respectively. Only Avian MST was measured in 3/27 samples in the Sheep and Beef category.

Table 3 Comparison of land use and dominant MST sources for *Campylobacter* in samples where *E. coli* exceeds 550 MPN/100 mL

	Samples with *Campylobacter*	Human	Human & Ruminant	Ruminant	Avian	Unidentified
Dairy	23	6	9	7	0	1
Urban	33	5	4	18	4	2
Sheep & Beef	27	6	11	6	3	1
Low impact	1	1				
Total sample numbers	**67**	**18**	**24**	**31**	**7**	**4**

In samples containing >550 *E. coli* MPN/100 mL, *Salmonella* was most frequently detected in samples with Human & Ruminant MST markers, 12/26. In total, 19 samples have a Human MST marker; 13 of which are Urban land use. Three samples had ruminant only MST Dairy and three in Sheep & Beef. One site, which was observed as Dairy only, had avian MST.

Table 4 Comparison of land use and dominant MST sources for *Salmonella* in samples where *E. coli* exceeds 550 MPN/100 mL

	Salmonella	**Human**	**Human & Ruminant**	**Ruminant**	**Avian**
Dairy	10		6	3	1
Urban	13	7	6		
Sheep & Beef	3			3	
Low impact	0				
Total sample numbers	**26**	**7**	**12**	**6**	

In conclusion, if only land observation were used to inform the source of faecal contamination, the ruminant contribution in Urban waterbodies and human contribution to Dairy and Sheep & Beef would be missed.

4.2.3 Mixtures of MST

Quantifying MST markers using qPCR has the advantage of being able to identify the significance of the faecal contamination. The prevalence of Avian MST in the samples (90%) is unsurprising as birds are ubiquitous and have freedom of movement. Measuring only Avian MST markers would, therefore, not be a robust sampling strategy (Figure 1). Mixtures of Avian and significant concentrations of Human MST markers occurred in 40 samples and 20 of these samples had significant concentrations of all three MST markers. There were fewer samples (n = 14) where both Avian MST and Ruminant MST markers were detected.

It would not be a robust sampling strategy to measure only MST markers associated with observed land use. Multiple MST markers are particularly useful where a source is unexpected in a designated land use category as exemplified by identification of human MST markers in Dairy or Sheep & Beef or Ruminant MST markers at Urban sites (Figure 1). In terms of management of water quality to protect public health, human faecal contamination has the higher risk because of the potential for the presence of viruses and needs to be prioritised.

In conclusion, human and ruminant MST markers need to be included in water quality investigations to ensure other significant sources of contamination are not overlooked.

4.3 CONCLUSION

This overview of the field data from the pathogen and indicator survey has highlighted the benefits of using MST in conjunction with *E. coli* as the indicator of a faecal contamination event (Leonard et al 2020, Leonard et al 2021). In this limited dataset, *E. coli* indicates a higher presence and higher median concentration of pathogens when the criterion of >550 *E. coli* MPN/100 mL is exceeded. Inclusion of MST markers allows sites which require further investigation to be identified and mitigations implemented.

Collection of data for the freshwater and indicator survey is continuing, and this larger data set will provide more robust analysis to support recommendations for water quality management.

The use of MST adds value in that it enables the actual faecal sources to be investigated beyond what is observed through sanitary surveys. Observed land use has the disadvantage of being a static indicator, whereas the potential for faecal contamination may be variable, or unobserved. The data clearly shows that the faecal source(s) may differ from that inferred from the observed land use. Information from MST markers gathered over multiple sampling occasions provides a powerful tool for identifying significant human and unexpected faecal sources. Comparisons of concentrations between different MST markers is not advised. Not

only because they are measuring different micro-organisms or microbial survival rates, but because the identification of human faecal sources is of significant public health importance. Human sources potentially present the greatest risk to human health. The information gained from these MST investigations enables water quality managers to target interventions more effectively.

5. SUMMARY

FIB alert water quality managers to faecal contamination in waterways and consequently to the potential public health risk. MST markers assist water quality managers to identify source(s) of faecal contamination because of their host specificity to animals.

However, MST markers can have different sensitivity and different non-target species specificity in different geographical areas. It is therefore important that MST markers are tested on faecal material from animal species in New Zealand. A summary of common MST markers used in New Zealand and their cross reactivity is given below.

- Human contamination
    - HF183 indicates human faecal contamination, or possibly cats or chicken, but not ruminants and is detected down to low levels of human contamination
    - high concentrations of BiADO indicates human faecal contamination but may also indicate possums or avian
    - high concentrations of crAssphage indicate human faecal contamination, or possibly cats or seagulls, but not other birds and not ruminants.
- Ruminant contamination
    - high concentrations of *Bacteroidetes* spp. (GenBac3) indicate faecal contamination.
    - high concentrations of the ruminant *Bacteroidetes* MST, BacR indicate the contamination is from ruminants and if the contamination is <42 days then the presence of CowM2 could indicate that the contamination source is bovine specific.
    - high ratio of BacR:GenBac3 (>17%) indicates the majority of contamination is ruminant.

In the field study, more pathogens, with the limited dataset, were measured when the FIB were elevated above the *E. coli* criterion of 550 MPN/100 mL and the likely sources of the pathogens was determined from MST. More data is required for robust statistical analysis. The field study highlights the value of a sampling strategy using multiple MST markers to ensure that there are no unobservable faecal inputs. There were many instances where land use was not relevant to the faecal source present at the time of sampling. Land use is static, but contamination sources vary temporally. Multiple MST are also required because all MST markers have a degree of cross-reaction to other non-host animals. This is the reason why routine monitoring for human contamination utilises three markers that target different microbes in the human intestinal environment.

Both FIB and MST markers have different decay rates to pathogens especially viruses and protozoa, although crAssphage has a longer decay rate compared to other commonly used human MST markers and therefore is a useful addition to the suite of human MST markers. While MST markers can be quantified, different markers cannot be compared as the markers each target different micro-organisms, or microbiological interactions. Not all sources are present at equal risk and investigation of significant concentrations of human MST markers should be a priority due to the potential risk from viruses.

FIB and MST are both useful tools for identifying health risk and management of water quality.

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