MICROBIAL INDICATORS AND PATHOGENS IN OUR WATER: WHERE THEY COME FROM AND WHAT THEY DO

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ABSTRACT

Contamination of drinking water and recreational waters with faecal microorganisms is of high public interest, with billions spent annually managing, preventing or treating this contamination. Globally contaminated water kills an estimated 1-2 million annually and in New Zealand it has been estimated that 100,000 become sick each year from waterborne microorganisms. The organisms causing disease include bacteria such as *Campylobacter jejuni*, *E. coli* O157, and *Salmonella*; protozoa such as *Cryptosporidium* and *Giardia*; and viruses such as norovirus and adenovirus. People who are infected suffer a range of illnesses, which can include diarrhea, fever, vomiting, reactive arthritis, and more serious complications.

The microbial water quality of our drinking water, and our recreational waters are assessed by measuring levels of the indicator bacteria *Escherichia coli* and/or enterococci. These bacteria usually do not cause illness themselves, but they are present in the faeces at high levels, and thus are typically associated with pathogens spread by the faecal oral route. These are useful indicators, and water containing these organisms should be treated as though it is contaminated with pathogens and appropriate action taken.

One key action is to identify the source of the contamination. There are a range of approaches which can be taken, but the most popular world-wide is the application of microbial source tracking methodology based on the detection of DNA from organisms specific to faecal sources. Human sewage or faeces is the highest risk source, and a number of markers are available to detect this source. Zoonotic sources of pathogens include cows, sheep, dogs, and wildfowl which again specific DNA markers are available. When a source of pollution is fresh, and dominated by a single source interpretation is relatively straight forward. However, mixed sources of pollution, aged or partially treated sources, and potential confounding non-faecal sources can make this challenging.

All sources of faecal pollution have a health risk associated with them, and a health risk may exist even in the absence of a detectable faecal source. Therefore, a full assessment may require the direct quantitation of pathogens in a water sample and/or a source. Molecular methods are becoming available which can make this more feasible, although there are issues related to sensitivity and specificity of some methods. Particularly where pathogens aren't detected, there needs to be a robust programme of sampling to ensure this actually reflects the true situation.

We live in a microbial world, and naturally water is an ecosystem for microorganisms. Reducing the disease burdens from contaminated water, requires a thorough understanding of the threat microorganisms can pose, and how changes in the

environment or in management practices can tip the balance from safe water into dangerous water.

KEYWORDS

Pathogens, E. coli, microbial source tracking, waterborne disease

PRESENTER PROFILE

Brent is a molecular microbiologist whose primary research interests include the application of genetic analysis techniques to understanding food and waterborne outbreaks (including outbreaks at Havelock North and Darfield), microbial water quality, faecal source tracking, and zoonoses.

1 INTRODUCTION

1.1 MICROBIAL WATER QUALITY

Drinking and recreational waters may be impacted by faecal contamination from a number of different sources, including the discharge of municipal sewage, seepage from septic tanks, agricultural effluents, stormwater and urban runoff, and direct deposition from birds or domestic or wild animals. The contamination of ways with faecal material may result in the introduction of enteric pathogens (disease-causing bacteria, viruses or protozoa that live in the gut), such as *Campylobacter*, *Salmonella,* norovirus, *Cryptosporidium* or *Giardia* (MfE and MoH, 2003; WHO, 2011; Wood et al., 2016). Human ingestion of contaminated water may result in pathogen ingestion and illness. Illness usually presents as self-limiting gastroenteritis (vomiting, diarrhoea) or respiratory or skin infections. The risk and severity of illness depends on the specific pathogen and dose ingested, and the overall health of the consumer; the risk is greatest for individuals with low immunity, including young children, the elderly, pregnant women, and people who are otherwise immunocompromised (MfE and MoH, 2003; Wood et al., 2016). The risk may also differ based on the source of contamination; faecal contamination of human origin is considered to pose the greatest risk to human health due to the host-specificity of any pathogens, particularly viruses, that are present. However, enteric pathogens from ruminant animals (e.g. cows and sheep) and wildfowl are also known to present a risk to human health (i.e. to be zoonotic) (Soller et al., 2010; Atwill et al., 2012; Devane and Gilpin, 2015).

Direct routine monitoring for the presence of pathogens in waterways is impractical, as pathogens tend to be present in the water at only low levels and are often unevenly distributed, making detection difficult. Further, specific testing for each potential pathogen is expensive and time-consuming, and some pathogens cannot be cultured within the laboratory (Field and Samadpour, 2007; Greening and Lewis, 2010). A simpler and accepted approach to assess microbiological water quality is to monitor the presence of indicator organisms. Indicator organisms are not usually pathogenic themselves, but are indicative of faecal contamination, and therefore the potential presence of faecal pathogens. The most commonly used indicators of faecal contamination are faecal coliforms, *E. coli* and enterococci – bacteria which live in the intestinal tract of humans

and warm-blooded animals, and are found in elevated concentrations in their faeces (MfE and MoH, 2003; Wood et al. 2016). Collectively, these bacteria are referred to as faecal indicator bacteria (FIB). In contrast with pathogen monitoring, the presence of FIB is quick and inexpensive to test. *E. coli* is the preferred indicator organism for monitoring freshwaters (MfE and MoH, 2003).

2 SOURCES OF FAECAL POLLUTION

2.1 ANIMAL FAECES

2.1.1 CATTLE

A number of studies have measured the presence and concentration of faecal indicators and pathogens in the faeces of dairy and beef cattle, and have demonstrated a link between cattle farming and degraded microbial quality of local surface and ground waters (Davies-Colley et al., 2004; Close et al., 2008; Moriarty et al., 2008). For example, *Campylobacter* has been reported in cattle faeces at sites throughout New Zealand, with the percentage of positive animals varying between 11 and 81% (Adhikari et al., 2004; Gilpin et al., 2008). Devane et al. (2005) reported that 98 and 94% of composite samples collected from five dairy and five beef cattle farms contained *Campylobacter*. Studies have also reported the presence of *Salmonella enterica* (Sinton et al., 2007), Shiga toxin-producing *E. coli* (STEC; Cookson et al., 2006), *Cryptosporidium* (Grinberg et al., 2005) and *Giardia* (Learmonth et al., 2003) in bovine faeces. In a survey of New Zealand dairy farms, Moriarty et al. (2008) reported median bacterial counts of 10^6 *E. coli* and 10^5 *Campylobacter* per gram of faeces, although counts were highly variable for individual samples. Low levels of STEC, *Cryptosporidium* and *Giardia* were also detected.

2.1.2 SHEEP

In New Zealand, an estimated 32 million sheep graze on open pasture (Moriarty et al. 2011c), and have been implicated as significant contributors to the microbial loading of freshwaters (MfE and MoH, 2003; Davies et al., 2004; Devane et al., 2005; McDowell, 2006). It has been suggested that in some instances, the total *E. coli* burden per hectare of pasture is higher for land being grazed by sheep than by cattle (Wilcock, 2006). Sheep are known to harbour a range of microbial pathogens, including *Campylobacter* (Milnes et al., 2008), STEC (Kudva et al., 1998), *Giardia* (Castro-Hermida et al., 2007), and *Cryptosporidium* (Castro-Hermida et al., 2007; Milnes et al. 2008). There is some evidence that many of the ovine *Cryptosporidium* and *Giardia* genotypes may not be zoonotic (Ryan et al. 2005).

Moriarty et al. (2011c) undertook a survey of microbial indicators and pathogens in the faeces of New Zealand sheep and lambs. They determined that lamb faeces contain 10-100 times the concentration of *E. coli*, enterococci and *Campylobacter* than sheep faeces. Further, the prevalence of *Campylobacter*, *Salmonella* and STEC was higher in lambs than in sheep. For example, *Campylobacter* was present in 81% and 30% of lambs and sheep, respectively, with mean concentrations of 10^5 and 10^3 per gram of faeces. Further, 29% and 4% of lamb and sheep samples were positive for *Cryptosporidium*, while mean *E. coli* loads were 10^8 per gram for lambs and 10^7 per gram for sheep.

2.1.3 OTHER RUMINANTS

Compared with other ruminants, information as to the microbial burden of equine faeces is limited. Several studies have enumerated *E. coli* in horse faces: Weaver et al. (2005) reported a mean concentration of 3.0×10^5 cfu/g wet weight, while Moriarty et al. (2015) reported a concentration of 1.2×10^5 cfu/g dry weight. Other studies have isolated potentially zoonotic strains of *Cryptosporidium* spp. and *Giardia* spp. (Grinberg et al., 2009), *Salmonella* spp. (Jay-Russell et al., 2014), STEC (Pritchard et al., 2009) and *Campylobacter* spp. (Moriarty et al., 2015). The prevalence of zoonotic microorganisms in horse faeces varies significantly between pathogens, as well as between studies.

Few studies have investigated the microbial content of deer faeces. Pattis et al. (2017) reported that in a survey of faecal samples from red deer, *E. coli* was present in all samples, with an average concentration of 10^8 cfu/g wet weight. *Campylobacter* was isolated in 13% of samples. *Yersinia* and *Cryptosporidium* have also been associated with deer populations (Ball and Till, 1998).

2.1.4 AVIAN FAECES

A range of potentially zoonotic pathogens have been isolated from the faeces of wildfowl. For example, *Campylobacter*, *Cryptosporidium*, *Bacillus cereus* and *Clostridium perfringens* have been recovered from New Zealand ducks (Murphy et al., 2005; Moriarty et al., 2011a). *Salmonella*, *Vibrio*, *Listeria* and *Campylobacter* have been recovered from various gull species (Moriarty et al., 2011a), and *Campylobacter* and *Cryptosporidium* from black swans (Moriarty et al., 2011a). *Salmonella*, *Giardia*, *Cryptosporidium* and *Campylobacter* have been isolated from Canada geese (Moriarty et al., 2011a); Moriarty et al. (2011a) reported that 40% of Canada geese faecal samples collected were positive for *Campylobacter*, at concentrations up to 10^5 MPN/g dry weight.

2.1.5 HUMAN SOURCES

Human sewage contains high concentrations of indicator organisms, including *E. coli* (approximately 10^6-10^8 per 100 ml). A range of pathogenic microorganisms, including *Campylobacter*, *Salmonella*, *Shigella*, norovirus, rotavirus, adenovirus, *Cryptosporidium* and *Giardia* may also be present if these are present in the source population.

Most human waste in New Zealand is treated by municipal sewage treatment systems before being discharged to the environment, typically a waterway or the coastal marine environment. Waste may also be treated in on-site septic systems. Untreated or partially-treated human waste may enter the environment through inadequate treatment, or via urban runoff or combined sewer overflows (CSO), where both sewage and stormwater flow in the same pipe to the treatment plant; after heavy rainfall, their combined volume may exceed the capacity of the plant and be discharged directly to the environment. Waste may also enter waterways from failing septic tanks (e.g. through leaking systems or ineffective treatment) or leaking sewerage pipes, and subsequent subsurface flow through the soil. A report prepared for the Ministry for the Environment (MfE, 2008) estimated that between 15 and 50% of septic tanks, particularly aging systems, are susceptible to failure.

Estimating the prevalence and abundance of pathogens in human sewage is complex, and dependent on whether the sewage is raw or treated, and the type of treatment that has been undertaken (Soller et al., 2010).

3 FAECAL SOURCE TRACKING

Whilst the detection of FIB provides an indication that water is contaminated with faecal material, and thus there is a risk of pathogens being present, it does not identify the source(s) of contamination. Discriminating between human and non-human sources of faecal contamination, and/or the subsequent identification of the animal species are essential components of effective water quality management (Cornelisen et al., 2011). Faecal source attribution allows for risk assessment and targeted mitigations. For example, human contamination is considered to pose a greater risk than wildfowl contamination. The 'toolbox' of analyses involved in determining the origin of faecal contamination is known as Faecal Source Tracking (FST), and includes microbial and chemical methods (Harwood et al., 2013).

Microbial methods look to identify the presence of microorganisms that are specific to the gut of a certain host animal. There is a wide range of microorganisms other than the traditional faecal indicators (i.e. coliforms, *E. coli* and enterococci), that are present in animal faeces, and some of these are specific to certain animals. Although these organisms are often difficult to culture in the laboratory, it is possible to extract the total DNA from a water sample and use polymerase chain reaction (PCR) to identify gene fragments ('markers') that are unique to these host-associated microorganisms. However, while many markers are strongly associated with an animal source, they each have a degree of non-specificity (Devane et al., 2013; Harwood et al., 2013). Chemical FST methods include analysis of faecal sterol and stanol fingerprints, which differ between human and animal sources, and compounds associated with anthropogenic pollution, such as caffeine, synthetic drugs (e.g. contraceptives) and fluorescent whitening agents (Scott et al., 2002).

4 CONCLUSIONS

We live in a microbial world, and naturally water is an ecosystem for microorganisms. Reducing the disease burdens from contaminated water, requires a thorough understanding of the threat microorganisms can pose, and how changes in the environment or in management practices can tip the balance from safe water into dangerous water.

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